PHYTOCHEMICAL, ANTIOXIDANT ACTIVITY AND ANTIBACTERIAL EVALUATION OF *FICUS RELIGIOSA*

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MASTER OF SCIENCE IN BOTANY

By

N. AMIRTHA ROSELIN

Reg.No. 19APBO01

DEPARTMENT OF BOTANY

ST. MARY'S COLLEGE (Autonomous)

THOOTHUKUDI -628001

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CERTIFICATE

This is to certify that this dissertation entitled, **PHYTOCHEMICAL, ANTIOXIDANT ACTIVITY AND ANTIBACTERIAL EVALUATION OF *FICUS RELIGIOSA*** submitted by **N.AMIRTHA ROSELIN Reg. No. 19APBO01** to ST. MARY'S COLLEGE (Autonomous), THOOTHUKUDI in partial fulfillment for the award of the degree of **"Master of Science in Botany"** is done by her under my supervision. It is further certified that this dissertation or any part of this has not been submitted elsewhere for any other degree.

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HOD

HOD of Botany
St. Mary's College (Autonomous)
Thoothukudi-628 001.

PRINCIPAL

St. Mary's College (Autonomous)
Thoothukudi - 628 001.

DECLARATION

I do here by declare that this dissertation entitled, "**PHYTOCHEMICAL, ANTIOXIDANT ACTIVITY AND ANTIBACTERIAL EVALUATION OF *FICUS RELIGIOSA***" submitted by me in partial fulfillment for the award of the degree of **Master of Science in Botany** is the result of my original and independent work carried out under the guidance of **Dr. A. Jacintha Tamil Malar M.Sc., M.Phil., Ph.D.** Assistant Professor, Department of Botany, St. Mary's College (Autonomous), Thoothukudi and it has not been submitted elsewhere for the award of any other degree.

N. Amirtha Roselin

Station : Thoothukudi **(N.Amirtha Roselin)**

Date : 15.04.2021

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CONTENT

INTRODUCTION

INTRODUCTION

Nature has bestowed us with colossal variety of plants which are medicinally useful. Medicinal plants are naturally gifted with valuable bioactive compounds which form the backbone of traditional medicines. *Ficus religiosa* is one of the important traditional medicine that has been used as a remedy for various diseases since antiquity and still offers a source of biologically active chemical compounds.

The genus *Ficus* consists of about 800 species and 2000 varieties which are widely distributed in India and throughout the world especially in tropical and sub-tropical regions. *Ficus religiosa* Linn is commonly known as Peepal belongs to family Moraceae. In India, since ancient times it has got great mythological, religious and medical importance. This is considered as the oldest tree in Indian art literature. There are many chemical compounds which have been extracted out from different plants as they as they have very important use in medicinal field. These compounds play a very important role in as medicinal usages is increasing worldwide very rapidly, it is very important to get alternate sources of drugs with effective results and with no side effects.

F. religiosa has got mythological, medical and religious importance since ancient times. According to religious point of view, *F. religiosa* is very important as it is believed that Gautam Buddha achieved enlightenment under this tree. Because of this, it is also named as "Bodhi tree or Bo tree". In medicinal field, *F. religiosa* is gaining great attention because it has many compounds which are beneficial in treatment of many diseases like diabetes, skin diseases, respiratory disorders, central nervous system disorder, gastric problems.

Ficus religiosa and its medicinal values have been well documented in traditional and folklore medicine. It is a constituent of Ayurvedic formulations namely Nalapamaradi

tailum, Nyagrodhadi churna, Chandanasavam Sari bady asavam, Shankha vati, Chandraprabha vati and Kaminividravan rasa. *F. religiosa* is native to Indian subcontinent and southeast Asia. It is found in India, Bangladesh, Nepal, Sri Lanka and Pakistan. It is known by more than 150 names and also has multiple names in the same language. Its synonyms include: Sanskrit - Ashwattha, English -Sacred fig , Marathi - Pimpala , Hindi - Peepal and Tamil - Achuvattam . *Ficus religiosa* is mostly planted near religious or spiritual places in Indian cities and villages as it holds great relevance in Indian culture, mythology and religion. It is considered sacred by followers of Hinduism, Buddhism and Jainism..

In Togo, plants are widely used in traditional medicine, especially in the rural areas. Among the plants used in Togolese pharmacopoeia, there is *Ficus* religiosa (Moraceae) commonly called "Petit sycamore" in French and "Wild fig" in English. This plant is usually found in tropical and subtropical areas along the rivers. The plant can grow up to 30-35 m of height while trunk diameter can reach 150 cm. The stem and leaves of *Ficus* are used as food in the northern Togo. In addition, several medicinal properties have been attributed to various parts of the plant since its roots, barks, leaves, and fruits are used to treat, relieve and heal several pathologies. Indeed, the roots are braised and crushed with grilled corn cobs, then the sieved are used against female infertility. The crushed and macerated fruits in water are used for the care of women during childbirth and after delivery (promotion of lactation). The beverage obtained by decoction after mixing roots and leafy twigs is used to treat eczema. Decocted trunk bark is used against amenorrhea, dysentery, hepatic, and cardiovascular pain. It is known that oxidative stress is involved in many inflammatory processes related to chronic diseases such as cardiac dysfunction, neurodegenerative diseases or diabetes. Indeed, a large number of phenolic compounds such as flavonoids, isoflavones, and phenolic acids have shown antioxidant activity. Moreover, the benefits of phenolic antioxidant compounds

from plants in prevention of chronic diseases have been reported.

Interest in medicinal plants with antibacterial properties has revived due to several problems associated with the use of antibiotics and development of resistant strains of infectious microorganisms. But still, there are small number of drugs and formulations available. In order to promote the use of medicinal plants as potential sources of antimicrobial compounds, it is pertinent to thoroughly investigate their activity and standardize the crude drug powder/herbal extract to monitor the quality. Literature survey reveals reports about antimicrobial, antioxidant activities of different parts of *F.religiosa* The ethno pharmacological knowledge supported by experimental base can be used as a discovery system for affordable, safer and newer drug formulations. In view of these facts, the present investigation is under taken

- To carry out preliminary phytochemical screening to establish the phytoconstituents present in the leaves of *Ficus religiosa.*
- To analyse the functional groups of *Ficus religiosa* by FT- IR.
- To evaluate quantitatively flavonoids, protein, carbohydrate, vitamin C and vitamin E content in the leaves of *Ficus religiosa.*
- To evaluate antioxidant DPPH radical scavenging activity of ethanol, water, chloroform, acetone, petroleum ether and hexane extracts of the leaves of *Ficus religiosa.*
- To evaluate the antibacterial activity of *Ficus religiosa.*

LITERATURE REVIEW

LITERATURE REVIEW

Herbs have always been the principal form of medicine in India. Medicinal plants have curative properties due to the presence of various complex chemical substances of different composition, which are found as secondary plant metabolites in one or more parts of these plants. *Ficus religiosa* (L.), commonly known as peepal belonging to the family Moraceae, is used traditionally as antiulcer, antibacterial, antidiabetic, in the treatment of gonorrhea and skin diseases. *F. religiosa* is a Bo tree, which sheltered the Buddha as he divined the "Truths." The present review aims to update information on its phytochemistry and pharmacological activities (Chandrasekar *et al.*,2019).

Medicinal plants play a vital role in improving health of people. Hundreds of medicinal plants have been used to cure various diseases since ancient times. *Ficus religiosa* (Peepal) has an important place among herbal plants. Almost every part of this tree i.e., leaves, bark, seeds and fruits are used in the preparation of herbal medicines. Therapeutic properties of this tree in curing a wide range of diseases can be attributed to its richness in bioactive compounds namely flavonoids, alkaloids, tannins, saponins, phenols etc. Its antimicrobial, anti-diabetic, anticonvulsant, wound healing, anti-inflammatory and analgesic properties have made it a popular herbal tree and its parts are placed as important ingredient in modern pharmacological industry. (Dimple *et al.*,2018).

In traditional medicine, medicinal plants have been used for the treatment of various diseases. *Ficus religiosa* is known to be a sacred plant in India. Since very ancient time, it has great medicinal and religious significance. In Ayurveda, Unani and Homeopathy, this plant serves as important source of medicine. The various parts of the plants like stem bark, fruits, buds, latex are used in treatment of different diseases like dysentery, mumps, jaundice, heart diseases, constipation, skin diseases, etc. According to

Ayurvedic system of medicine, *F. religiosa* (Peepal tree) is well known to be useful in diabetes. Since last couple of years, it has also been investigated for the presence of various phytoconstituents (phenolics, sterols, flavonoids etc). *F.* religiosa showed diverse range of pharmacological activities like, anticonvulsant, antidiabetic, anti-inflammatory, antimicrobial, analgesic, wound-healing, antioxidant, acetylcholinesterase, proteolytic, and anti-amnesic. The present review is to compile up-to-date information of this plant that covers its natural phytochemical, biochemical, ethnobotanical and pharmacological significance (Bhagyawant, 2014).

Ficus religiosa Linn is a large evergreen tree found throughout India, wild as well as cultivated. It is popular indigenous system of medicine like Ayurveda, Siddha, Unani and Homeopathy. In traditional system of medicine, various parts such as stem, leaves, are used in diabetes, vomiting, burns, gynecological problems, dysentery, diarrhea, nervous disorders, tonic and astringent. Phytochemical investigation of plant barks, showed the presence carbohydrates, protein, flavonoids (Sharma, 2013).

Herbal medicine is one of the oldest valuable bestowals that was given to mankind. Many plants and herbs hold their prestigious position in the field of medicine among which *Ficus religiosa* belonging to the family Moraceae is the vital one. It is found all over India, as it is one of the sacred tree worshipped by Hindus. The different parts of the *F. religiosa* species tree namely stem, leaves, are used as chief indigenous medicines to cure various ailments. Recent studies have reported that *F. religiosa* is used in the traditional medicine to relieve about 50 types of disorders including diabetes, diarrhea, epilepsy inflammatory disorders, and gastric problems, sexual and infectious disorders (Arumugam *et al.*, 2012).

With the concern of adverse effects of allopathic medicines and development of resistance by bacteria, search for the new antibiotics is necessary. Traditional medicines offer better alternatives but their standardization is difficult. It is popular in the indigenous systems of medicine like Ayurveda, Siddha and Unani. *Ficus religiosa* is an important Indian traditional medicine. It is known to be effective for more than 50 diseases such as cancer, inflammation, ulcer, convulsion and diabetes (Pankaj ,2019).

Ficus religiosa is known to be a sacred plant in India and since ancient times it is widely being used to treat various ailments likes kind diseases, heart diseases, diabetes, vomiting, burns, nervous disorder, constipation, dysentery, snake bite and important constituent of various traditional herbal preparations like shankhavati,chandraprab havati and kaminivin dravanrasa (Satish *et al.*, 2014).

F. religiosa is a traditional religious plant in India and is used in treatment of several health ailments as a home-based remedy either singly or in combination with other herbs (Herry, 2016). *F. religiosa*. is widely branched tree with leathery, heart-shaped, long tipped leaves. It is one of the most versatile plants having a wide variety of medicinal activities, therefore, used in the treatment of several types of diseases, *e.g.*, diarrhea, diabetes, urinary disorders, burns, hemorrhoids, skin diseases, convulsions, tuberculosis, fever, paralysis, oxidative stress, bacterial infections, etc. (Singh *et al.*,2016).

Ficus religiosa has been extensively used in traditional medicine for a wide range of ailments. Its bark, fruits, leaves, roots, latex and seeds are medicinally used in different forms, sometimes in combination with other herbs (Aiyegoro and Okoh, 2009). The therapeutic utilities of *F.religiosa* have been indicated in traditional systems of medicine like Ayurveda, Unani, Siddha, etc. (Singh *et al.*, 2011). *F. religiosa* has been reported to have medicinal properties like antibacterial, anti-diabetic, anti-amnesic, anti-ulcer and anti-oxidant properties (Gautam *et al.*, 2014).

The entire parts of the *F. religiosa* exhibit a wide spectrum of medicinal importance as an anticancer, antioxidant, antidiabetic, antimicrobial, anticonvulsant, anthelmintic, antiulcer, antiasthmatic, anti-amnesic etc (Makhija *et al.*, 2010). Stem can be used in treatment of urinary disorders and problems of digestive system. The dried powder of fruits has been used in treatment of respiratory problems like asthma (Panchawat, 2014).

The Bark is cooling and astringent and is useful in inflammation and glandular swellings of neck. The paste of powdered bark is used in cases of anal fistula and as absorbent for inflammatory swellings and also used in burns (Warrier *et al.*, 1995). The bark of *Ficus religiosa* is reported to possess antiulcer and wound healing activities (Khan *et al.*, 2011,). Bark is used in diabetes, diarrhea, leucorrhea, anxiety, for vaginal and other urinogenital disorders and to improve the complexion (Pandit *et al.*, 2010). Powder of stem bark of *F. religiosa* is considered more effective if taken with honey, before or after meal (Anupama, 2014).

The leaves contain phytochemicals such as flavonoids, terpenoids, tannins etc., which are effective in curing ailments like hiccups, vomiting, gonorrhea etc. (Bhalerao and Sharma, 2014). The leaves alone are used to treat constipation. The leaves used together with young shoots are act as strong laxative. In Nepal leaf juice with honey is used for multipurpose such as for diarrhoea, asthma, cough, earache, toothache, and migraine, in gastric problems and in hematuria (Kunwar and Bussmann, 2006).

The fruit powder enhances fertility rate and is used in the treatment of dysentery, uterine troubles, ulcers, biliousness, bitter tonic, in blood diseases. The ripe fruit is used as tonic, alexipharmic, suitable for burning sensation, biliousness and diseases of blood and heart (De Feudis *et al.*, 2003). The seeds and fruits are digestive, laxative and refrigerant. The

dried fruit, pulverized and taken in water for a fortnight removes asthma. The ripe fruit is cold in potency and good for burning sensation. It act as cardiac tonic and is useful to cure the diseases of Vagina. It also cures vomiting, anorexia and edema (Singh *et al.*, 2008).

Phytochemical :

Medicinal plants play a vital role in improving health of people. Hundreds of medicinal plants have been used to cure various diseases since ancient times. *Ficus religiosa* (Peepal) has an important place among herbal plants. Almost every part of this tree i.e. leaves, stem are used in the preparation of herbal medicines. Therapeutic properties of this tree in curing a wide range of diseases can be attributed to its richness in bioactive compounds namely flavonoids, protein, carbohydrates, vitamin E, vitamin C, etc. Its antimicrobial, analgesic properties have made it a popular herbal tree and its parts are placed as important ingredient in modern pharmacological industry (Ashwani Kumar 2018).

The aqueous extract of the bark, leaves, stem and fruits of *Ficus religiosa* were screened for their phytochemical and antimicrobial activity and showed the presence of carbohydrates, protein, flavonoids and vitamin c, vitamin E. The disc diffusion method was used to screen for the antimicrobial activity of the pathogens. (Rathish *et al.*,2012).

Phytochemical analysis indicated the presence of glycosides, alkaloids, saponins, tannins, flavonoids, vitamin c , vitamin E and phenols in the extracts. Chemical analysis showed that *Ficus religiosa* contained carbohydrates, protein, flavonoids. Previous pharmacological studies revealed that *Ficus religiosa*. (Ali Esmail *et al.*,2017). *F.* religiosa is rich in tannins, saponins, flavonoids, steroids, terpenoids and cardiac glycosides, wax etc (Singh *et al.*, 2015).

The barks of *Ficus religiosa* L., were investigated for invitro antibacterial activity and phytochemical analysis. The various solvents extract like aqueous, methanol, chloroform, petroleum ether and hexane were screened for The preliminary phytochemical analysis of the methanol extracts of both the plants showed the presence of carbohydrates, flavonoids, protein, vitamin c and vitamin E (Uma,2009). The bark contains phytochemicals like tannins, saponins, flavonoids etc. which show beneficial effects in health conditions such as diarrhea, dysentery, inflammation, bacterial infections, bleeding and paralysis (Singh and Jaiswal, 2014).

Phytochemicals constituents were abundant in methanolic leaves extracts of *F. religiosa* The characterization of functional groups were analyzed using FT-IR spectroscopy (Gopukumar *et al.*,2016). The leaves have been reported to have bioactive compounds (campestrol, stigmasterol, isofucosterol, tannins, arginine, serine, aspartic acid, glycine, threonine, alanine, proline, tryptophan, tyrosine, methionine, valine, isoleucine) which help in preventing gastric problems. (Rutuja *et al.*, 2015).

Rajiv and Sivaraj (2012) studied different parts of the *Ficus religiosa* mainly bark, fruit, leaves, stem by making aqueous extracts. The aqueous extracts of selected parts were used to screen for their phytochemical and antimicrobial activity and showed presence of many important phytochemicals such as alkaloids, phenols, sugar, terpenoids, glycosides, flavonoids and tannins.

Antioxidant:

The ethanolic extract of leaves of *Ficus religiosa* was evaluated for antioxidant (DPPH) activity. The tested extract of different dilutions in the range 200 µg/ml to 1000 µg/ml shows antioxidant activity in a range of 6.34% to 13.35% (Charde *et al.*,2010). *Ficus religiosa*, plant from Ficus species used traditionally for the treatment of various

ailments. Shaikh, (2018) investigated in vitro antioxidant activity of three plants from Ficus species and effect of extracting solvents, total flavonoids and phenolics content on its in vitro activity.

Zeb, (2016) evaluated different solvent fractions of stem bark of *Ficus religiosa* L. for antioxidant potential of the extracts by DPPH radical scavenging assay. The plant showed significant antifungal activity with highest activity observed for the n-butanol fraction showing 66 to 77.5% inhibition of fungal growth at the tested concentrations. Least active was the ethyl acetate fraction with % inhibition ranging from 47.5% to 62.5% at the tested concentrations. The extracts also showed significant antioxidant activity with highest activity of 91.71% observed for dichloromethane fraction.

Measurement of total phenolic content of the methanolic extract of *Ficus religiosa* fruit was achieved using Folin<Ciocalteau reagent containing 0.2%w/w of phenolic content, which was found significant. The results obtained in this study clearly indicate that *Ficus religiosa* fruits have a significant potential to use as a natural antioxidant agent. The overall results of this study indicates that the various extract conc. from *Ficus religiosa* fruits have interesting antioxidative properties and represent a potential source of medicine for the treatment of inflammatory diseases (Permender ,2010).

Antioxidant activity were determined by spectrophotometric method while the alkaloid content was evaluated by titrimetric method. The amount of total phenolic in extracts and fractions were estimated in comparison to gallic acid, whereas total flavonoids, tannins and saponins were estimated corresponding to quercetin, tannic acid and saponin respectively. 2, 2-diphenylpicryl hydrazyl radical (DPPH)* and phosphomolybdate methods were used to evaluate the antioxidant activities of leaf and stem bark of *F. religiosa*. Phytochemical screening revealed the presence of flavonoids,

saponins, terpenoids/steroids, alkaloids for both extracts of leaf and stem bark of *F.* religiosa. The phenolic content of *F.religiosa* was most abundant in leaf ethanol crude extract as 3.53 ± 0.03 mg/g equivalent of gallic acid. Total flavonoids and tannins content were highest in stem bark aqueous ethanol fraction of F. religiosa estimated as 3.41 ± 0.08 mg/g equivalent of quercetin and 1.52 ± 0.05 mg/g equivalent of tannic acid respectively. The hexane leaf fraction of *F. religiosa* the utmost saponin and alkaloid content as 5.10 ± 0.48 mg/g equivalent of saponins and0.171 ± 0.39 g of alkaloids. Leaf aqueous ethanol fraction of *F.* religiosa. showed high antioxidant activity (IC50 value of 63.092 µg/mL) and stem ethanol crude extract (227.43 ± 0.78 mg/g equivalent of ascorbic acid) for DPPH and phosphomolybdate method respectively and the least active was found to be the stem hexane fraction using both methods (313.32 µg/mL; 16.21 ± 1.30 mg/g equivalent of ascorbic acid (Taiw, 2020).

Anti bacterial activity:

The aqueous, methanol and chloroform extracts of the leaves of *Ficus religiosa* were evaluated for their antibacterial and antitumor activities. These extracts showed an elevated level of antibacterial activity and reduced antifungal activity. The most sensitive organisms *S. typhi*, *P. vulgaris*, *S. typhimurium*, and *E. coli* were inhibited even at lowest concentrations of the chloroform extracts. Aqueous and methanolic extracts were found to be less active. The antitumor activity conducted by crown gall potato disc assay proved that all the three extracts are efficient in reducing the tumors formed (Khan et al.,2011).

Rathish *et al* (2012) evaluated antimicrobial activity of *Ficus religiosa* against *Escherichia coli, Enterobacter aerogenes, Pseudomonas aeruginosa, Aeromonas hydrophila, Staphylococcus aureus, Streptococcus pyogenes, Aspergillus niger* and *Candida albicans* at various concentrations. The highest zone of inhibition (10 mm -15 mm in diameter) was observed in 100 mg/ml concentration in all tested microbes.

The extracts *Ficus religiosa* were subjected for antimicrobial activity against E.*coli* using agar well diffusion method. Aqueous and methanolic extract showed a zone of inhibition of 10 mm and 12 mm respectively (Shivali *et al.*, 2017). The leaf extracts were screened for antimicrobial activity against *Escherichia coli, Bacillus subtilis*, minimum inhibitory concentration was determined for ethanolic extract against S. *aureus* and B. *subitilis*. In all the extracts, significant antimicrobial activity was observed against S. *aureus* and B. *subtilis*, MIC of ethanolic extract was found to be 25 mg/mL and 12 mg/mL against S. *aureus* and B. *subtilis* respectively. It presents scientific proof of its antimicrobial activity and different quality control parameters in herbal drug standardization (Pankaj, 2019).

The antimicrobial activity of ethanolic extracts of *F. religiosa* (leaves) was examined using the agar well diffusion method. The test was performed against four bacteria: *Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa* and against two fungi: *Candida albicans* and *Aspergillus niger*. The results showed that 25mg/ml of the extract was active against all bacterial strains and effect against the two fungi was comparatively much less (Farrukh *et al.*, 2003).

The antibacterial potential of *Ficus religiosa* was investigated by Hemaiswarya *et al.* (2009). According to this study the chloroform extract of the leaves of *Ficus religiosa* inhibited the growth of various Salmonella species, P. *vulgaris, E. coli, B. Subtilis and K.*

Pneumonia etc which revealed the antibacterial potential of the plant. Uma *et al.* (2009) evaluated the extracts (methanol, aqueous, chloroform) of the bark of *Ficus religiosa* and reported that it has inhibitory effect on the growth of three enteroxigenic E. coli, isolated from the patients suffering from diarrhoea.

Ramakrishnaiah and Hariprasad (2013) investigated the antimicrobial activity of *Ficus religiosa* by measuring the zone of inhibition (ZoI) produced by two types of solvent extracts namely methanol and diethyl ether extracts of bark and leaves, on three bacteria (two Gram negative bacteria (*E.coli and Pseudomonas aeruginosa*), one Gram positive bacteria (Staphylococcus aureus) and one fungus (*Aspergillus niger*). Swami and Bisht (1996) proved that the furanocoumarins (bergapten and bergaptol) isolated from the bark of *F.religiosa* had activity against *S.aureus, E.coli, Penicillium gluacum* and even a protozoan namely *Paramecium* (Swami and Bisht, 1996). The activity of chloroform extract of fruits was investigated against *P.aureus, A.chroococcum, K.pneumonia, S.lactic* and *B.megaterium* (Mousa *et al.*, 1994).

Salem *et al.* (2013) reviewed the different antimicrobial activities and phytochemical composition of extracts of *Ficus* spp. To study the antimicrobial activities, different antimicrobial methods such as disc and well diffusion, minimum inhibitory concentration (MIC), minimum bacterial concentrations (MBC) were used for the evaluation of different extracts. This review gives the idea of which solvent works potentially against different pathogenic microorganisms. Aqueous extracts showed high antimicrobial activity against *B.subtilis* and multi drug resistant *P.aeroginosa*. Ethanolic leaves extract was successful to inhibit wide range of microorganisms which mainly includes *B.subtilis, S.aureus, P.aeroginosa, E.coli* but failed to show any action against *C.albicans* and *A.niger*. The fruit extracts have high potential towards antibacterial activity but no antifungal activity

The acetone, methanol and the ethyl acetate extracts of the bark powder of *Ficus religiosa* were checked for antibacterial activity against some medically important bacteria *Pseudomonas aeruginosa, Escherichia coli, Proteus vulgaris, Bacillus subtilis* and *Staphylococcus aureus*. The antimicrobial assay was performed by agar disc diffusion assay. It was observed that methanol extracts had activity against *B.subtilis, E.coli, P.vulgaris, S.aureus* whereas acetone extracts showed more antibacterial activity only against *B.subtilis, E.coli*. The ethyl actetate extracts were found to have no activity against any of the tested bacteria (Manimozhi et al., 2012).

Tambekar *et al.* (2013) carried out the studies on antimicrobial potential and phytochemical analysis of medicinal plants from Lonar Lake. . In case of *Ficus religiosa* aqueous and ethanolic leaves extracts showed antibacterial activity against *E.coli and P.vulgaris* . Nair and Chanda (2007) conducted studies and found that the aqueous and ethanol extract of bark had activity against *P.mirabilis, S.aureus, A.fecalis* and *S. typhimurium*. A study was carried out by Supriya and Harshita (2013) in which extracts of dried powdered leaves of *Ficus religiosa* in petroleum ether, chloroform, methanol and water was made. These extracts were then subjected for in vivo antimicrobial activity against *E.coli* and *S.aureus* by cup plate diffusion technique in which wells were bore in the agar plates that were flooded with the bacterial culture and the extract was filled into these wells.

MATERIALS AND METHODS

MATERIALS AND METHODS

Collection and identification of plant materials

The leaves of *Ficus religiosa* were collected from Thoothukudi. The collected samples were cut into small fragments and shade dried until the fracture is uniform and smooth. The dried leaf material was granulated or powdered by using a blender, and sieved to get uniform particles by using sieve No. 60. The final uniform powder was used for extraction of active constituents of the plant materials.

Morphological studies:

Morphology parameters like plant height, leaves size and shape, phyllotaxy, inflorescence, size of flowers, colour were noted.

Qualitative analysis:

Preparation of the plant extracts:

5 gram of *Ficus religiosa* leaf samples are extracted separately with aqueous, ethanol, petroleum ether, hexane, acetone and chloroform by maceration pubescence (24 hrs for each solvent) with constant shaking, the homogenates are filtered through Whatman No 2 paper and the extract are stored at 4°c the extract thus obtained are used for various analyses.

Preliminary phytochemical Screening of different extracts (Harbrone, 1998)

The qualitative phytochemical test for alkaloids, flavonoids, tannins, sterols, saponins, quinones, phenols, Terpenoids, coumarins, betacyanins, anthraquinone, carbohydrates, glycosides, proteins are carried out in the concentrated extract using the standard procedures to identify the constitutes in the leaf extract of *Ficus religiosa*. The chemical test for various phytoconstituents in the extract are carried out as described below.

Phytochemical analysis.

Test for tannins (Ciulei I.)

To 1 ml of the extract, 2 ml of 5% FeCl3 was added. A dark blue or green -black indicates the presence of tannins.

Test for saponins (kokate,1999)

Foam test the crude extract is mixed with 5 ml of distilled water and shaken vigorously, resulting in the formation of a stable foam which is a positive indication for saponins.

Test for Flavonoids (Savithramma *et al.*,)

For identification of flavonoids, 2ml of plant extract, 1ml of 2N sodium hydroxide (NaOH) was added. Formation of yellow colour indicates the presence of flavonoids.

Test for Coumarins (Harbrone JB)

For identification of coumarins, 1ml of plant extract, 1ml of 10% NaOH was added. Formation of yellow colour indicates the presence of coumarins.

Test for Terpenoids (Harbrone JB)

For identification of terpenoids, 0.5 ml of the plant extract, 2ml of chloroform along with concentrated Sulphuric acid. Formation of red brown colour at the interface indicates the presence of Terpenoids.

Test for Quinines (P. D. Egwaikhide and C. E. Gimba)

A small amount of extract was treated with concentrated HCl and observed for theformation of yellow colour precipitate.

Test for Alkaloids (E. C. G. Clarke)

Wagner's test

A fraction of extract was treated with Wagner's reagent (1.27 g of iodine and 2 g of potassium iodide in 100 mL water) and observed for the formation of reddish brown

colourprecipitate. There was a formation of reddish brown colour confirming the presence of alkaloid.

Test for Sterols (P. D. Egwaikhide and C. E. Gimba)

Extract (1 mL) was treated with chloroform, acetic anhydride and drops of H_2SO_4 was added and observed for the formation of dark pink or red colour. No dark pink or red colour precipitate, absence of sterols.

Test for Carbohydrate (Harbrone JB)

Fehling's test

5 ml of Fehling's solution was added to 0.5 mg of extract and boiled in a water bath. The formation of yellow or red precipitate indicates the presence of reducing sugars.

Test for Glycosides (E. C. G. Clarke)

0.5 mg of extract was dissolved in 1 ml of water and then aqueous NaoH solution was added. Formation of yellow colour indicates the presence of glycosides.

Test for Protein (Harbrone JB)

Ninhydrin test:

0.5 mg of extract was taken and 2 drops of freshly prepared 0.2% ninhydrin reagent was added and heated. The appearance of pink or purple colour indicates the presence of proteins, peptides or amino acids. Test for phenol (Harbrone JB) To 1 ml of the extract, 2 ml of distilled water was added and followed by few drops of 10% aqueous ferric chloride. Appearance of blue or green colour indicates the presence of phenols.

QUANTITATIVE ESTIMATION

Total Soluble Protein (Lowry *et.al.*,1951)

Requirements

- Alkaline copper reagent
- Solution A- 20% Sodium carbonate in 0.1 N sodium hydroxide

- Solution B- 1% Sodium potassium tartarate
- Solution C- 0.5% copper sulphate

To prepare 100 ml alkaline copper reagent, 98 ml of solution A, 1ml of B and 1 ml of C were mixed together freshly. Folin-ciacalteau reagent (commercial reagent was diluted in the ratio of 1:1 with distilled water at the time of use).

Procedure

100 mg of sample was homogenized in 10 ml of distilled water and filtered through a muslin cloth and centrifuged at 3000 rpm for 10 minutes. To the supernatant 10% trichloro acetic acid (TCA) was added in 1:1 ratio and left in an ice bath for 30 minutes to precipitate protein. Then centrifuged at 3000 rpm for 5 minutes and discarded the supernatant. The precipitate was dissolved in 0.1N sodium hydroxide and diluted to a known volume.

To 0.5 ml of protein extract, 5 ml of alkaline copper reagent was added. After thorough mixing 0.5 ml of folinciocalteau reagent was added and allowed to stand for 30 minutes; the blue colour appeared and absorbance was measured at 650 nm using UV visible spectrophotometer (Model No: UV 2371). The analysis was performed in triplicates and the amount of protein was calculated and expressed as mg/g DW. Bovine serum albumin (BSA) was used as standard.

Estimation of Carbohydrates Phenol - Sulpuric Acid Method (Dubois *et al.*, 1956)

Requirements

- 5% phenol (5 ml phenol + 95 ml distilled water)
- 96% Sulphuric acid (96% sulpuric acid + 4 ml distilled water)

Procedure:

100 mg of sample was grounded with 10 ml distilled water. It was then filtered and centrifuged. The filtrate was collected. To 0.1 ml of the filtrate, 0.9 ml of distilled

water, 1 ml of 5% phenol and 5 ml of 96% H_2SO_4 were added. After 30 minutes absorbance was measured at 490 nm using UV visible spectrophotometer (Model No: UV 2371). The analysis was performed in triplicates and the results were expressed as mg/g sample. Glucose was used as standard.

Total flavonoid (Zhinshen *et al.*, 1999)

Reagent

- 5% Sodium nitrate
- 10% Aluminum chloride
- 1N Sodium hydroxide
- Quercetin standard

Procedure

100 mg of sample was homogenized with 10 ml of distilled water and filtered through muslin cloth. 0.5 ml of extract was added with 2.5 ml distilled water and mixed . After 6 minutes, 0.15 ml NaNO was added of and again after 6 minutes, 0.3 ml of 10% AlCl3 was added. After 5 minutes 1 ml of 1M NaNH and 0.5% ml of water were added . Following thorough mixing of the solution, absorbance against blank was determined at 510 nm. Quercetin was used as standard and the results were expressed as mg quercetin equivalents (QE)/g dry weight

Vitamin-C (Ascorbic acid) – Baker and Frank, (1968)

Reagent

- 5% of TCA
- Indophenol reagent (20 mg of dichlorophenol indophenol was dissolved in 10 ml of warm distilled water).

- 20 mg of dichlorophenol indophenols ws dissolved in 10ml of warm distilled water
- DT reagent (2 g of 2, 4 dinitrophenyl hydrazine and 1 g of thiourea were dissolved in 100 ml of 9 N sulphuric acid).
- 85% sulphuric acd
- L – ascorbic acid – standard

100 mg of each sample was homogenized with 10 ml of 5% trichloro acetic acid (TCA). The homogenate was centrifuged at 3000 rpm. To 2 ml of protein free supernatant, 1 drop of indophenol reagent and 0.5 ml of DT reagent were added and incubated at 10°C for 1 hour. Then cooled in ice bath and 2.5 ml of 85% sulphuric acid was added. After intermittent shaking for 30 minutes (until red colour appeared), 30 absorbance was measured at 540 nm. L-ascorbic acid was used as standard and the results were expressed as mg/g DW.

Vitamin E (Tocopherol (Rosenberg, 1992)

The sample (2.5 g) was homogenized in 50 ml of 0.1 N sulphuric acids and allowed to stand overnight. The content in the flask was shaken vigorously and filtered through Whatman No.1 filter paper. Aliquots of the filtrate was used for estimation. Into stoppered centrifuge tubes, 3 ml of extract and 3 ml of water were pipetted out separately. To both the tubes, 3 ml of ethanol and 3 ml of xylene were added, mixed well and centrifuged. Xylene (2.0 ml) layer was transferred into another stoppered tube. To each tube, 2.0 ml of dipyridyl reagent was added and mixed well. The mixture (3 ml) was pipetted out into a cuvette and the extinction was read at 460 nm. Ferric chloride solution (0.66 ml) was added to all the tubes and mixed well. The red colour developed was read exactly after 15 minutes at 520 nm.

FT-IR analysis

A little powder of plant specimen was mixed with KBr salt, using a mortar and pestle, and compressed into a thin pellet. Infra -red spectra were recorded as KBr pellets on a Thermo Scientific NicotiS5ID1 transmission, between 4000-400 cm-1(Kareru *et al.*, 2008).

ANTIOXIDENT ACTIVITY

Crude samples extracts were prepared by pouring 100ml of distilled water in a conical flask containing 10g of each samples separately in the ratio of 10:1 (V/W). after 24 hours, the mixture was filtrated through whatman no:1 filter paper and the filtrate was evaporated to dryness. Crude (aqueous) extracts of all samples (1mg/ml) were used for the determination of free radical scavenging activity.

Free radical scavenging assay (Hatano *et al.*, 1998).

Free radical scavenging assay was measured by 2-2 Diphenyl, 1-picryl hydrazine (DPPH) method proposed by with slight modifications. 1ml of aliqut of test sample was added to 3ml of 0.004% DPPH solution prepared in methanol. The mixture was vortexed for 1min and kept at room temperature for 30 minutes in darkness the absorbance was read at 517 nm. Allow absorbance of the reaction mixture indicated a high free radical scavenging activity. Ascorbic acid was used as standard.

DPPH scavenging activity(%)

A control –A test / A control * 100 Where, A control is the absorbance of the DPPH solution without test solution. A test is the absorbance of DPPH with the test solution. Aqueous extract was used as blank.

HYDROGEN PEROXIDE SCAVENGING ACTIVITY (Chandrika *et al.*, 2007).

Requirements:

- Phosphate buffer
- H_2O_2
- Ascorbic acid

Procedure:

Samples were dissolved in 0.5 ml of 0.1 M phosphate buffer (pH 7.4), mixed with 0.5 ml of 20 mM H_2O_2 solution and measured at 230 nm. Ascorbic acid and phosphate buffer were used as positive and negative controls respectively. The activity was calculated according to the following equation:

H_2O_2 scavenging activity (%) = $(1 - A1/A0) \times 100$

Where A1 is the absorbance of the test sample and A0 is the absorbance of negative control.

ANTIBACTERIAL ASSAY

Extraction of plant materials

The plant powder was extracted with methanol, acetone and water. 25 gms of plant powder was extracted with methanol, acetone and water solution individually in soxhlet apparatus continuously for about 4-6 hours, which was again concentrated till it become semisolid. It was evaporated to dryness and stored at 0° C, until the time of the experiment

Bacterial strains used

The test organisms were obtained from the Department of Microbiology; St. Mary's College (Autonomous), Thoothukudi. One gram positive bacteria *Bacillus subtilis* and one gram negative bacteria *Escherichia coli*, were used in the present study.

Broth Medium:

- Nutrient broth Himedia MOOI
- Nutrient broth 1.3 gm
- Distilled water 100 ml

2-3 ml of sterilized broth medium was taken in the sterilized culture tube. The inoculating loop was flamed and after a few minutes a loopful bacterial colony was transferred to the broth medium. This microbe culture was incubated at room temperature for 24 hours.

Agar medium:

- Nutrient agar Himedia MOOI
- Nutrient agar 2.8 gm
- Distilled water 100 ml

To prepare the agar medium all the above ingredients were dissolved and sterilized.

Disc diffusion method

Anti- bacterial activity was evaluated by agar disc diffusion method (Kirby-Bauer *et al.*, 1986). Test solutions were prepared with known weight of methanol, acetone and water extracts dissolved in 5% dimethyl sulphoxide (DMSO). What man No. 1 filter paper disc (5mm) were impregnated with 20 of these extracts and allowed to dry at room temperature. The spread plates were prepared by proper concentration of inoculate. Each sample loaded discs was placed in the seeded agar plate. 24-48 hours of + 37oc incubation, the diameter of the inhibition zone was for positive control, streptomycin discs (100 g/ml) was used, whereas for negative control; respective solvents loaded on the sterile discs.

RESULT AND DISCUSSION

RESULT AND DISCUSSION

In the present investigation, leaf of *Ficus religiosa* are subjected to macroscopic analysis, preliminary qualitative phytochemical analysis, quantitative analysis, FTIR analysis, antioxidant activity and antimicrobial activity.

Systematic position:

In Linnaeus system of Classification, the systematic position of *Ficus religiosa* is as follows.

***Ficus religiosa* (Linn.)**

Kingdom : Plantae

Class : Dicotyledons

Order : Rosales

Family : Moraceae

Genus : Ficus

Species : religiosa

Macroscopic Characters:

Habit: Tree is semi-evergreen tree that has up to 98 ft tall and a trunk of about 9.8 ft. (Plate-1).

Leaves: The leaves are cordate in shape with a distinctive extended drip tip; they are 10–17 centimetres long and 8–12 centimetres broad, with a 6–10 centimetres petiole.

Inflorescence: Hypanthodium, globosely turbinate, androgynous sessile in axillary pairs, the apex truncate or subacute, with few brown spots, basal bracts 3, unequal, slightly imbricate.

Flowers: Flowers unisexual; inflorescence a syconia, sessile, axillary, in pairs, obovoid or globose, twig wall thick; basal bracts 3, 3-5 mm long, ovate-obtuse, silky-puberulous, persistent, orifice, closed by 3 apical bracts in a disc 2-3 mm wide; internal bristles none;

Plate 1: Ficus religiosa

Fruit: The fruits are small figs 1–1.5 centimetres in diameter, green ripening to purple.

Flowering and Fruiting Time: Flowering occurs in February; onset of fruits start in summers and ripening is complete before the onset of rainy season

Vernacular names: Arasamaram

Distribution:

Ficus religiosa is native to most of the Indian subcontinent – Bangladesh, Nepal, Pakistan and India including the Assam region, Eastern Himalaya and the Nicobar Islands, as well as part of Indochina – the Andaman Islands, Myanmar and Peninsular Malaysia.

Preliminary Phytochemical Screening:

The presence of different phytochemical constituents in aqueous acetone, ethanol, chloroform, petroleum ether, hexane extract of leaf of *Ficus religiosa* are evaluated qualitatively and present in (Table 1). The phytochemical such as alkaloids, anthraquinones, betacyanin, saponin, protein, steroids, tannins, carbohydrates, coumarins, flavonoids, phenols and quinines are present in ethanol leaf extract of *Ficus religiosa* .

In the current study ethanol extract of leaves of *Ficus religiosa* contains Tannins, saponin, phenol, sterol, protein, terpenoids, carbohydrates, protein and glycoside. Prakash *et al.* (2017) reported that ethanol extract of *Ficus religiosa* contains alkaloids, saponins, phenols, flavonoids, protein, tannins and terpenoids.

The Petroleum ether extract of *Ficus religiosa* contains of flavonoids, Glycosides, coumarins, Quinones and betacyanin. Gupta, (2020) revealed the presence of flavonoids, alkaloids, tannins, coumarins, terpenoids, proteins and amino acids in aqueous, petroleum ether extracts.

Alkaloids commonly are concentrated in particular organs such as the leaves, bark or roots. Alkaloids play an important role in the defence systems against pathogens and animals. The applications of alkaloids are not limited to biological control of herbivores but also have pharmacological, veterinary and medical importance. Alkaloids belonging to beta- carboline group possess antibacterial, anti - HIV and anti-parasitic activity (Pater *et al.,* 2011) . Alkaloids are present in a wide range of plant families and have a variety of biological effects (Kurel, 2019).

Terpenes also act as diuretics and helps in relieving gastrointestinal spasms. Terpenes are added to creams and ointments to relieve pain and itching terpenes also possess antibacterial properties thus, helps to fight microorganisms resistant to antibiotics such as yeast and other fungi. Terpenes like menthol when consumed as a tea aids to

Table 1: Phytochemical Screening of different extracts of *Ficus religiosa*

S.NO	Phytochemical	Solvents					
		Ethanol	Water	Chloroform	Acetone	Petroleum Ether	Hexane
1	Alkaloids	-	+	+	+	-	-
2	Flavonoids	-	+	+	-	+	+
3	Tannins	+	+	-	+	-	-
4	Phenols	+	+	-	+	-	-
5	Terpenoids	+	+	+	-	-	-
6	Sterol	+	-	-	+	-	-
7	Quinones	-	+	+	-	+	+
8	Betacyanin	-	+	-	+	+	-
9	Coumarins	-	-	+	-	+	+
10	Saponin	+	-	+	-	-	-
11	Anthraquinone	-	+	+	+	-	-
12	Carbohydrates	+	+	+	-	-	-
13	Protein	+	-	-	-	-	-
14	Glycoside	-	+	-	-	+	+

Table 2: Amount of flavanoid, carbohydrates , protein present in the leaves of *Ficus religiosa*

Sample	*Ficus religiosa*		
Leaf	Amount of flavanoid (mg (QE) / g)	Amount of proteins (mg (QE) / g)	Amount of carbohydrate(mg (QE) / g)
	0.691 ± 0.151	5.405 ± 3.415	0.855 ± 0.195

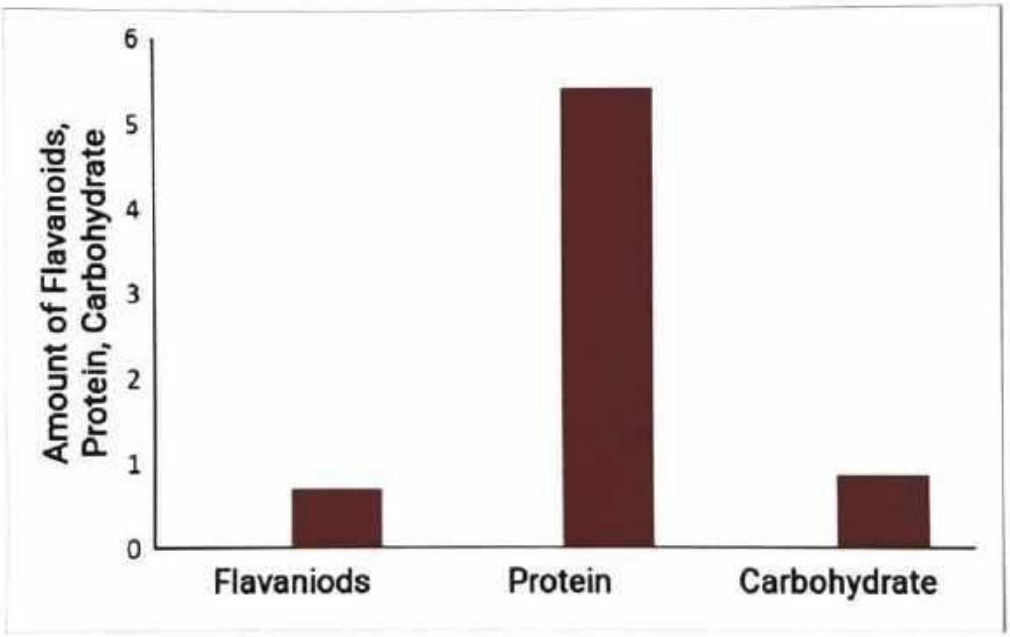


Figure1. Amount of Flavanoid, Carbohydrates, Protein present in the leaves of *Ficus religiosa*

Table 3: Amount of vitamin C and vitamin E present in the leaves of *Ficus religiosa*

Sample	*Ficus religiosa*	
Leaf	Amount of vitamin C (mg (QE) /g)	Amount of vitamin E (mg (QE) /g)
	5.952 ± 2.749	10.732 ± 0.755

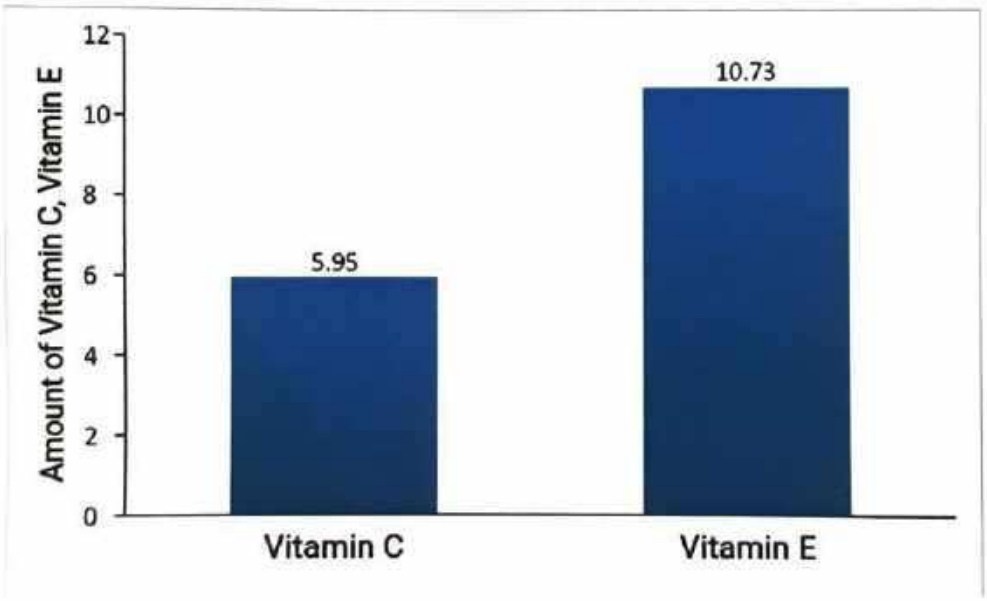


Figure 2: Amount of Vitamin C and Vitamin E present in the leaves of *Ficus religiosa*

reduce flatulence and indigestion (Destinney *et al.*, 2019). The coumarins acts anticoagulants, which block multiple steps in the coagulation cascade. Coumarins can be used treat the side effects caused by radiotherapy (Esra *et al.*, 2020).

Betacyanins are a class of red and yellow indolent- derived pigments found in plants of the caryophyllales, where they replace anthocyanins pigments. Betacyanin are antioxidant, anti - inflammatory and detoxifying agents that are richer in beets than other plant foods. The antioxidant properties beneficial in the prevention of cancer and cardiovascular diseases.

Plate sterols are secondary metabolites occurring in plants in small quantities with the highest concentration in vegetable oils. They reduce total and LDL cholesterol levels the plasma by inhibiting its absorption from thesmall intestine. Hence, they lower the antherosclerotic risk and offer protection against cardiovascular diseases (Nigon *et al.*, 2001). Phytosterols may attenuate the inflammatory activity of immune cells. including macrophages and neutrophils (Lanlan *et al.*, 2019).

Total Flavonoids, Proteins, Carbohydrates, Vitamin C, Vitamin E

The flavonoids, proteins, carbohydrates contens of leaves of *Ficus religiosa shows* in (Figure. 2) is flavonoid (69 mg QE / g) protein (5.40 mg QE /g) carbohydrates (0.85 mg QE / g) in *Ficus religiosa*. The present of flavonoid in the leaf of *Ficus religiosa* could accurent for its use as an anti-inflammatory agent (Vishal *et al.*, 2011) and for treatment of diarrhoea (Lee, 2015) fever reducing, pain relieving and anticancer activities. The results of quantitative phytochemical screening shows *Ficus religiosa* has more protein, carbohydrate, flavanoid, vitamin -E and vitamin-C.

FT- IR analysis

FT- IR analysis is proved to be a reliable and sensitive ethanol as it provided a unique fingerprint for the biomolecules. It has been used as a requisite ethanol to identify the various function groups responsible for medicinal properties in the herbal drug (Cheikh *et al.,2019).* Most researchers applied FT- IR spectrum as a tool for discriminating closely associated species (Rodriguez *et al.,* 2011). As the chemical functional group are responsible for the absorption of the radiation at different frequencies, the frequencies are helpful for the identification of the chemical makeup of the sample (Priori,1994).

The FT- IR spectroscopic analysis of the present of phytocomponents in leaf ethanolic extract of *Ficus religiosa* (Fig 3) confirms the presence of alcohol, urethane, alkane, ester, carbonyl, guanidine, aromatic , nitro group, acid, acetals, ether, sulphonic acid, alkyl halide, and halogen compound corresponding to the major peak values at 3747.43, 3443.66, 2919.06, 2849.63, 1806.21, 1743.53, 1644.20, 1514.98, 1418.55, 1317.29, 1243.04, 1163.00, 1032.81, 893.94, 781.12 , 669.25, 595.00, 517.85 cm- 1.

Table 4: FT- IR Peak values and functional groups of *Ficus religiosa*

PEAK VALUE	BOND	FUNCTIONAL GROUP
3747.43	STRONG, ALCOHOL GROUP	O- H
3443.66	MEDIUM,URETHANES	N- H
2919.06	STRONG, ALKANE	C-H
2849.63	MEDIUM , ESTER	C- H
1806.21	STRONG	C=O
1743.53	STRONG, CARBONYL	C=O
1644.20	MEDIUM, ATOMATIC ,AMINES	N- H
1514.98	STRONG , NITROMATIC	N- O
1418.55	MEDIUM, AROMATIC	C=C
1317.29	STRONG, NITRO GROUP	N=O
1243.04	STRONG, ALHYL ETHER	C-O
1163.00	STRONG, ALCOHOL	C-O
1032.81	STRONG, ALCOHOL	C-O
893.94	WEAK, PYRANOSE COMPOUNDS	C-H
781.12	STRONG, ALIPHATIC ,CHLOROFORMATE	C-O
669.25	STRONG, PRIMARY AMINES	N- H
595.00	STRONG, HALO BOND	C-I
517.85	STRONG, ALKYL HALIDE	

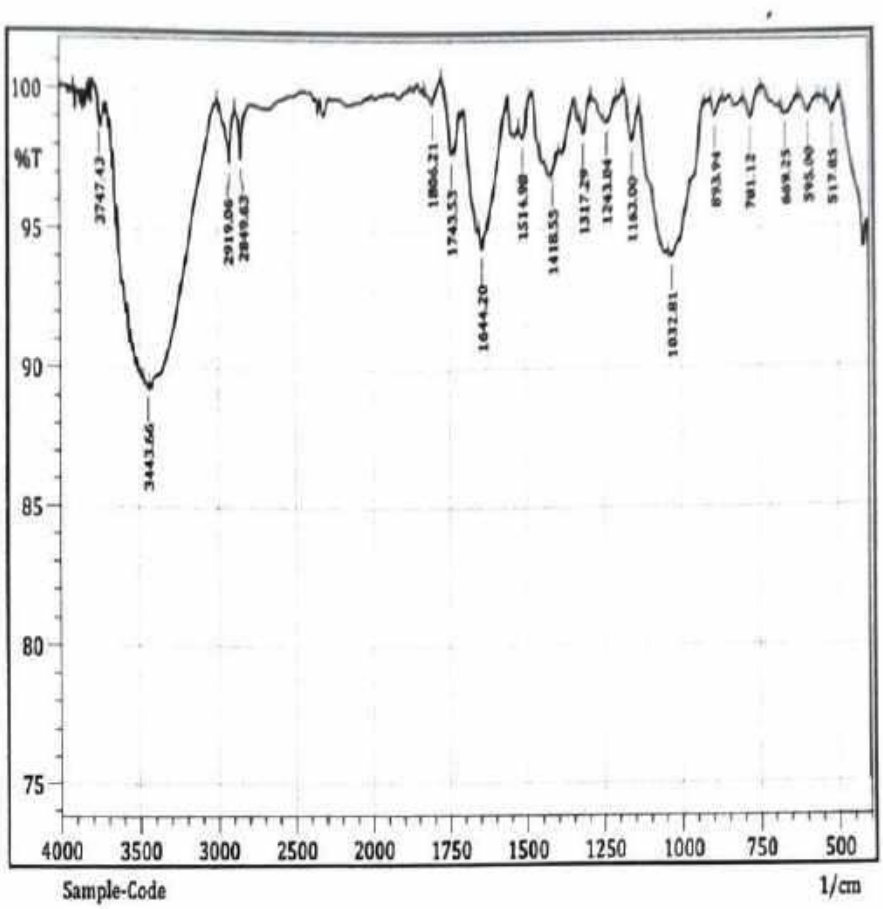


Fig 4: FT- IR Spectrum of leaf of *Ficus religiosa*

ANTIOXIDENT:

DPPH scavenging activity :

The results of anti – oxidant DPPH scavenging activity shows low percentage in *Ficus religiosa.*

hydrogen peroxide scavenging activity:

The results of anti – oxidant hydrogen peroxide scavenging activity shows more percentage in *Ficus religiosa.*

Table 5: Amount of DPPH scavenging activity and hydrogen peroxide scavenging activity present in the leaves of *Ficus religiosa*

Sample	DPPH scavenging activity	hydrogen peroxide scavenging activity
Ficus religiosa leaves	0.849 ± 9.645	8.887 ± 5.405

Figure 3: Amount of DPPH scavenging activity and hydrogen peroxide scavenging activity present in the leaves of *Ficus religiosa*

Antibacterial activity

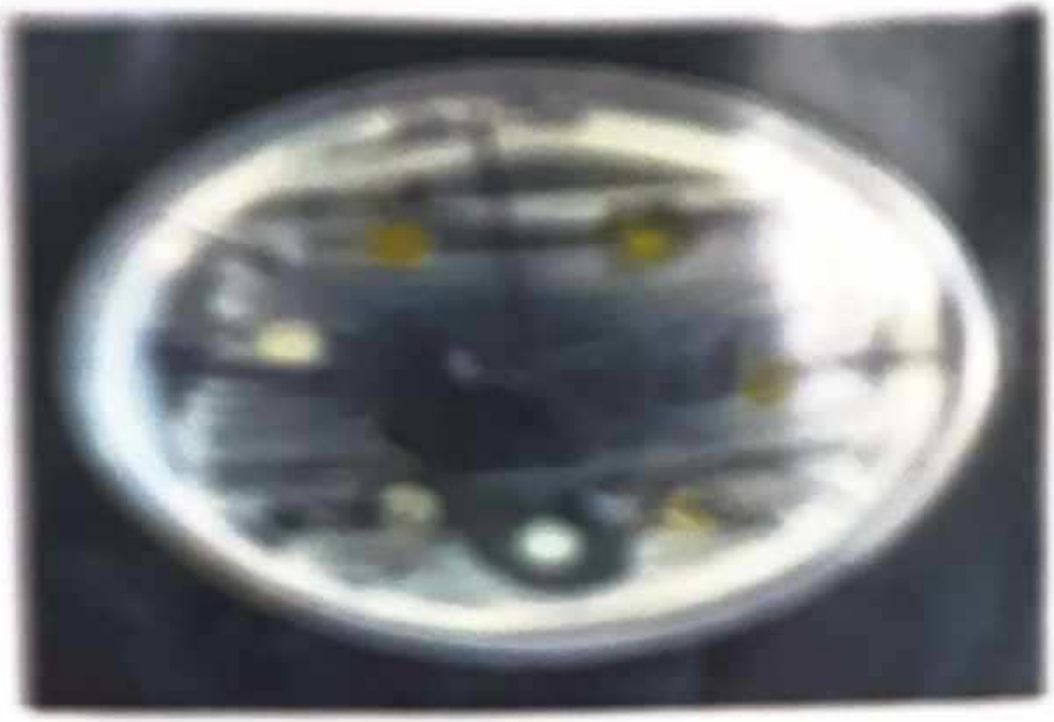
Antibacterial properties of medicine plants are being increasing reported from different parts of the world. The world health organization estimation that plant extracts of their active constituents are used as folk medicine in traditional therapies of 80% of the world's population (*Tedros et al.,2019).* In this study we analysed the antibacterial activity of ethanol and methanolic extracts of *Ficus religiosa* on *E.coli* and *B. subtilis.*

Life threatening disease and high rate of mortality occur in animal and human population due to bacterial infection. Many bacteria both Gram positive and Gram negative contaminate food, water, air, soil, ect, and cause biological / microbial pollution. *B.subtilis* is responsible for causing food borne gastroenteritis, *E. coli,* cause disease like mastitis, abortion and upper respiratory complication (Hazard and Defense, 2007*).*

The ethanol, methanol, chloroform, petroleum ether, hexane, acetone and water extracts of two selected plants are tested against pathogenic microbes *E. coli* and *B. subtilis*. The results of antibacterial activity of different plants extracts are shown in (Fig.6 & 7). The antibacterial activity of thesis plant extracts ranges from 7 mm to 12 mm against with the presence of the secondary metabolites through different mechanism.

The methanolic extract of *Ficus religiosa* showed highest antibacterial activity against both the tested organism (plate 2). These activities may be due to the phytochemical such as tannin, saponin and glycosides which could serve as the lead to the isolation of chemotherapeutic agent. This is an indication that the extracts possess substances that can inhibit the growth of some microorganism.

Dereje et al. (2021) reviewed that methanol extraction yielded higher antimicrobial activity than other extract. The variation of antibacterial activity among different crude extracts of this investigation might be due to distribution of varied



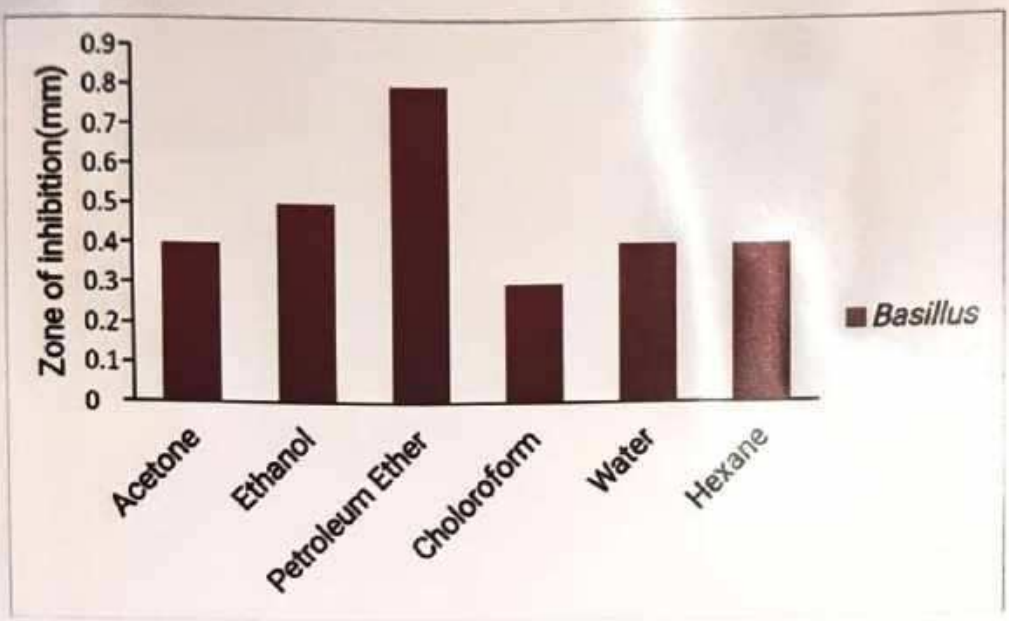


Figure 5: Antibacterial activity of *Ficus religiosa* against *Bacillus* .

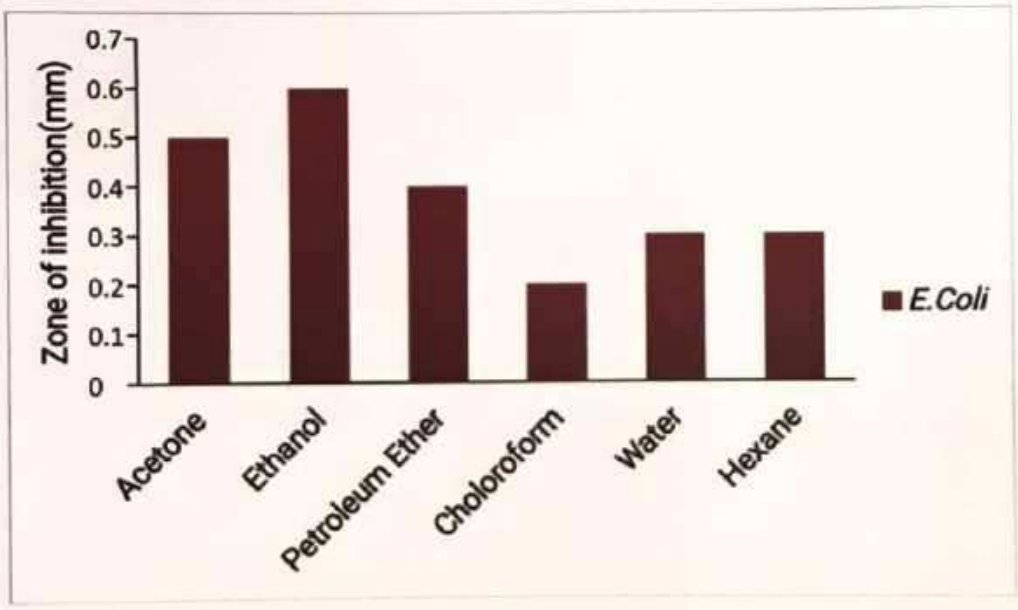


Figure 6: Antibacterial activity of *Ficus religiosa* against *E.Coli*

antimicrobial similarly, Shisir *et al.* (2019) reviewed that different extracts of plants show different antimicrobial activities on an organism. The present study showed that Gram negative bacteria were more sensitive to the tested methanolic and Ethanol extracts as compare to the Gram-positive bacteria.

Some of the extracts has a good potential for therapeutic uses against some pathogens. It appears that extracts with high antimicrobial activity against Gram-negative bacteria do not necessarily have activity against other Gram-negative bacteria compared to Gram-bacteria. This may mean that the activity is not related to the differences in cell wall structure. Because there is a such a wide range of MICs for different strains of the same bacterial species (*Faraja et al.*, 2018).

SUMMARY AND CONCLUSION

SUMMARY AND CONCLUSION

The thesis entitled "Phytochemical, Antioxidant activity and Antibacterial Evaluation of *Ficus religiosa*" deals with a systematic evaluation of macroscopic characters, phytochemical and antibacterial activity of leaves of *Ficus religiosa* belongs to family Moraceae.

The present work is focused on the following aspects of the medicinal plants,

- macroscopical characters of *Ficus religiosa*.
- preliminary phytochemical screening to establish the phytoconstituents present.
- functional groups of the selected plants by FTIR.
- antibacterial potential of *Ficus religiosa* against *Escherichia coli* and *Bacillus subtilis*.

Plants are becoming potential source for phytoconstituents with varied pharmacological activities. Identification of such plants of potential use in medicine is of significance and as a prelude to this, it becomes necessary to examine the various pharmacognostical characters of the plant before further investigation. Macroscopical study had provided a characteristic identity of leaf of *Ficus religiosa*.

Basic phytochemical screening was performed using suitable reagent to detect the presence of secondary metabolites, in the ethanol, petroleum ether, acetone, hexane, water and chloroform leaf extracts of selected plants. The phytochemical screening revealed chemical constituents that form the foundation of their pharmacological activity. Antioxidants are quantitatively estimated using standard procedure. The antioxidants were high in the *Ficus religiosa*. The DPPH in vitro assays indicate that these plant extracts are a significant source of natural antioxidant, which might be helpful in preventing the various diseases associated with oxidative stresses.

The result of the antimicrobial screening on the methanolic extract of *Ficus religiosa* showed high inhibitory activity towards *E coli*. Almost all the extracts showed activity against at least one organism tested, which indicates that the test samples contain biologically active ingredients. The knowledge of exact mode of inhibition of specific compounds which are present in the plant extract, may contribute to the successful utilization of such natural compounds for the treatment of infectious bacterial disease.

The findings of the present investigation suggests that the organic solvent extraction was suitable to verify the antimicrobial properties of medicinal plants and they supported by many investigations. The obtained results may provide a support to use of the plant in traditional medicine. It also justifies the claimed uses of leaves in the traditional system of medicine to treat various infectious diseases caused by the microbes. Further laboratory and clinical studies of this plant was required in order to understand better antibacterial principles which will allow the scientific community to recommend their use as an accessible alternative to other synthetic drugs.

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SCREENING OF PHYTOCHEMICALS AND ANTI-OXIDANT ACTIVITY OF *VICOA INDICA* (L.) DC.

Dissertation submitted to

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Submitted by

ANTONY FELICS, J. (19APBO03)

RESEARCH DEPARTMENT OF BOTANY,

ST. MARY'S COLLEGE (AUTONOMOUS),

(Re-accredited with 'A' Grade by NAAC)

THOOTHUKUDI - 628 001

APRIL – 2021

CERTIFICATE

This is to certify that this project work entitled **"Screening of Phytochemicals, Anti-oxidant activity and Green synthesis of Silver nanoparticles using *Vicoa indica* (L.) DC."** is a bonafide record carried out by **J. Antony Felics** (19APBO03) under my guidance for the partial fulfillment for the requirements for the degree of **MASTER OF SCIENCE IN BOTANY** during 2020-2021, submitted to St. Mary's College (Autonomous), Thoothukudi - 628 001. This project has not formed the basis for the award of any Degree, Diploma, Fellowship or any other similar title and that the dissertation represents independent and original work on the part of the candidate under my guidance.

Signature of the Guide

M. Glory
Head of the Department

HOD of Botany
St. Mary's College (Autonomous)
Thoothukudi- 628 001

15/4/2021
Signature of the Examiner

Lucia Rose
Signature of the Principal

St. Mary's College (Autonomous)
Thoothukudi - 628 001.

DECLARATION

Hereby declare that the dissertation entitled **"Screening of Phytochemicals, Anti-oxidant activity and Green synthesis of Silver nanoparticles using *Vicoa indica* (L.) DC."** is the original work and it has not been submitted for the award of any Degree, Diploma, Fellowship or any other similar title and that the dissertation represents independent and original work on the partial fulfillment of the requirements for the degree of **MASTER OF SCIENCE IN BOTANY** during 2020-2021, submitted to St. Mary's College (Autonomous), Thoothukudi - 628 001.

Place: Thoothukudi - 628 001

Date : 15.04.21

J. Antony Felics

J. ANTONY FELICS (19APBO03)

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Place: Thoothukudi - 628 001 J. Antony Felics
Date**:**

CONTENTS

CHAPTER I

INTRODUCTION

I. INTRODUCTION

Plant biodiversity has contributed in many ways to the development of human culture for the livelihood, nutrition and health. Plants have been used in traditional medicine for several thousand years. The knowledge of medicinal plants has been accumulated in the course of many centuries based on different medicinal system such as Ayurveda, Unani and Siddha. In India, it was reported that traditional healers use 2,500 plant species and 100 species of plants serve as regular source of medicine World Health Organization (WHO) estimated that about 80% of the population in the developing countries depends directly on plants for the medicine. 25% of the medical drugs are based on plants and the derivatives. Out of the 20,000 medicinal plants listed by the WHO globally, India's contribution is 15-20%. In India, about 2,000 drugs used are of plant origin. Less than 10% of the medicinal plants traded in the country are cultivated, about 90% are collected from the wild (Singh *et al.,* 2011).

Plants are rich source of secondary metabolites with interesting biological activities. In general, the secondary metabolites are an important source with a variety of structural arrangements and properties. The increasing recognition of herbal medicine as an alternative form of health care, the screening of medicinal plants for active compounds has become very significant. A large number of medicinal plants and the purified constituents have shown beneficial therapeutic potentials. In order to promote the use of medicinal plants as potential sources of chemical compounds, it is important to thoroughly investigate the composition and activity and thus validate the use (Osaka *et al.,* 2006).

Phytochemicals are biologically active, naturally occurring chemical compounds found in plants which provide health benefits for humans further than those attributed to macronutrients and micronutrients (Hasler and Blumberg, 1999). They protect plants from disease, damage and contribute to the plant's color, aroma and flavor.

In general, the plant chemicals that protect plant cells from environmental hazards such as pollution, stress, drought, UV exposure and pathogenic attack are called as phytochemicals (Gibson *et al.,* 1998). Recently, it is clearly known that they have roles in the protection of human health, when the dietary intake is significant. More than 4,000 phytochemicals have been cataloged and are classified by protective function, physical characteristics and chemical characteristics and About 150 phytochemicals have been studied in detail (Meagher and Thomson, 1999). In wide ranging dietary phytochemicals are found in fruits, vegetables, legumes, whole grains, nuts, seeds, fungi, herbs and spices. Broccoli, cabbage, carrots, onions, garlic, whole wheat bread, tomatoes, grapes, cherries, strawberries, raspberries, beans, legumes, and soy foods are common sources (Moorachian, 2000).

Phytochemicals accumulate in different parts of the plants, such as in the roots, stems, leaves, flowers, fruits or seeds. Many phytochemicals, particularly the pigment molecules are often concentrated in the outer layers of the various plant tissues. Levels vary from plant to plant depending upon the variety, processing, cooking and growing conditions (Costa *et al.,* 1999). Phytochemicals are also available in supplementary forms, but evidence is lacking that they provide the same health benefits as dietary phytochemicals. These compounds are known as secondary plant metabolites and have biological properties such as antioxidant activity, antimicrobial effect, modulation of detoxification enzymes, stimulation of the immune system, decrease of platelet aggregation and modulation of hormone metabolism and anticancer property (King and Young, 1999).

Naturally, there are more than thousand known and many unknown phytochemicals. It is well known that plants produce these chemicals to protect themselves, but recent researches demonstrate that many phytochemicals can also protect human against diseases. Phytochemicals are not essential nutrients and are not required by the human body for sustaining life, but have important properties to prevent or to fight some common diseases.

Many of these benefits suggest a possible role for phytochemicals in the prevention and treatment of disease, because of this property. Many researchers have been performed to reveal the beneficial health effects of phytochemicals (Narasinga, 2003).

An assessment of the previous trends and impact of research into the phytochemistry on medicinal plants of the world is quite desirable before considering recent trends. After centuries of empirical use of herbal preparation, the first isolation of active principles alkaloids such as morphine, strychnine, quinine etc. in the early 19^{th} century marked a new era in the use of medicinal plants and the beginning of modern medicinal plants research. Emphasis shifted away from plant derived drugs with the tremendous development of synthetic pharmaceutical chemistry and microbial fermentation after 1945. Plant metabolites were investigated from a phytochemical and chemotaxonomic view during this period.

Over the last decade, however, interest in drugs of plant and probably animal origin has grown steadily. Utilization of medicinal plants has almost doubled in Western Europe during that period. Ecological awareness, the efficacy of a good number of phytopharmaceutical preparations, such as ginkgo, garlic or valerian and increased interest of major pharmaceutical companies in higher medicinal plants as sources for new lead structures has been the main reasons for this renewal of interest. With the development of chemical science and pharmacognosy physicians began to extract chemical products from medicinal plants (Hamburger and Hostettmann, 1991).

A few examples of the products extracted from medicinal plants are in 1920, quinine was isolated from Cinchona by the French pharmacist, Peletier and Caventou. In the mid nineteenth century, a German chemist, Hoffmann obtained aspirin from the bark of the willow. With the active principles in medicinal plants identified and isolated, plant based prescriptions began to be substituted more and more with pure substances, which were more powerful and easier to prescribe and administer (Harvey, 2000).

Phytomedicine almost went into extinction during the first half of the 21st century due to the use of the 'more powerful and potent synthetic drug'. However, because of the numerous side effects of these drugs, the value of medicinal plants is being rediscovered as some of them have proved to be as effective as synthetic medicines with fewer or no side effects and contraindications. It has been proved that although the effects of natural remedies may seem slower, the results are sometimes better on the long run especially in chronic diseases.

According to Showkat *et al.* (2011), free radicals, the partially reduced metabolites of oxygen and nitrogen, are highly toxic and reactive. The most common reactive oxygen species are superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), peroxyl radical (ROO) and highly reactive hydroxyl radical (OH). Oxidation process is one of the most vital routes for producing free radicals in food, drugs and living systems. Antioxidants are the substances that when present in low concentration significantly delay or reduce the oxidation of the substrate. Antioxidants protect the body from damaging oxidation reactions by reacting with free radicals and other reactive oxygen species within the body and hindering the process of oxidation. Hence, diseases associated with free radicals can be avoided by antioxidant therapy which gained an immense importance.

Current research is now directed towards finding naturally occurring antioxidants particularly of plant origin. Currently available synthetic antioxidants like butylated hydroxyl anisole (BHA), butylated hydroxy toluene (BHT), tertiary butylated hydroquinone and gallic acid esters have been suspected to cause negative health effects. Hence, strong restrictions have been placed on their application and there was a trend to substitute them with naturally occurring antioxidants. Furthermore, these synthetic antioxidants also show low solubility and moderate antioxidant activity (Pourmorad *et al.*, 2006).

More than 1200 plants has been reported as medicinal agents in various scientific and popular literatures, as plant drugs are generally considered to be less toxic with lesser or rare side effects than those of synthetic ones (Pari and Umamaheswari, 2000). There are several plant genus which are reputedly known for their contribution to traditional as well as modern medicines.

Vicoa indica (L.) Dc. is a herbal plant belonging to the family Asteraceae. It is used by tribal population in India (especially northern states), acting as a contraceptive agent and female anti-fertility drug (Dhall ans Dogra, 1988). The ethnobotanical views show the infusion of whole plants were used in abortion (Tayade and Patil, 2005), roots are remedy to cough and jaundice (Oudhia, 2003), anti-inflammatory, analgesic properties (Krishnaveni *et al.,* 1997), antiviral constituents (Chowdhury *et al.,* 1990) and pre-clinical toxicity studies (Gandhi *et al.,* 1985). Considering the above views, the present investigation was indented to carry out the following objectives.

- Preliminary phytochemical anasysis of *Vicoa indica* (L.) Dc.
- To isolate and analyze the phytochemicals present in *Vicoa indica* (L.) Dc. using UV-Visible spectrophotometer, FT-IR and GC-MS.
- To study the anti-oxidant activity of *Vicoa indica* (L.) Dc.

CHAPTER II

REVIEW OF LITERATURE

II. REVIEW OF LITERATURE

For improved "quality of life" there is significant increase in demand of herbal medicines for primary health care, because of their effectiveness, safety and minimal or no side effects. The synthetic drugs although effective against various health related disorders, produce some severe side effects, which culminate in deterioration of human health (Harvey, 2008). In order to overcome these side effects, scientists have focused their research on medicinal herbs (Petrovska, 2012). Moreover, the herbal formulations also offer remedy for age-related disorders like osteoporosis, immune disorders, memory loss, etc., for which very few modern medicines are available (Kamboj, 2000). The medicinal plants are a major source of biodynamic compounds of therapeutic value and have been known for their health benefits in Ayurveda, Unani and traditional system of medicines. Herbal molecules are safe and have the potential to overcome the resistance produced by the pathogens, as they exist in a pooled form of more than one molecule in the protoplasm of the cell (Lai and Roy, 2004). Nowadays, almost all the dreadful diseases including cancer, AIDS, kidney damage, cardiovascular diseases and many more are curable by the use of medicinal herbs (Kolasani *et al.,* 2011).

PHYTOCHEMICAL STUDIES

The importance of plants is known to us well. The plant kingdom is a treasure house of potential drugs and in the recent years there has been an increasing awareness about the importance of medicinal plants. Drugs from the plants are easily available, less expensive, safe, efficient and rarely have side effects. The plants which have been selected for medicinal use over thousands of years constitute the most obvious choice of examining the current search for therapeutically effective new drugs such as anticancer drugs, antimicrobial drugs (Phillipson and Wright, 1996), antihepatotoxic compounds. According to World Health Organization (WHO), medicinal plants would be the best source to obtain variety of drugs.

About 80% of individuals from developed countries use traditional medicines, which has compounds derived from medicinal plants.

However, such plants should be investigated to better understand their properties, safety, and efficiency (Arunkumar and Muthuselvam, 2009). Medicinal plants contain some organic compounds which provide definite physiological action on the human body and these bioactive substances include tannins, alkaloids, carbohydrates, terpenoids, steroids and flavonoids (Edoga *et al.,* 2005). These compounds are synthesized by primary or rather secondary metabolism of living organisms. Secondary metabolites are chemically and taxonomically extremely diverse compounds with obscure function. They are widely used in the human therapy, veterinary, agriculture, scientific research and countless other areas (Vasu *et al.,* 2009). A large number of phytochemicals belonging to several chemical classes have been shown to have inhibitory effects on all types of microorganisms *in vitro* (Cowan, 1999).

Plant products have been part of phytomedicines since time immemorial. This can be derived from barks, leaves, flowers, roots, fruits, seeds (Criagg and David, 2001). Knowledge of the chemical constituents of plants is desirable because such information will be value for synthesis of complex chemical substances (Mojab *et al.,* 2003; Parekh and Chanda, 2008).

Seven medicinal plants such as *Bryophyllum pinnatum, Ipomea aquatica, Oldenlandia corymbosa, Ricinus communis, Terminalia bellerica, Tinospora cordifolia* and *Xanthium strumarium* were investigated the presence of phytochemicals and to determine the total phenolic and flavonoid contents of the selected medicinal plants. Soxhlet apparatus was used for the organic solvent extraction. Solvents used were water, methanol, ethanol and acetone. Total phenolic contents of the aqueous extracts of the plants were determined by the Folin-Ciocalteus reagent method whereas total flavonoid contents of the aqueous extracts were determined by the Aluminium chloride method. Proteins, carbohydrates, phenols, tannins, flavonoids, saponins, were detected in all of the plants tested. Total phenolic contents

obtained were 18.4mg/gm, 18.8mg/gm, 11.6mg/gm, 29.2mg/gm, 29.6mg/gm, 40.8mg/gm, 12.8mg/gm, 71.6mg/gm of the extract and total flavonoid contents obtained were 8.4mg/gm, 37.6mg/gm, 4.4mg/gm, 6mg/gm, 42.8mg/gm, 18mg/gm, 6mg/gm, 28.8mg/gm of the extract for the plants *Bryophyllum pinnatum* (Leaves), *Ipomea aquatica* (Leaves), *Oldenlandia corymbosa* (Whole plant), *Ricinus communis* (Roots), *Terminalia bellerica* (Leaves), *Tinospora cordifolia* (Leaves), *Tinospora cordifolia* (Stem), and *Xanthium strumarium* (Leaves) respectively. The findings provided evidence that crude aqueous and organic solvent extracts of these tested plants contain medicinally important bioactive compounds and it justifies their use in the traditional medicines for the treatment of different diseases (Yadav and Munin, 2011).

Satheesh *et al.* (2012) investigated the presence of various phytochemicals from the ethanolic, aqueous and chloroform extracts of *Punica granatum* peel, whole fruit and seeds. The three different extracts from peel were found to contain triterpenoids, steroids, glycosides, flavonoids, tannins, carbohydrates and vitamins. The three different extracts from whole fruit were found to contain triterpenoids, steroids, glycosides, saponins, alkaloids, flavonoids, tannins, carbohydrates and vitamins. The three different extracts from seeds were found to contain triterpenoids, steroids, glycosides, saponins, alkaloids, tannins, carbohydrates vitamins. The generated data from the three different extracts of *Punica granatum* peel, whole fruit and seeds provided the basis for its wide uses in the traditional and folk medicines.

Amin *et al.* (2013) screened the qualitative and quantitative analysis of the major bioactive constituents of medicinally important plant *Taraxacum officinale* in its aqueous and methanol extract of root, stem and flower. Saponins, flavonoids, alkaloids and phenols were highly concentrated in the stem, root and flower with the higher concentration of flavonoids in the flower extracts. Phenols and steroids were also found present in the investigated plant

parts. The percentage value of plant extracts in water and methanol are stem (water extract 21%, methanol 18%), root (water extract 22%, methanol 17.8%), flower (water extract 19%, methanol 16%). The significance of the plant in traditional medicine and the importance of the distribution of these chemical constituents are discussed with respect to the role of the plant in ethnomedicine in Kashmir region of India.

Victor and Chidi (2009) studied tannins, saponins, phlobatannins, flavonoids, anthraquinones, terpenoids, steroids, alkaloids, carbohydrates and glycosides distribution in four medicinal plants belonging to different families. The medicinal plants investigated were *Carica papaya*, *Ocimum gratissimum*, *Adenia cissampeloides* and *Cymbopogan citratus*. All the plants were found to contain tannins, flavonoids, terpenoids, steroids and carbohydrates while anthraquinones were absent in all. Alkaloids were absent in both *O. gratissimum* and *C. citratus*. Glycosides were absent in only *C. papaya*, saponins were absent in only *O. gratissimum* while phlobatannins were absent in only *C. citratus*. The extraction of oils was carried out by solvent extraction and steam distillation methods and the percentage yield of extracts by each method determined. Solvent extraction method gave percentage yield of 7.40, 6.30, 6.75 and 5.63% for *C. papaya*, *O. gratissimum*, *A. cissampeloides* and *C. citratus* respectively. For steam distillation, *C. papaya*, *O. gratissimum*, *A. cissampeloides* and *C. citratus* gave percentage yield of 5.60, 5.80, 5.44 and 3.82% respectively.

Anubha (2013) dealt with the phytochemical studies of leaves of different medicinal plants like *Alstonia scholaris*, *Catharanthus roseus*, *Nerium oleander*, *Tabernaemontana divaricata, Thevetia neriifolia*, *Withania somnifera, Adhatoda vasica, Cannabis sativa, Solanum nigrum, Plumeria alba and Achranthus asperatc*. Methanolic (90%) extracts of leaf powders have been screened for qualitative determination of different secondary metabolites like cardiac glycoside. alkaloids, flavonoids, tannins, glycoside, reducing sugar, saponin and terpenoids. All phytochemicals such as alkaloids, flavonoids, tannins, reducing sugars,

saponins, flavonoid and terpenoides were present in all selected plant species. Tannin were absent in *Adhatoda vasica* and *Cannabis satvia.*

Sudipa *et al.* (2013) carried out the phytochemical studies of leaves of different medicinal plants like *Andrographis paniculata* of the family Acanthaceae, *Bauhinia acuminata* of the family Caesalpiniaceae, *Clerodendrum indicum* and *Clerodendrum siphonanthus* of the family Verbenaceae, *Nerium odorum* of the family Apocynaceae and *Sida humilis, Sida veronicaefolia* and *Sida cordata* of the family Malvaceae. Methanolic (90%) extracts of leaf powders have been screened for qualitative determination of different secondary metabolites like alkaloids, flavonoids, tannins, reducing sugars, amino acids and lignins by specific chemical color reaction tests.

The phytochemical analysis of leaf extracts in aqueous, methanol, acetone, petroleum ether and chloroform extracts of indigenous medicinally important plants of *Holoptelea integrifolia* and *Celestrus emarginata* were investigated. The phytochemical analysis revealed the presence of alkaloids, saponins, tannins, flavonoids, terpenoids, coumarins, quinines, cardiac glycosides, xanthoproteins, glycosides, steroids, phenols, resins, carboxylic acid group in varying concentrations. This research supported the local use of the leaf extract of the plant *Holoptelea integrifolia* for pediculoses and *Celestrus emarginata* for increasing male sex vigour**.** This plant belongs to family Euphorbiaceae and Celestracea respectively (Suman *et al.,* 2013).

Dhawale (2013) analyzed seven medicinal plants from Yavatmal District. The plants were *Argimone Mexicana, Carea arborea, Caesalpinia pulcherima, Mimosa pudica, Ocimum canum, Phyllanthus emblica and Zizipus jujube.* Qualitative phytochemical analysis of these plants confirmed the presence of various phytochemicals like alkaloids, flavonoids, steroids and terpenoid. The presence of these phytochemicals can be correlated with medicinal potential of these plants.

Venkata *et al.* (2013) reported 84 methanolic extracts prepared from the 54 Indian plants belonging to 33 different families collected from the forest located in Eastern Ghats of India. A qualitative preliminary phytochemical screening was performed on aforesaid extracts for the presence of alkaloids, flavonoids, steroids and terpenoids. Each analysis was carried out in triplicate, which resulted a total of 22, 19, 37 and 30 plant species were found to give positive results for alkaloids (41%), flavonoids (35%), steroids (69%) and terpenoids (56%), respectively.

Studies carried out during the past few decades have shown that the phytochemicals have an important role in preventing chronic diseases like cancer, diabetes and coronary heart disease. The major classes of phytochemicals with disease preventing functions are dietary fibre, antioxidants, anticancer, detoxifying agents, immunity potentiating agents and neuropharmacological agents. Each class of these functional agents consists of a wide range of chemicals with differing potency. Some of these phytochemicals have more than one function. There is, however, much scope for further systematic research in screening Indian medicinal plants for these phytochemicals and assessing their potential in protecting against different types of diseases (Mamta *et al.,* 2013).

Qualitative analysis of phytochemical constituents such as tannins, phlobatannins, saponins, flavonoids, steroids, alkaloids, quinones**,** coumarin, terpenoids and cardiac glycosides and quantitative analysis of total phenolics, alkaloids, saponins and flavonoids was performed by the well known tests protocol available in the literature. The phytochemical screening revealed the extract richness in tannins, phlobatannin, saponins, flavonoids, steroids and alkaloids. Quantitative analysis of phenolics, alkaloids, saponins and flavonoids had revealed that *Mentha spicata* possessed maximum phenolic (18.41%), *Gmelina arborea* highest alkaloids (5.66%) and flavonoids (22.80%) and *Trigonella foenum-graecum* highest saponin (50.12%) contents (Anjali and Sheetal, 2013).

An RP-HPLC method with photodiode array detection has been developed for the determination of major constituent berberine from *Berberis aristata* and *Berberis tinctoria*. Berberine was isolated from the plant extract on semi-preparative HPLC and separated on HPLC by using an isocratic mode consisting of 0.1% trifloroacetic acid: acetonitrile (60:40 v/v) at a flow rate of 1mL/min. Under these conditions, a plot of integrated peak area versus concentration of berberine was found be linear over the concentration range of 0.2µg/mL to 150µg/mL. The limit of detection was 1ng on column and limit of quantification was 2ng on column for berberine. The berberine content in *B. aristata* and *B. tinctoria* was found to be 3.18% and 1.46% respectively (Hemant *et al.,* 2013).

Different extract of *Convolvulus pluricaulis* (methanol, ethanol and water) were prepared and tested of scopoletin. The maximum scopoletin content was observed in 50% ethanolic extract followed by methanol and water extracts. It was 0.1738%, 0.0932% and 0.0435% in ethanol, methanol and water extract respectively. A simple HPLC was developed for the determination of scopoletin in *Convolvulus pluricaulis*. Shankhpushpi is an astringent, hot aphrodisiac and a nervine tonic. It improves strength, digestive power, helpful in epilepsy, insomnia, heart disease and hemetemesis. Analytic separation and quantification were achieved by high performance liquid chromatography and UV detection at 366 nm. The method involves the use of C_{18} column (Phenomenex, 250 mm × 4.6 mm, 5µm) with isocratic mixture of methanol and water containing 0.1% v/v formic acid in the ration of 30:70. Linearity was observed in the range of 20-100ppm with correlation coefficient of 0.9961. Relative standard deviation of linearity of the method was found to be 1.29%. Detection limit was 5.0ppm and quantification limit was 7.5ppm. The repeatability of the method was found to be 0.71%. Recovery values from 99.10 to 100.1% indicated best accuracy of the method (Upadhyay *et al.,* 2013).

A Rapid and specific reversed phase high pressure liquid chromatography (RP-HPLC) method for the quantitative analysis of flavonoid quercetin in the extract of *Solanum trilobatum* and various dietary sources. The flavonoid was analyzed on a kromasil Rp C_{18} column. Using a mobile phase consisted of methanol-acetonitrile-water (60:20:20 v/v/v) under the following conditions. Detected the wavelength at 262nm and the flow rate was at 1.1ml/min. The sensitivity 0.05 AUFS and the volume of injecting sample is 10µl. The HPLC system was operated at ambient temperature (28°C). The stop time was set at 6min. The standard quercetin was diluted using the mobile phase at a known concentration of 0.5mg/ml; the sample was filtered through sample filter contain 0.45µm prous nylon membrane filter paper. The filtrate was introduced on to a reverse phase analytical column. The content of quercetin which is present in *Solanum trilobatum* and in various dietary sources (onion, green apple, lemon, green tea) was between 2.03 to 2.30min. Recovery of the flavonoid quercetin was 21.1% to 98.6%. The method was applied to the quantitative analysis of flavonoid in *Solanum trilobatum* and various dietary sources and was found to be simple rapid and efficient. The HPLC method showed an excellent performance in separating the flavonoid quercetin in dietary sources and in medicinal plants (Phani *et al.,* 2010).

Antony *et al.* (2013) carried out to characterize the bioactive constituents present in different leaf extracts of *Stylosanthes fruticosa* using UV-VIS, FTIR and GC-MS. The crude extracts were scanned in the wavelength ranging from 200-1100nm by using Perkin Elmer spectrophotometer and the characteristic peaks were detected. For GC-MS analysis, 10g sample is extracted with 30ml ethanol, filtered in ash less filter paper with 2g sodium sulphate and the extract is concentrated to 1ml by bubbling nitrogen into the solution. The compound detection employed the NIST Ver.2.0 Year 2005 library. The biological activities were based on Dr. Duke's phytochemical and ethnobotanical databases by Jim Duke of the Agricultural Research Service/USDA. The UV-VIS profile showed different peaks ranging

from 400-700nm with different absorption respectively. The FTIR spectrum confirmed the presence of secondary alcohols, phenols, alkanes, alkenes, carboxylic acids, aromatics, nitro compounds and amines in different extracts. The results of the GC-MS analysis provide different peaks determining the presence of 21 phytochemical compounds with different therapeutic activities. The major phyto constituents were Trans-5-Hexyl-1,4-dioxane-2-carboxylic acid (9.26%), dodecadienoic acid, Methy-ester (6.58%) and Nonanoic acid Methyl ester (6.58%).

The leaves of *Achyranthes aspera* were screened for the presence of the phytochemical composition using UV-Visible spectroscopic and Tourier Transform Infrared spectroscopic analysis. Estimation of total phenolic content was performed by Folin-Ciocalteau reagent method. *Achyranthes aspera* leaves showed the presence of carbohydrates, phenolic compounds, oil and fats, saponins, flavonoids, alkaloids and tannins as major phytochemical groups. Fourier Transform Infrared spectroscopic analysis of the leaves powder showed the presence of –OH group for phenolic compounds and UV-Visible spectroscopic analysis exhibits the presence of flavonol derivatives, carotenoids and b-cryptoxanthin epoxide as major phenolic compounds (Priya *et al.,* 2012).

The bioactive components of *Physalis minima* leaves have been evaluated using GC-MS, HPLC, UV-Visible and FTIR. The chemical compositions of the extract of *Physalis minima* leaves were investigated using perkin-elmer gas chromatography-mass spectrometry, while the mass spectra of the compounds found in the extract was matched by the National Institute of Standards and Technology (NIST) library. GC-MS analysis of extract of *Physalis minima* leaves revealed the existence of heneicosanoic acid (25.22%), hepta-2,4-dien (27.41%), octadecanoic acid, stearic acid (31.19%) and octadeca-9,12-dienoic acid (32.02%). HPLC profiles of *Physalis minima* reported to contain four phenolic compounds, namely ellagic acid (4.13min), catechol (3.59min), gallic acid (4.12min) and catechin (7.41min). The

UV-Visible profile showed the peaks at 315.09nm, 408.09nm and 676.50nm with the absorption 0.247, 0.106 and 0.003 respectively. The results of FTIR analysis confirmed the presence of phenol, alkanes, aldehyde, secondary alcohol, amino acid, aromatic amines and halogen compound (Karpagasundari and Kulothungan, 2014).

The extraction of bark powder of *Litsea glutinosa* and its preliminary phytochemical screening was studied by Pragna *et al.* (2012). The bark powder was subjected to methanolic extraction and further explored for the phytochemical constituents using TLC and GC-MS. Preliminary phytochemical screening revealed the presence of alkaloids, flavonoides, glycosides, phenols, tannins and saponins. The extract was further subjected to separation using TLC and fractions were evaluated using GC-MS. GC-MS analysis of the total methanolic extract showed the presence of oliec acid, tricosene, erucic acid, tetra decanoic acid, pyrrolidinone, piperidine, eicosanoic acid like major phytochemicals. Alkaloid fraction was found to be rich in therapeutically potential compounds like eicosane, pieprizine, pyridine, thio-coumarin, tetrahydroisoquinoline. Apart from this various androstane, androsta-trione, pregnene like phytoestrogens were also observed. TLC of various subfractions of alkaloids revealed that the plant is rich in variety of potential therapeutic phytochemicals.

Antony *et al*. (2013) carried out the characterization of the bioactive constituents present in different leaf extracts of *Stylosanthes fruticosa* using UV-Visible spectroscopy, FT-IR and GC-MS. The crude extracts were scanned in the wavelength ranging from 200-1100nm by using Perkin Elmer spectrophotometer and the characteristic peaks were detected. For GC-MS analysis, 10g sample was extracted with 30ml methanol, filtered in ash less filter paper with 2g sodium sulphate and the extract was concentrated to 1ml by bubbling nitrogen into the solution. The compound detection employed the NIST Ver.2.0 year 2005 library. The UV-Visible profile showed different peaks ranging from 400-700nm with different

absorption respectively. The FT-IR spectrum confirmed the presence of secondary alcohols, phenols, alkanes, alkenes, carboxylic acids, aromatics, nitro compounds and amines in different extracts. The results of the GC-MS analysis provide different peaks determining the presence of 21 phytochemical compounds with different therapeutic activities. The major phytoconstituents were trans-5-Hexyl-1,4-dioxane-2-carboxylic acid (9.26%), dodecadienoic acid, methy-ester (6.58%) and nonanoic acid, methyl ester (6.58%).

Ragavendran *et al.* (2011) studied FT-IR spectra of various extract (aqueous, ethanol and aqueous ethanol) of *Aerva lanata*. The vibrational assignments, intensities and wave number of dominant peak were obtained from absorption spectra. Probable assignments of the bands were made with respect to the components present in various extracts. By the analysis, functional groups such as aminoacids, amides, amines, carboxylic acid, carbonyl compounds, organic hydrocarbons, halogens were present in all the three extracts. The result indicated that the aqueous, ethanol and aqueous ethanol extract of the plant having high therapeutic value.

Neha and Jyoti (2013) characterized the bioactive constituents present in flower extracts of *Bougainvillea glabra* using UV-Visible and FT-IR. The extract was examined under Visible and UV light for the proximate analysis. The crude extracts of *Bougainvillea glabra* were scanned in the wavelength ranging from 200-1100 nm by using Perkin Elmer spectrophotometer system and the characteristic peaks were detected. FT-IR method was performed on a Perkin Elmer spectrophotometer system which was used to detect the characteristic peak values and their functional groups. As the result UV-Visible profile showed the peaks at 324.00nm and 290.00nm for flavonoid and FT-IR spectra showed the peak at 3364.58cm^{-1} for OH group.

Praveen and Rajesh (2019) estimated the phytochemical components and total flavanoid content assay of chloroform, methanol and ethanolic solvent extracts from *Ocimum*

sanctum (basil) leaf and stem. The basil leaf and stem was extracted by maceration process using ethanol, chloroform, and methanol. The Chloroform, ethanol and methanol extract were screened of phytochemical content including identification of flavonoid, alkaloid, polyphenols, glycosides, tannin, saponin etc. Estimation of total flavonoids content was based on aluminium chloride method in the sample extract by spectrophotometrically. Phytochemical screening test showed that the presence of diterpines, saponins, proteins, flavonoids, amino acids, carbohydrates, alkaloids in leaves and stem parts when extracted with methanolic and ethanolic solvents. Chloroform extract of basil leaf and stem does not show the presence of any phytochemicals. Higher flavonoid component were present in methanolic extract of *Ocimum sanctum* leaves than ethanolic solvent extract. In the study, *Ocimum sanctum* has phytochemicals properties in the leaves and stem which are used in curing the ailments and higher flavonoid content indicated the natural antioxidant activity signifying their medicinal importance and potent source in pharma industries.

ANTI-OXIDANT ACTIVITY

Antioxidant based drug formulations are used for the prevention and treatment of complex diseases like atherosclerosis, stroke, diabetes, Alzheimer's disease and cancer (Devasagayam *et al.,* 2004). Compounds responsible for such antioxidant activity can be isolated and used for prevention and treatment of free radical-related disorders (Middleton *et al.,* 2000). Extract of *Selaginella tamariscina* contained a strong antioxidant activity that was able to reduce blood sugar levels and function as a lipid peroxide serum and increase insulin serum (Miao *et al.,* 1996). Antioxidant activity of two phloroglucinol derivatives from *Dryopteris crassirhizoma* exhibited significant antioxidant activity as assessed by DPPH radical scavenging assay *in vitro* (Lee *et al.,* 2003).

Antioxidant activity of aqueous extract and ethyl acetate fraction of *Equisetum telmateia* was evaluated by DPPH, TEAC and TBARS assays. Significant antioxidant

activity was detected in the ethyl acetate fraction. Analysis of aqueous extract and ethyl acetate fraction by HPLC-PADESI/MS allowed the identification of the major phenolic compounds as flavan-3- ol, kaempferol and phenolic acid derivatives (Helena *et al.,* 2005). Free radical scavenging activity of aqueous extract of *Pteris multifida* was evaluated using DPPH, hydroxyl radical and reducing power assay (Wang *et al.,* 2006).

Hort *et al*. (2008) studied the antioxidant and hepatoprotective activity of *Cyathea phalerata* hydroalcoholic extract and fractions obtained by treatment with organic solvents of increasing polarity. Ethyl acetate fraction of the crude extract displayed the best antioxidant and hepatoprotective activities. The flavonoids present in ethyl acetate fraction of *Cyathea phalerata* could be responsible for these activities.

Mimica *et al.* (2008) evaluated antioxidant activity and phenolic composition of three different extracts of field horsetail *Equisetum arvense* by measuring the total reducing power, inhibition of lipid peroxidation, free radical scavenging capacity viz., DPPH radical and nitric oxide and total flavonoid content. In addition, they determined the phenolic constituents of each extract, the antioxidant activity of ethanolic crude extract and fractions of *Microgramma vacciniifolia* using DPPH assay. The ethyl acetate fraction of *Microgramma vacciniifolia* showed good activity in DPPH assay.

Kunnathupara *et al*. (2016) studied quantitative phytochemical analysis, *in vitro* anti-oxidant potential and gas chromatography-mass spectrometry studies in ethanolic extract of *Azolla microphylla.* The quantitative phytochemical and *in vitro* anti-oxidant analyses were performed using standard procedures. GC-MS analysis displayed the presence of 21 bioactive compounds, each belonging to various categories of phytochemicals such as chalcones, terpenoids, fatty acids, coumarins and steroids. The results indicated *Azolla microphylla* can be used as an effective scavenger of free radicals and has the potential to be used as a natural anti-oxidant which is attributed to the rich presence of secondary metabolites.

Daonian *et al*. (2010) studied the antioxidant and hepatoprotective activity of *Arachniodes exilis* by different assays viz., reducing power, lipid peroxidation, DPPH, ABTS, superoxide anion, hydroxyl radicals and hydrogen peroxide. The aqueous extract of *Davallia solida* rhizome contains high content of phenolic compound and showed a strong DPPH scavenging activity (Chen *et al.,* 2008). Li *et al.* (2010) determined the central composite design combined with response surface methodology to optimize the parameter of ultrasonic-assisted extraction of total flavonoids from *Selaginella doederleinii.* They observed maximum flavonoids 4.414 mg/g from 70% ethanolic extracts obtained from 50 min at 65°C extraction. The free radical scavenging activity of *Selaginella doederleinii* was ranged from 20.22 to 46.64 mg/ml.

Lai and Lim (2011) studied the antioxidant activity of selected ferns found in Malaysia. Methanolic extracts of fifteen fern species were screened and the results showed very high total phenolic content, above 2000 mg GAE/100g fresh leaves. The ferns with strong antioxidant properties were *Cyathea latebrosa, Cibotium barometez, Drynaria quercifolia, Blechnum orientale* and *Dicranopteris linearis*. These ferns exhibited strong DPPH radical scavenging activity (ascorbic acid equivalents 2866-3111 mg/100g), ferric ion reducing power (963-1417 mg GAE/100g) and inhibition of lipid peroxidation. Strong chelating activity was found in *Pteris vittata* and *Pteris venulosa*.

Rajurkar and Kunda (2012) studied the antioxidant activity of ethanolic extract of *Adiantum capillus-veneris* and compared to the standard ascorbic acid. The results exhibit low IC_{50} values 0.3986 mg/g for DPPH assay and 0.695 mg/g for ABTS assay. Paulsamy *et al.* (2013) reported the antioxidant activities *of Actiniopteris radiata* and *Equisetum ramosissimum* methanolic extracts. The results were found to have potent antioxidant activity against DPPH with the IC_{50} value of 93.48 and 78.58 respectively. *Actiniopteris radiata* had the highest values for ABTS+ radical (2523.11 µTE/g) and reducing power assay (0.853

absorbance at 700 µg/ml). *Equisetum ramosissimum* exhibited higher iron chelating activity (41.18% at 5000 µg/ml).

Jaishee and Chakraborty (2014) determined the antioxidant activity of *Pteris biaurita* by DPPH free radical scavenging activity, hydrogen peroxide scavenging assay, nitric oxide scavenging assay, superoxide scavenging and ferric reducing antioxidant power assay. All the tested three extracts showed appreciable antioxidant activity in a dose-dependent manner.

Jinu *et al.* (2014) evaluated the polyphenolic composition and antioxidant properties of methanolic extract of *Drynaria quercifolia* rhizome. The extract yielded total phenolic content of 240 ± 0.01 mg (GAE)/100g of fresh mass and total flavonoid content of 150 ± 0.02 mg (QE)/100g of fresh mass. The extract of *Drynaria quercifolia* rhizome exhibited remarkable scavenging capacity towards DPPH, OH, NO, H_2O_2 and $ABTS^+$. The antioxidant capacities of the extract were comparable and stronger than that of the antioxidant standard, butyl hydroxy toluene. Ethyl acetate fraction of *Cheilanthes albomarginata* showed the strongest DPPH radical scavenging (82.54 ± 0.48%), hydrogen peroxide scavenging (3.41 ± 0.21 mg/ml) and nitrite scavenging activity (61.39%). The highest phenolic content was found in the ethyl acetate fraction followed by the butanol fraction (Lamichhane *et al.,* 2014).

Mathad *et al.* (2015) evaluated the in vitro antioxidant property and phytochemical constituents of *Actiniopteris radiata*. The scavenging activity of DPPH, H_2O_2 and reducing power including phenolics, flavonoids, alkaloids and tannin contents were also determined. The extracts exhibited scavenging activity towards all radicals tested due to the presence of relatively high alkaloids and flavonoids content.

CHAPTER III

MATERIALS AND METHODS

III. MATERIALS AND METHODS

COLLECTION OF PLANT MATERIAL

The plant materials for the present study were collected from Kamanaickenpatti, located in Thoothukudi district, Tamil Nadu, India, during the month of December, 2020. The collection of the *Vicoa indica* (L.) Dc. (Plate-1) belonging to Asteraceae. The collected materials were washed thoroughly with tap water to remove the epiphytes. The samples were packed separately in polythene bags in wet conditions and brought to the laboratory, once again thoroughly washed with distilled water to remove the sediment dust particles on the surface of the plant materials. They were stored in 5% formalin solution.

PHYTOCHEMICAL ANALYSIS

PRELIMINARY PHYTOCHEMICAL ANALYSIS OF *VICOA INDICA* (L.) DC.

For the preliminary phytochemical analysis, the different extracts (methanol, acetone, chloroform, ethyl acetate and benzene) of *Vicoa indica* (L.) Dc. were tested for the presence of alkaloids, anthocyanin, anthraquinones, cardiac glycosides, coumarin, diterpenes, flavonoids, glycosides, phenols, phlobatannins, phytosteroids, quinones, saponins, tannins and terpenoids. Preliminary phytochemical screening of the extracts was carried out according to the standard methods (Harborne, 1998).

PREPARATION OF EXTRACTS

For the preparation of different extracts, the plant specimens were washed thoroughly and placed on blotting paper and spread out at room temperature in the shade condition for drying. The shade dried samples were grounded to fine powder using a tissue blender. The powdered samples were then stored in the refrigerator for further use. 30g powdered samples were packed in Soxhlet apparatus and extracted with methanol, acetone, chloroform, ethyl acetate and benzene for 8h separately.

TEST FOR ALKALOIDS

1ml of 1% HCl was added to the 2ml of extract in a test tube and was treated with few drops of Mayer's reagent. A creamy white precipitate indicates the presence of alkaloids.

TEST FOR ANTHOCYANIN

2ml of extract was added with 1ml of 2N NaOH and heated for 5min. the formation of bluish green colour indicated the presence of anthocyanin.

TEST FOR ANTHRAQUINONES

2ml extract was mixed with benzene and 1ml 10% ammonia solution was added. The presence of a pink, red or violet color indicates the anthraquinones.

TEST FOR CARDIAC GLYCOSIDES

Take 2ml extract, 2ml of glacial acetic acid, 1ml of Conc. sulphuric acid and few drops of 5% ferric chloride. The formation of brown ring indicates the presence of cardiac glycosides.

TEST FOR COUMARINS

1ml of extract was added with 1ml of 1N NaOH. The test tubes were kept in boiling water bath for few minutes and shaken well. The appearance of yellow colour indicates the presence of coumarins.

TEST FOR DITERPENES

1ml of extract was added to 1ml of distilled water and 10 drops of copper acetate solution. A emerald green color indicates the presence of diterpenes.

TEST FOR FLAVONOIDS

A few drops of 1% NH_3 solution was added to 2 ml of extract in a test tube. A yellow Coloration indicates the presence of flavonoids.

TEST FOR GLYCOSIDES

2ml of 50% H_2SO_4 was added to 2ml of extract. 10ml of Fehling's solution was added and boiled. A brick red precipitate indicates the presence of glycosides.

TEST FOR PHENOLS

To 1ml extract, add 2ml distilled water followed by few drops of 10% Ferric chloride. The formation of blue or black colour indicates the presence of phenolic groups.

TEST FOR PHLOBATANNINS

1ml extract was added with 1% aqueous HCl and then boiled. Red precipitate indicates the presence of phlobatannins.

TEST FOR PHYTOSTEROIDS

1ml of extract added to 1ml $CHCl_3$ and few drops of Conc. H_2SO_4. Golden red colour or Brown colour indicates the presence of phytosteroids.

TEST FOR QUINONES

1ml of plant extract added with 1ml of alcoholic KOH. Red to blue colour indicates the presence of quinones.

TEST FOR SAPONINS

2ml of extract was shaken vigorously with 5ml distilled water to obtain stable persistent foam. The formation of emulsion indicates the presence of saponins.

TEST FOR TANNINS

To 2ml extract, 1ml of distilled water and 1-2 drops of ferric chloride solution was added and observed for brownish green or a blue black coloration indicates the presence of tannins.

TEST FOR TERPENOIDS

2ml extract was mixed with 2ml of $CHCl_3$ in a test tube. 3ml Conc. H_2SO_4 was added carefully along the wall of the test tube to form a layer. An interface with a reddish brown coloration confirms the presence of terpenoids.

UV-VISIBLE SPECTRAL ANALYSIS

The crude extracts of methanol, acetone, chloroform, ethyl acetate and benzene of *Vicoa indica* (L.) Dc. containing the bioactive compound was analyzed spectroscopically for further confirmation. The various crude extracts of *Vicoa indica* (L.) Dc. were scanned in a wavelength ranging from 200-900nm using a Shimazdu spectrophotometer and characteristic peaks were detected.

FTIR ANALYSIS

FTIR analysis of the methanol, acetone, chloroform, ethyl acetate and benzene extracts of *Vicoa indica* (L.) Dc. was performed using Perkin Elmer Spectrophotometer system, which was used to detect the characteristic peaks and their functional groups. The peak values of the FTIR were recorded. Each and every analysis was repeated twice and confirmed the spectrum.

GC-MS SPECTRUM ANALYSIS

The GC-MS analysis of various crude extracts of *Vicoa indica* (L.) Dc. was carried out using GC model Clarus 680, Mass Spectrometer Clarus 600 (EI) Perkin Elmer, Gas Chromatograph equipped and coupled to a mass detector TurboMass 5.4.2 spectrometer with an Elite-5MS, (100% Dimethyl ply siloxane), 30.0m × 250µm df capillary column. The instrument was set to an initial temperature of 60°C and maintained at this temperature for 2min. At the end of this period, the oven temperature was raised upto 300°C, at the rate of an increase of 10°C/min and maintained for 6min. Injection port temperature was ensured as 250°C and Helium flow rate as 1ml/min. The ionization voltage was 70eV. The samples were

injected in split mode as 10:1. Mass Spectral condition solvent delay 2min, transfer temperature 240°C, source temperature 240°C and scaning range was set at 50-600Da. The chemical constituents were identified by GC-MS.

Interpretation of mass spectrum of GC-MS was conducted using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The spectrum of the known component was compared with the spectrum of the known components stored in the NIST library. The name, retention time, molecular weight and molecular formula of the components of the test materials were ascertained.

ANTIOXIDANT ACTIVITY

DPPH FREE RADICAL SCAVENGING ASSAY

Methanolic extract of *Vicoa indica* (L.) Dc. was analyzed for the antioxidant activity based on the scavenging activity of the 1,1-diphenyl-2-picryl hydrazyl (DPPH) free radical using the method of Mensor *et al.* (2001). DPPH is a stable free radical and acts as a scavenger for other radicals. Rate reduction of a chemical reaction using DPPH is a useful indicator of the radical state of a reaction. Methanolic extract were prepared in triplicates at different concentrations (100-500µg/ml) and transferred into 1ml of 0.3mM methanolic DPPH solution (Sigma Aldrich). Samples were left to stand for 30 minutes in the light and the absorbance was measured at 517nm, zeroing the spectrophotometer with a methanol blank. The DPPH radical had a dark violet colour solution, and once neutralized, became pale yellow allowing visual monitoring of the radical reaction. Ascorbic acid was used as a positive control from Sigma was also used for a comparison. The percentage of inhibition was calculated using the following equation:

$$\text{Inhibition Percentage} = \frac{\text{Absorbance of Blank} - \text{Absorbance of Sample}}{\text{Absorbance of Blank}} \times 100$$

CUPRAC Assay

The CUPRAC (Cupric Reducing Antioxidant Capacity) method was also applied for the determination of anti-oxidant activity of *Vicoa indica* (L.) Dc. Copper chloride ($CuCl_2$) solution (0.01M) was prepared by dissolving 0.426g $CuCl_2$ in water and diluting the solution to 250ml. Ammonium acetate (NH_4Ac) buffer (pH 7, 1.0M) was made by dissolving 19.27g of NH_4Ac in water, and diluting this solution to 250ml. Neocuproine (Nc) solution (0.075M) was prepared fresh by dissolving 0.039g Nc in 96% ethanol and diluting to 25ml with ethanol. The isolated extracts were prepared separately in triplicates at different concentrations (100-500µg/ml) and added into a solution containing 1ml $CuCl_2$, 1ml NH_4Ac, 1ml neocuproine and 0.1ml water. Test samples were incubated for 10 minutes at room temperature and the final absorbance was measured at 450nm, zeroing the spectrophotometer with water blank.

CHAPTER IV

RESULTS AND DISCUSSION

IV. RESULTS AND DISCUSSION

PHYTOCHEMICAL ANALYSIS

PRELIMINARY PHYTOCHEMICAL ANALYSIS OF *VICOA INDICA* (L.) DC.

In the preliminary phytochemical analysis of *Vicoa indica* (L.) Dc., fifteen different types of secondary metabolites (alkaloids, anthocyanin, anthraquinones, cardiac glycosides, coumarin, diterpenes, flavonoids, glycosides, phenols, phlobatannins, phytosteroids, quinones, saponins, tannins and terpenoids) were tested in five different extracts (methanol, acetone, chloroform, ethyl acetate and benzene). Thus, out of (1x5x15) 75 tests for the presence or absence of the compounds, 46 tests gave positive results and the remaining gave negative results. The 46 positive results showed the presence of alkaloids, flavonoids, glycosides, phenols, saponins and tannins being found in four extracts, anthocyanin, diterpenes, phytosteroids and terpenoids in three different extracts, followed by anthraquinones, cardiac glycosides, coumarins, phlobatannins and quinones found in only two extracts.

Among the five different extracts, methanol extract showed the presence of the maximum number (13) of compounds. Next to methanol, acetone and chloroform extracts showed the presence nine compounds each, ethyl acetate extract with eight compounds, followed by benzene extract showed the presence of seven compounds (Table-1).

UV-VISIBLE SPECTRUM ANALYSIS OF *VICOA INDICA* (L.) DC.

The UV-Visible spectrum of the different extracts of *Vicoa indica* (L.) Dc. was selected at the wavelength of 200nm to 900nm due to the sharpness of the peaks and proper baseline. The methanol spectrum of *Vicoa indica* (L.) Dc. showed the compounds separated at the nm of 663, 607, 540, 460, 420 and 300 with the absorption of 3.374, 1.589, 1.653, 4.000, 4.000 and 0.361 respectively (Table-2 & Figure-1).

Acetone spectrum of *Vicoa indica* (L.) Dc. showed the compounds separated at the nm of 662, 414 and 376 with the absorption of 0.249, 0.708 and 0.521 respectively (Table-2 & Figure-2). Likewise, chloroform extract of *Vicoa indica* (L.) Dc. showed the compounds separated at the nm of 665, 406, 261, 254, 248 and 243 with the absorption of 0.090, 0.799, 1.962, 2.340, 2.201 and 2.161 respectively (Table-2 & Figure-3).

FTIR SPECTRUM ANALYSIS OF *VICOA INDICA* (L.) DC.

The FTIR spectrum was used to identify the functional group of the active components based on the peak value in the region of infra red radiation. The various crude extracts of *Vicoa indica* (L.) Dc. was passed into the FTIR and the functional groups of the components were separated based on its peak ratio.

As illustrated in Table-3 & Figure-4, FTIR spectrum of methanol extract of *Vicoa indica* (L.) Dc. showed different peaks at 719.40, 956.63, 1274.86, 1377.08, 1463.87, 2850.59 and 2920.03cm^{-1}. It was confirmed the presence of functional groups such as phenols (OH out- of-plane deformation), vinyl (CH_2 out-of-plane wag), ethers (C-O-C antisym stretching), isopropyl (CH_3 deformation), aliphatic (CH_3 antisym deformation), alkane (C-H stretching) and aliphatic (CH antisym and sym stretching).

FTIR spectrum of acetone extract of *Vicoa indica* (L.) Dc. showed different peaks at 640.32, 1037.63, 1076.21, 1228.57, 1384.79, 1735.81, 2850.59 and 2921.96cm^{-1}. It was confirmed the presence of functional groups such as esters (O-C-O bending), cyclic alcohols (C-O stretching), sulfonic acids (SO_3 sym stretching), amines (C-C-N bending), sulfonyl chlorides (SO_2 antisym stretching), aldehydes (C=O S stretching), alkane (C-H stretching) and aliphatic (CH antisym and sym stretching) respectively (Table-4 & Figure-5).

The chloroform extract of FTIR spectrum of *Vicoa indica* (L.) Dc. showed different peaks at 723.26, 1222.79, 1382.87, 1463.87, 1544.88, 1712.67 and 2923.88cm^{-1}. It was confirmed the presence of functional groups such as alkenes (CH=CH plane deformation), vinyl ethers (CH_2 out-of-plane wag), sulfonyl chlorides (SO_2 antisym stretching), aliphatic (CH_3 antisym deformation), aromatic nitro (NO_2 antisym stretching), ketones (C=O Stretching) and aliphatic (CH antisym and sym stretching) respectively (Table-5 & Figure-6).

GC-MS SPECTRAM ANALYSIS OF *VICOA INDICA* (L.) DC.

The compounds present in the different extracts of *Vicoa indica* (L.) Dc. were identified after the comparison of the mass spectra with NIST library by GC-MS analysis. The active principles with their retention time (RT), molecular formula, molecular weight and area percentage in the different extracts of *Vicoa indica* (L.) Dc. were presented.

GC-MS profile of methanol extract of *Vicoa indica* (L.) Dc. was found to have 26 major peaks which showed the presence of 26 compounds. The identified compounds in methanol extract were aromandendrene (0.211%), β-pinene (0.213%), Z,z-6,28-heptatriactontadien-2-one (2.789%), 2-hexadecanol (0.311%), tetradecanoic acid, 10,13-dimethyl-, methyl ester (4.702%), hexadecanoic acid, ethyl ester (1.41%), elimicin (0.325%), 1-hexyl-2-nitrocyclohexane (19.00%), methyl 1,3-octadecenoate (2.206%), octadecanoic acid (0.117%), 1-pentanol,2,2,4 trimethyl- (0.009%), thujone (0.11%), bicyclo[4.1.0] heptan-3-ol,4,7,7-trimethyl-(1a,3a,4a,6a,)- (0.22%), palmitaldehyde (0.078%), tetradecanoic acid, ethyl ester (CAS) (0.775%), methyl heneicosanoate (0.264%), trans-5-hexyl-1,4-dioxane-2-carboxylic acid (1.113%), tetradecanoic acid (0.009%), vitamin E (0.11%), (2-propyl-1,3-dioxolan-2-yl)acetic acid (0.23%), 1,2-benzenedicarboxylic acid, bis (2-methylprophyl) (0.161%), β-sitosterol (23.399%) and 3-penten-2-one, 4-(2,2,6-trimethyl-7-oxabicyclo[4.1.0]hept-1-yl)-, (e)- (4.308%), vitamin E (1.21%), n-hexadecanoic acid (1.28%) and stigmasterol (3.21%). The spectrum profile of GC-MS confirmed the presence of twenty

six major components with retention time of 14.49min, 15.45min, 16.40min, 16.85min, 17.34min, 17.83min, 18.02min, 18.30min, 18.98min, 19.63min, 20.11min, 20.81min, 21.40min, 22.62min, 23.03min, 24.04min, 24.58min, 26.14min, 26.46min, 27.06min, 27.96min, 28.58min, 28.88min, 28.67min, 28.75min and 31.00min respectively (Table-6 & Figure-7).

As illustrated in Table-7 & Figure-8, the acetone extract of *Vicoa indica* (L.) Dc. showed 22 major peaks which indicated the presence of 22 compounds by GC-MS spectrum. The known compounds in methanol extract were aromandendrene (3.421%), heptadecane (1.25%), 1,2-benzenedicarboxylic acid, butyl octyl ester (0.42%), capric acid methyl ester ester (12.11%), 2-(2-nitroallyl)-cyclohexanone (10.02%), hexadecanoic acid, ethyl ester (0.32%), benzeneacetic acid, 2,5-dihydroxy- [synonyms: homogentisic acid] (9.65%), hexadecanoic acid fatty acid (0.028%), 1,14-tetradecanediol (1.2%), α.-pyrrolidone, 5-[3-hydroxybutyl]- (8.21%), furfural (0.03%), palmitaldehyde (2.102%), β-bourbonene (0.28%), trans-anethole (1.21%), 6-undecyn-5-ol (2.156%), methyl 10-thia-octadecanoate (4.21%), trimethyl[4-(1,1,3,3, tetramethylbutyl) phenox] silane (2.56%), 2-furancarboxaldehyde (14.553%), β-sitosterol (1.121%), 9,12-octadecadienoic acid, methyl ester (0.01%), erythrodiol (0.10%) and methyl-octadecanoate (0.001). The spectrum profile of GC-MS confirmed the presence of twenty two major components with retention time of 14.44min, 15.42min, 16.42min, 16.86min, 17.36min, 17.83min, 18.21min, 19.59min, 19.94min, 20.81min, 22.01min, 22.65min, 23.97min, 24.64min, 26.13min, 26.52min, 27.13min, 28.04min, 28.69min, 30.21min, 30.80min and 31.39min respectively.

As presented in Table-8 & Figure-9, there are 21 different major peaks identified by GC-MS spectrum in chloroform extract of *Vicoa indica* (L.) Dc. which indicated the presence of 21 compounds. The existing compounds in methanol extract were asarone (1.205%), 2-phenoxysulfonyl-acetimidic acid methyl ester (0.314%), 1H-cycloprop[e]azulene (2.789%), 3,7,11,15-tetramethyl-2-hexadecen-1-ol (0.673%), ethyl 15-methyl-hexadecanoate (28.561%), n-hexadecanoic acid (0.316%), cis-1-chloro-9-octadecene (1.078%), N-propyl 11-octadecenoate (23.660%), thujol (0.625%), bicyclo[4.1.0]heptane, 7-pentyl- (1.455%), 1-octacosanol (0.101%), 1,2-benzenedicarboxylic acid, mono(2-ethylhexyl) ester (7.375%), 16-heptadecenal (0.264%), 2H-pyran, 2-(7- heptadecynyloxy) tetrahydro- (0.611%), methyl tricosanoate (0.016%), 2,4,6-cycloheptatrien-1-one, 3,5-bis-trimethylsilyl- (0.113%), 2-furancarboxaldehyde (0.431%), tridecanol, 2-ethyl-2- methyl- (0.316%), γ.-sitosterol (10.600%), erythrodiol (0.12%) and 2-pentanethiol (0.34%). The spectrum profile of GC-MS confirmed the presence of twenty one major components with retention time of 14.52min, 16.15min, 16.46min, 16.90min, 18.03min, 18.29min, 19.19min, 19.63min, 20.52min, 21.36min, 21.96min, 22.68min, 23.85min, 24.68min, 26.20mimn, 26.57min, 27.16min, 28.11min, 28.76mmin, 30.30min and 30.89min respectively.

ANTI-OXIDANT ACTIVITY OF *VICOA INDICA* (L.) DC.

DPPH Assay

The DPPH free radical scavenging activity has been used by various researchers as quick and reliable parameters to assess the *in vitro* antioxidant activity. In the assay, DPPH radicals reacted with suitable reducing agents and then electrons became paired off and the solutions lose its colour and became yellow. The ability of a compound to act as donor for hydrogen atoms or electrons was measured. The percentages of anti-oxidant property of the methanol extract was compared with the natural anti-oxidant ascorbic acid at the concentrations ranging from 100-500µg.

The methanol extract of *Vicoa indica* (L.) Dc. showed the antioxidant potential in concentration dependent pattern as shown in Table-9. Ascorbic acid had over 90% scavenging activity at a concentration of 100µg, whereas the tested methanol extract of *Vicoa indica* (L.) Dc. required a concentration of 500µg to reach a similar percentage. The methanol extract of *Vicoa indica* (L.) Dc. showed the percentage of DPPH free radical scavenging activity at 100µg, 200µg, 300µg, 400µg and 500µg were 19.18, 33.61, 51.87, 62.34 and 77.62% respectively.

Among the various concentration tested, 500µg methanol extract had the strongest scavenging ability while 100µg showed the lowest. Natural anti-oxidants are widely utilized in the food and medicine industries as they counteract the cellular free radicals. Anti-oxidant capacity is important marker for assessing medicinal bioactive components. The important property of an anti-oxidant is its ability to scavenge free radicals. DPPH radical scavenging is one of the most commonly used method for assessment of antiradical activity of medicinal plants. DPPH contains an odd electron which gives absorption maximum at 517nm and is purple in color. When free radical scavenging anti-oxidants donates their hydrogen to free radical, it becomes paired with hydrogen and formed reduced form of DPPH. After reduction, the color of DPPH is changed from purple to yellow.

In the current study, the DPPH radical scavenging method was used to evaluate the antioxidant capacity of the plant extracts, because the use of DPPH radical provides an easy, rapid and convenient method. Free radical scavenging activity of *Vicoa indica* (L.) Dc. was evaluated in the methanol extract. Five different concentrations of extract prepared with methanol were assessed for DPPH inhibition at different concentrations (100-500µg/ml). Percentage inhibition of DPPH radical with selected *Vicoa indica* (L.) Dc. has been presented in table and figure. The present study explained that when the concentration of methanol extracts increased, the antioxidant property of *Vicoa indica* (L.) Dc. also increased.

CUPRAC Assay

The reducing power of *Vicoa indica* (L.) Dc. was determined on copper ions using the CUPRAC assay. All samples exhibited the ability of reducing copper ions from Cu(II) to Cu(I) in a concentration dependent manner. 500µg of *Vicoa indica* (L.) Dc. possessed the highest percentage of reducing activity when compared to the other concentrations. At the concentration of 100µg, 200µg, 300µg, 400µg and 500µg of the methanolic extract of *Vicoa indica* (L.) Dc. showed the reducing capacity of 11.18, 19.65, 28.41, 31.63 and 38.42% respectively (Table-10). These results related to those obtained from the DPPH assay in which 500µg showed the highest total anti-oxidant capacity, followed by 400µg, 300µg, 200µg and 100µg of the selected plant parts.

Plate-1a: Natural habit of *Vicoa indica* (L.) Dc.

Plate-1b: *Vicoa indica* (L.) Dc. - A single plant

Table-1: Preliminary phytochemical analysis of *Vicoa indica* (L.) Dc.

Tests	SOLVENTS				
	Methanol	Acetone	Chloroform	Ethyl acetate	Benzene
Alkaloids	+	+	+	-	+
Anthocyanin	+	+	-	+	-
Anthraquinone	-	-	+	-	+
Cardiac Glycosides	-	+	-	+	-
Coumarins	+	-	+	-	-
Diterpenes	+	-	-	+	+
Flavonoids	+	+	-	+	+
Glycosides	+	+	+	+	-
Phenols	+	+	-	+	+
Phlobatannins	+	-	+	-	-
Phyto steroids	+	+	+	-	-
Quinones	+	-	+	-	-
Saponins	+	+	-	+	+
Tannins	+	-	+	+	+
Terpenoids	+	+	+	-	-

Table-:2 UV Visible spectrum of various extracts of *Vicoa indica* (L.) Dc.

SOLVENTS	Nm	Abs
Methanol	663	3.374
	607	1.589
	540	1.653
	460	4.000
	420	4.000
	300	0.361
Acetone	662	0.249
	414	0.708
	376	0.521
Chloroform	665	0.090
	406	0.799
	261	1.962
	254	2.340
	248	2.201
	243	2.161

Figure 1: UV-Visible spectrum of methanol extract of *Vicoa indica* (L.) Dc.

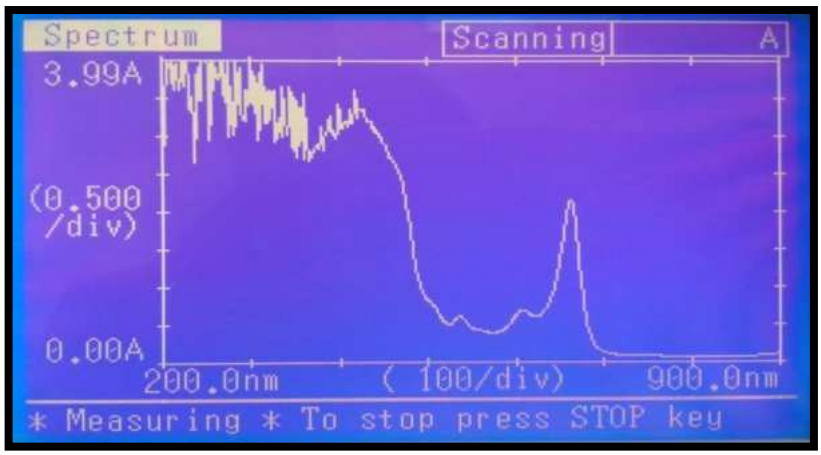

Figure 2: UV-Visible spectrum of acetone extract of *Vicoa indica* (L.) Dc.

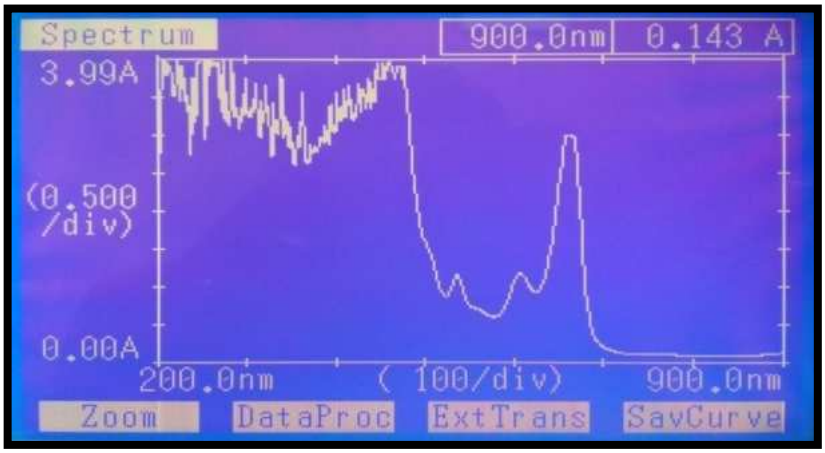

Figure 3: UV-Visible spectrum of chloroform extract of *Vicoa indica* (L.) Dc.

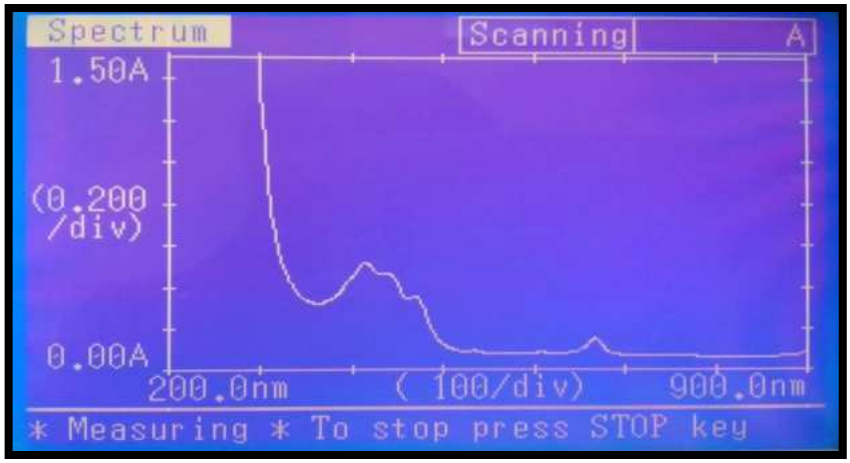

Figure-4: FTIR spectrum of methanol extract of *Vicoa indica* (L.) Dc.

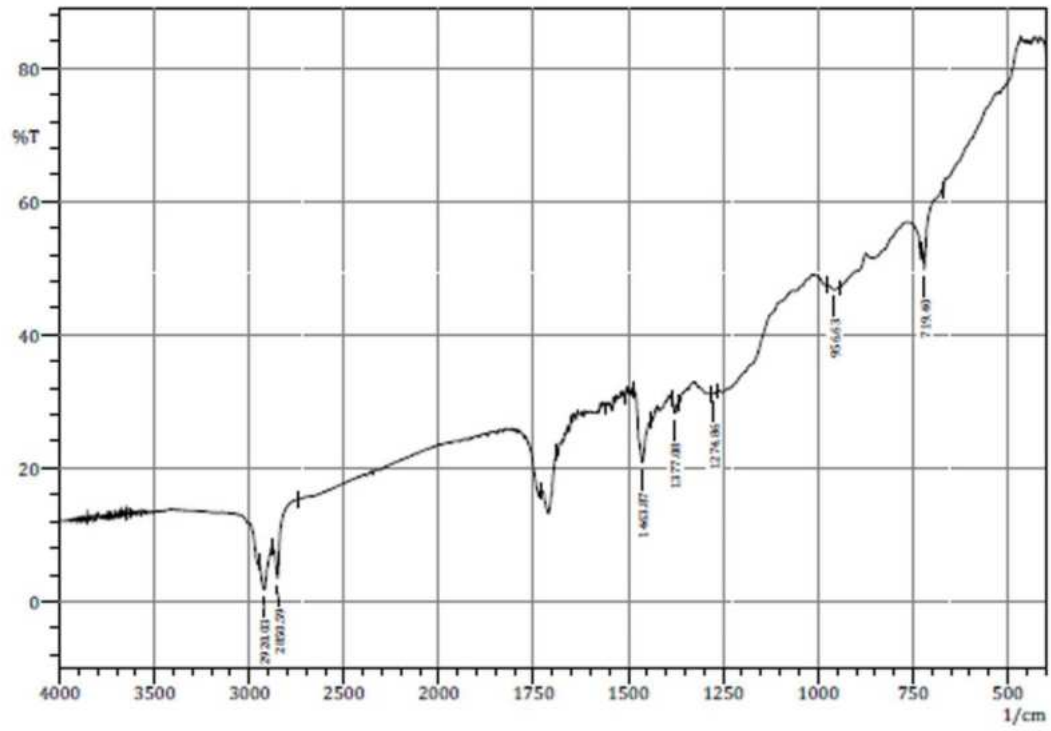

Table-3: FTIR spectrum of methanol extract of *Vicoa indica* (L.) Dc.

Peak values (cm^{-1})	Functional groups	Assignment
719.40	Phenols	OH out- of-plane deformation
956.63	Vinyl	CH_2 out-of-plane wag
1274.86	Ethers	C-O-C antisym stretching
1377.08	Isopropyl	CH_3 deformation
1463.87	Aliphatic	CH_3 antisym deformation
2850.59	Alkane	C-H stretching
2920.03	Aliphatic	CH antisym and sym stretching

Figure-5: FTIR spectrum of acetone extract of *Vicoa indica* (L.) Dc.

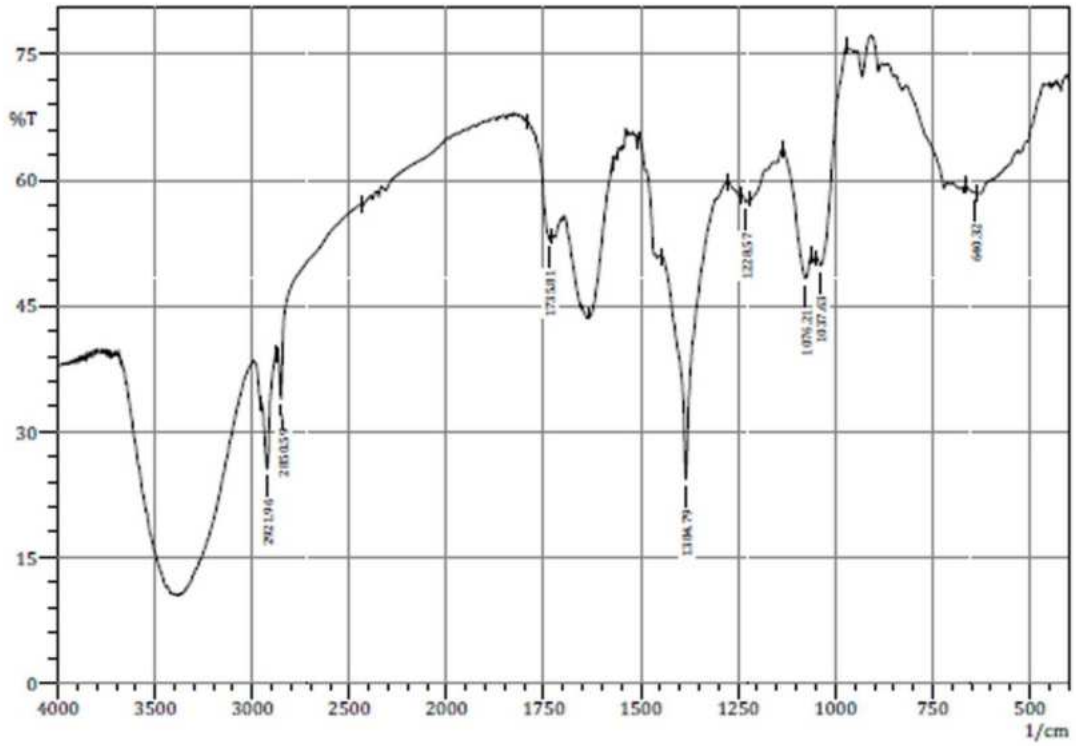

Table-4: FTIR spectrum of acetone extract of *Vicoa indica* (L.) Dc.

Peak values (cm^{-1})	Functional groups	Assignment
640.32	Esters	O-C-O bending
1037.63	Cyclic alcohols	C-O stretching
1076.21	Sulfonic acids	SO_3 sym stretching
1228.57	Amines	C-C-N bending
1384.79	Sulfonyl chlorides	SO_2 antisym stretching
1735.81	Aldehydes	C=O S stretching
2850.59	Alkane	C-H stretching
2921.96	Aliphatic	CH antisym and sym stretching

Figure-6: FTIR spectrum of chloroform extract of *Vicoa indica* (L.) Dc.

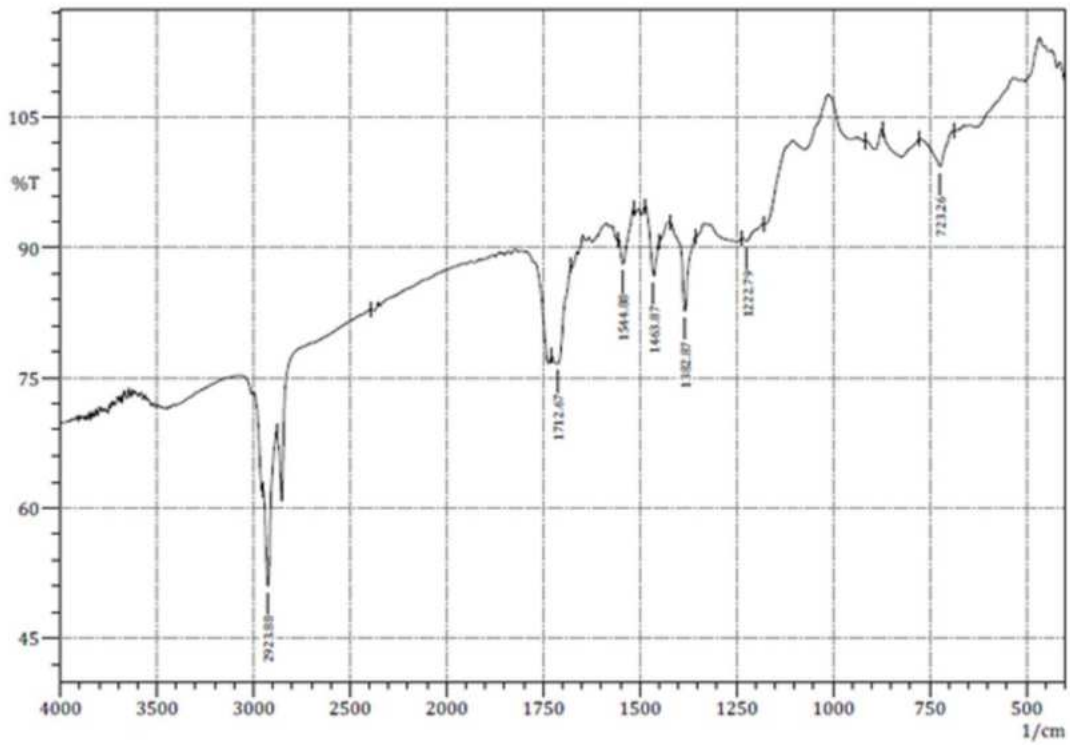

Table-5: FTIR spectrum of chloroform extract of *Vicoa indica* (L.) Dc.

Peak values (cm^{-1})	Functional groups	Assignment
723.26	Alkenes	CH=CH plane deformation
1222.79	Vinyl ethers	CH_2 out-of-plane wag
1382.87	Sulfonyl chlorides	SO_2 antisym stretching
1463.87	Aliphatic	CH_3 antisym deformation
1544.88	Aromatic nitro	NO_2 antisym stretching
1712.67	Ketones	C=O Stretching
2923.88	Aliphatic	CH antisym and sym stretching

Figure-7: GC-MS profile of methanol extract of *Vicoa indica* (L.) Dc.

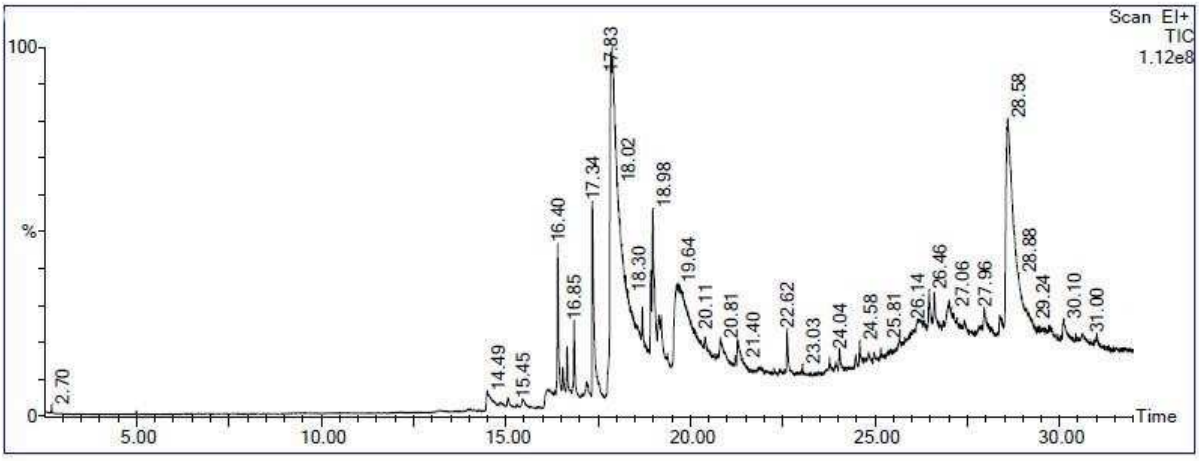

Table-6: GC-MS profile of methanol extract of *Vicoa indica* (L.) Dc.

SN	RT	Name of the compounds	MF	MW	PA (%)
1.	14.49	Aromandendrene	$C_{15}H_{24}$	204.35	0.211
2.	15.45	β-Pinene	$C_{10}H_{16}$	136.25	0.213
3.	16.40	Z,z-6,28-Heptatriactontadien-2-one	$C_{37}H_{70}O$	530	2.789
4.	16.85	2-Hexadecanol	$C_{16}H_{34}O$	242	0.311
5.	17.34	Tetradecanoic acid, 10,13-dimethyl-, methyl ester	$C_{17}H_{34}O_2$	270	4.702
6.	17.83	Hexadecanoic acid, ethyl ester	$C_{16}H_{32}O_2$	256.42	1.41
7.	18.02	Elimicin	$C_{12}H_{16}O_3$	208	0.325
8.	18.30	1-Hexyl-2-nitrocyclohexane	$C_{12}H_{23}NO_2$	213.31	19.06
9.	18.98	Methyl 1,3-octadecenoate	$C_{19}H_{36}O_2$	296	2.206
10.	19.63	Octadecanoic acid	$C_{18}H_{36}O_2$	284	0.117
11.	20.11	1-pentanol,2,2,4 Trimethyl-	$C_8H_{18}O$	130.2	0.009
12.	20.81	Thujone	$C_{10}H_{16}O$	152	0.11
13.	21.40	Bicyclo[4.1.0] heptan-3-ol,4,7,7-trimethyl-(1a,3a,4a,6a,)-	$C_{10}H_{18}O$	154	0.22
14.	22.62	Palmitaldehyde	$C_{16}H_{32}O$	240	0.078
15.	23.03	Tetradecanoic acid, ethyl ester (CAS)	$C_{16}H_{32}O_2$	256	0.775
16.	24.04	Methyl heneicosanoate	$C_{22}H_{44}O_2$	340	0.264
17.	24.58	Trans-5-hexyl-1,4-dioxane-2-carboxylic acid	$C_{11}H_{20}O_4$	216	1.113
18.	26.14	Tetradecanoic acid	$C_{14}H_{28}O_2$	228	0.009
19.	26.46	Vitamin E	$C_{29}H_{50}O_2$	430	0.112
20.	27.06	(2-propyl-1,3-dioxolan-2-yl)aceticacid	$C_8H_{14}O_4$	174	0.234
21.	27.96	1,2-Benzenedicarboxylic acid, bis (2-methylprophyl)	$C_{16}H_{22}O_4$	278	0.161
22.	28.58	β-sitosterol	$C_{29}H_{50}O$	414	23.39
23.	28.88	3-Penten-2-one, 4-(2,2,6-trimethyl-7-oxabicyclo[4.1.0]hept-1-yl)-, (e)-	$C_{14}H_{22}O_2$	222.3	4.308
24.	29.24	Vitamin E	$C_{29}H_{50}O_2$	430	1.21
25.	30.10	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.2	1.28
26.	31.00	Stigmasterol	$C_{29}H_{48}O$	412	3.21

SN : Serial Number; RT: Retention Time; MF: Molecular Formula;
MW: Molecular Weight; PA: Peak Area.

Figure-8: GC-MS profile of acetone extract of *Vicoa indica* (L.) Dc.

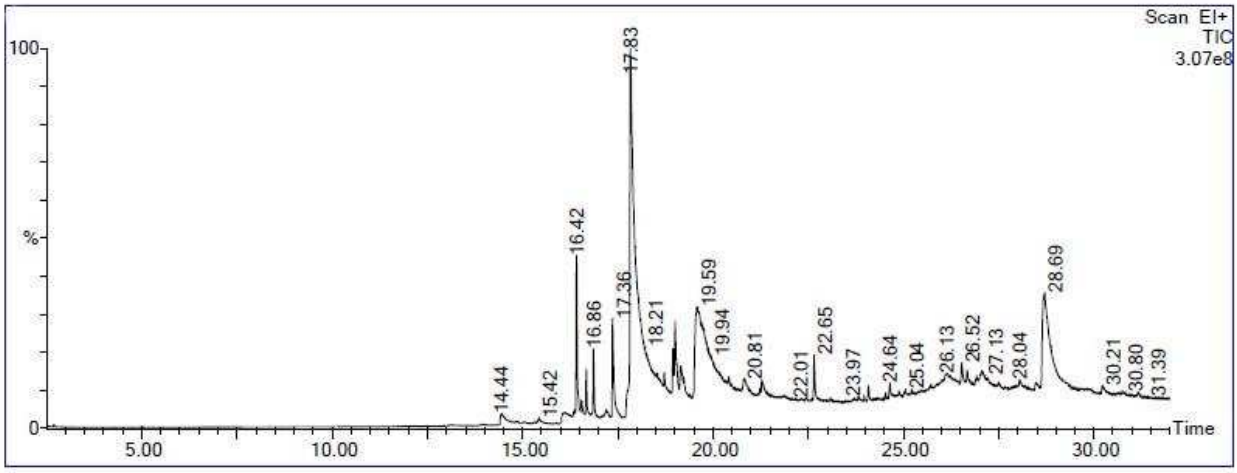

Table-7: GC-MS profile of acetone extract of *Vicoa indica* (L.) Dc.

SN	RT	Name of the compounds	MF	MW	PA (%)
1.	14.44	Aromandendrene	$C_{15}H_{24}$	204	3.421
2.	15.42	Heptadecane	$C_{17}H_{36}$	240.4	1.25
3.	16.42	1,2-Benzenedicarboxylic acid, butyl octyl ester	$C_{20}H_{30}O_4$	334	0.42
4.	16.86	Capric acid methyl ester Ester	$C_{11}H_{22}O_2$	186	12.11
5.	17.36	2-(2-Nitroallyl)-cyclohexanone	$C_9H_{13}NO_3$	183	10.02
6.	17.83	Hexadecanoic acid, ethyl ester	$C_{16}H_{32}O_2$	256.4	0.32
7.	18.21	Benzeneacetic acid, 2,5-dihydroxy- [synonyms: Homogentisic acid]	$C_8H_8O_4$	168	9.65
8.	19.59	Hexadecanoic acid Fatty acid	$C_{18}H_{36}O_2$	284	0.28
9.	19.94	1,14-tetradecanediol	$C_{14}H_{30}O_2$	230.3	1.2
10.	20.81	α-Pyrrolidone, 5-[3-hydroxybutyl]-	$C_8H_{15}NO_2$	157.2	8.21
11.	22.01	Furfural	$C_5H_4O_2$	96.08	0.03
12.	22.65	Palmitaldehyde	$C_{16}H_{32}O$	240	0.078
13.	23.97	β-Bourbonene	$C_{10}H_{12}O$	148	0.28
14.	24.64	Trans-Anethole	$C_{11}H_{20}O$	168	1.21
15.	26.13	6-Undecyn-5-ol	$C_{18}H_{36}O_2S$	316	2.156
16.	26.52	Methyl 10-thia-octadecanoate	$C_{17}H_{30}OSi$	278	4.21
17.	27.13	Trimethyl[4-(1,1,3,3, Tetramethylbutyl) phenox] Silane	$C_6H_6O_2$	110	2.56
18.	28.04	2-Furancarboxaldehyde	$C_{29}H_{50}O$	414	14.553
19.	28.69	β-sitosterol	$C_{18}H_{36}O_2$	284	1.121
20.	30.21	9,12-Octadecadienoic acid, methyl ester	$C_{30}H_{50}O_2$	442	0.01
21.	30.80	Erythrodiol	$C_{15}H_{24}$	204	0.1
22.	31.39	Methyloctadecanoate	$C_{19}H_{38}O_2$	298.5	0.001

SN : Serial Number; RT: Retention Time; MF: Molecular Formula;
MW: Molecular Weight; PA: Peak Area

Figure-9: GC-MS profile of chloroform extract of *Vicoa indica* (L.) Dc.

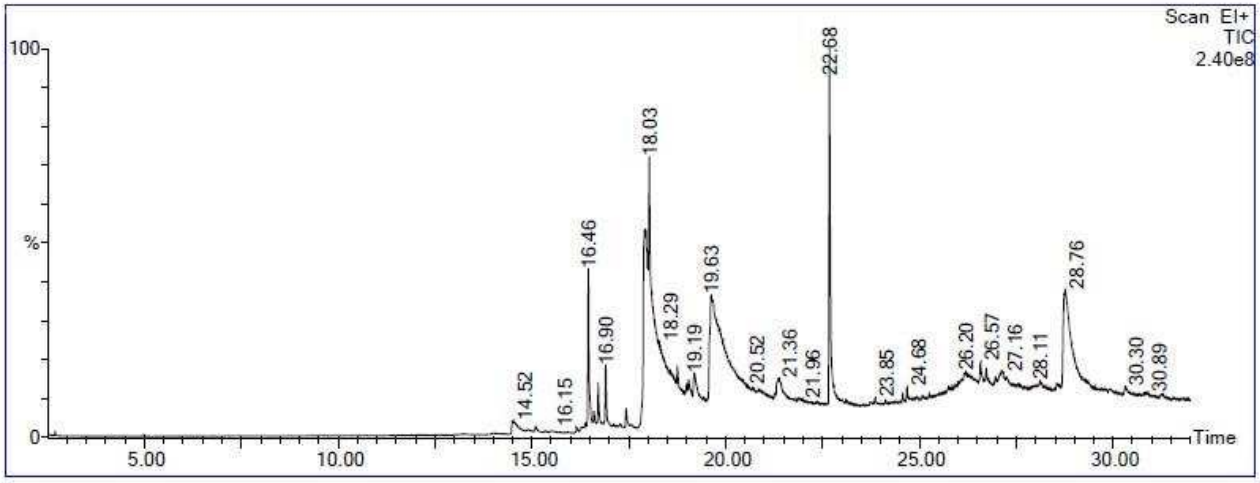

Table-8: GC-MS profile of chloroform extract of *Vicoa indica* (L.) Dc.

SN	RT	Name of the compounds	MF	MW	PA (%)
1.	14.52	Asarone	$C_{12}H_{16}O_3$	208	1.205
2.	16.15	2-Phenoxysulfonyl-acetimidic acid methyl ester	$C_9H_{11}NO_4S$	229	0.314
3.	16.46	1H-Cycloprop[e]azulene	$C_{15}H_{24}$	204.3	2.789
4.	16.90	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296	0.673
5.	18.03	Ethyl 15-methyl-hexadecanoate	$C_{19}H_{38}O_2$	298	28.561
6.	18.29	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	0.316
7.	19.19	Cis-1-chloro-9-octadecene	$C_{18}H_{35}Cl$	286	1.078
8.	19.63	N-propyl 11-octadecenoate	$C_{21}H_{40}O_2$	324	23.660
9.	20.52	Thujol	$C_{10}H_{18}O$	154.2	0.625
10.	21.36	Bicyclo[4.1.0]heptane, 7-pentyl-	$C_{12}H_{22}$	166	1.455
11.	21.96	1-Octacosanol	$C_{28}H_{58}O$	410	0.101
12.	22.68	1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester	$C_{16}H_{22}O_4$	278	7.375
13.	23.85	16-Heptadecenal	$C_{17}H_{32}O$	410	0.264
14.	24.68	2H-Pyran, 2-(7- heptadecynyloxy)tetrahydro-	$C_{22}H_{40}O_2$	336	0.611
15.	26.20	Methyl 10-thia-octadecanoate	$C_{17}H_{30}OSi$	278	4.21
16.	26.57	2,4,6-Cycloheptatrien-1-one, 3,5-bis-trimethylsilyl-	$C_5H_{12}S$	104.2	0.113
17.	27.16	2-Furancarboxaldehyde	$C_6H_6O_2$	110.1	0.431
18.	28.11	Tridecanol, 2-ethyl-2- methyl-	$C_{16}H_{34}O$	242.4	0.316
19.	28.76	γ-sitosterol	$C_{29}H_{50}O$	414	10.600
20.	30.30	Erythrodiol	$C_{30}H_{50}O_2$	442	0.12
21.	30.89	2-Pentanethiol	$C_5H_{12}S$	104.2	0.34

SN : Serial Number; RT: Retention Time; MF: Molecular Formula;
MW: Molecular Weight; PA: Peak Area

Table-9: Anti-oxidant effects on DPPH free radical by various concentrations of methanol extracts of *Vicoa indica* (L.) Dc. and Vitamin C

Concentration (µg)	Percentage of anti-oxidant effect on DPPH	
	Vitamin C	Methanolic extract
100	90.61±1.64	19.18±1.65
200	92.99±1.17	33.61±1.98
300	95.86±1.21	51.87±2.76
400	99.18±2.13	62.34±1.83
500	99.85±3.62	77.62±2.11

Table-10: CUPRAC assay by various concentrations of methanol extract of *Vicoa indica* (L.) Dc. and Vitamin C

Concentration (µg)	Percentage of anti-oxidant effect on CUPRAC	
	Vitamin C	Methanolic extract
100	92.21±1.65	11.18±1.09
200	94.67±1.19	19.65±1.01
300	98.49±2.43	28.41±0.54
400	99.54±1.65	31.63±1.12
500	99.79±1.69	38.42±1.87

CHAPTER V

SUMMARY AND CONCLUSION

V. SUMMARY AND CONCLUSION

The present study deals with the screening of phytochemicals and antioxidant using the selected plant namely *Vicoa indica* (L.) Dc. Fifteen different types of secondary metabolites (alkaloids, anthocyanin, anthraquinone, cardiac glycosides, coumarins, diterpenes, flavonoids, glycosides, phenols, phlobatannins, phytosteroids, quinones, saponins, tannins and terpenoids) were tested in five extracts namely methanol, acetone, chloroform, ethyl acetate and benzene. The various solvent extracts of *Vicoa indica* (L.) Dc. showed the presence of alkaloids, anthocyanin, anthraquinone, cardiac glycosides, coumarins, diterpenes, flavonoids, glycosides, phenols, phlobatannins, phytosteroids, quinones, saponins, tannins and terpenoids.

The UV-Visible spectrum of the different extracts of *Vicoa indica* (L.) Dc. was selected at the wavelength of 200nm to 900nm due to the sharpness of the peaks and proper baseline. The various extracts of *Vicoa indica* (L.) Dc. (methanol, acetone and chloroform) showed the compounds separated at the nm of 665, 663, 662, 607, 540, 460, 420, 414, 406, 376, 300, 261, 254, 248 and 243 with the absorption of 3.374, 1.589, 1.653, 4.000, 4.000, 0.361, 0.249, 0.708, 0.521, 0.090, 0.799, 1.962, 2.340, 2.201 and 2.161 respectively.

FTIR spectrum of methanol, acetone and chloroform extracts of *Vicoa indica* (L.) Dc. showed the presence of functional groups such as amines, ethers, esters, phenols, alkenes, alkyl chlorides, 1,2,4 trisubst benzenes, alkyl chlorides, 1,2,4 trisubst benzenes, vinyl, cyclic alcohols, organophosphorus, sulfonyl acids, siloxanes, vinyl ethers, isopropyl, sulfonyl chlorides, aliphatic, aromatic nitro, primary alkyl amide, amino acids, ketones, aldehydes, phosphines, alkane and alcohols and phenols respectively.

The compounds present in the methanol extract of *Vicoa indica* (L.) Dc. were identified after the comparison of the mass spectra with NIST library by GC-MS analysis. GC-MS spectrum of methanol extract of *Vicoa indica* (L.) Dc. showed the presence of aromandendrene, β-pinene, Z,z-6,28-heptatriactontadien-2-one, 2-hexadecanol, tetradecanoic acid, 10,13-dimethyl-, methyl ester, hexadecanoic acid, ethyl ester, elimicin, 1-hexyl-2-nitrocyclohexane, methyl 1,3-octadecenoate, octadecanoic acid, 1-pentanol,2,2,4 trimethyl-, thujone, bicyclo[4.1.0] heptan-3-ol,4,7,7-trimethyl-(1a,3a,4a,6a,)-, palmitaldehyde, tetradecanoic acid, ethyl ester (CAS), methyl heneicosanoate, trans-5-hexyl-1,4-dioxane-2-carboxylic acid, tetradecanoic acid, vitamin E, (2-propyl-1,3-dioxolan-2-yl)aceticacid, 1,2-benzenedicarboxylic acid, bis (2-methylprophyl), β-sitosterol, 3-penten-2-one, 4-(2,2,6-trimethyl-7-oxabicyclo[4.1.0]hept-1-yl)-, (e)-, n-hexadecanoic acid, stigmasterol, heptadecane, 1,2-benzenedicarboxylic acid, butyl octyl ester, capric acid methyl ester ester, 2-(2-nitroallyl)-cyclohexanone, hexadecanoic acid, ethyl ester, benzeneacetic acid, 2,5-dihydroxy-, hexadecanoic acid fatty acid, 1,14-tetradecanediol, α-pyrrolidone, 5-[3-hydroxybutyl]-, furfural, palmitaldehyde, β-bourbonene, trans-anethole, 6-undecyn-5-ol, methyl 10-thia-octadecanoate, trimethyl[4-(1,1,3,3, tetramethylbutyl) phenox] silane, 2-furancarboxaldehyde, 9,12-octadecadienoic acid, methyl ester, erythrodiol, methyloctadecanoate, asarone, 2-phenoxysulfonyl-acetimidic acid methyl ester, 1H-cycloprop[e]azulene, 3,7,11,15-tetramethyl-2-hexadecen-1-ol, ethyl 15-methyl-hexadecanoate, cis-1-chloro-9-octadecene, N-propyl 11-octadecenoate, thujol, bicyclo[4.1.0]heptane, 7-pentyl-, 1-octacosanol, 1,2-benzenedicarboxylic acid, mono(2-ethylhexyl) ester, 16-heptadecenal, 2H-pyran, 2-(7- heptadecynyloxy)tetrahydro-, methyl 10-thia-octadecanoate, 2,4,6-cycloheptatrien-1-one, 3,5-bis-trimethylsilyl-, 2-furancarboxaldehyde, tridecanol, 2-ethyl-2- methyl-, γ-sitosterol, erythrodiol and 2-pentanethiol.

Crude methanolic extract of *Vicoa indica* (L.) Dc. at various concentrations (100-500µg) were tested for antioxidant activity via the DPPH assay. The percentage of anti-oxidant property of the methanolic extract at concentrations ranging from 100-500µg, The percentage of scavenging activity of DPPH by methanolic extract of *Vicoa indica* (L.) Dc. at 100µg, 200µg, 300µg, 400µg and 500µg were 19.18, 33.61, 51.87, 62.34 and 77.62% respectively.

Methanolic crude extract of *Vicoa indica* (L.) Dc. at various concentrations (100-500µg) were tested for antioxidant activity via the CUPRAC assay. The percentage of scavenging activity of CUPRAC by methanolic extract of *Vicoa indica* (L.) Dc. at 100µg, 200µg, 300µg, 400µg and 500µg were 11.18, 19.65, 28.41, 31.63 and 38.42% respectively.

From the present study, it was concluded that *Vicoa indica* (L.) Dc. has a number of phytochemical compounds which can be used for medicinal purposes. The plant extracts are also capable of anti-oxidant activity.

CHAPTER VI

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VI. REFERENCE

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SYNTHESIS OF PLANT EXTRACTS MEDIATED METAL NANOPARTICLES AND THEIR UV-VISIBLE SPECTROSCOPY CHARACTERIZATION

A short- term project submitted to

ST. Mary's College (Autonomous)

affiliated to

Manonmaniam Sundaranar University, Thirunelveli

in partial fulfilment of the requirements for the degree of

Master of Science in Botany

BY

G. ASLIN BEMISHA, B.Sc.,

Reg.No.19APBO05

DEPARTMENT OF BOTANY

ST.MARY'S COLLEGE (AUTONOMOUS)

Re-accredited with ''A++'' Grade by NAAC

Thoothukudi-628 001

2019-2021

CERTIFICATE

It is certified that this short-term project work entitled **"SYNTHESIS OF PLANT EXTRACTS MEDIATED METAL NANOPARTICLES AND THEIR UV-VISIBLESPECTROSCOPY CHARACTERIZATION"** submitted by **G. ASLIN BEMISHA** in partial fulfilment of M.Sc. degree in botany to **St. Mary's College (Autonomous),** Thoothukudi affiliated toManonmaniam Sundaranar University, Tirunelveli is based on the results of studies carried out by her under my guidance and supervision. It is further certified that this short-term project work at any part thereof has not been submitted elsewhere for any other degree.

M. Glory
HEAD OF THE DEPARTMENT

HOD of Botany
St. Mary's College (Autonomous)
Thoothukudi- 628 001.

G.F.L 15/4/2021
GUIDE

Lucia Rose
PRINCIBLE

St. Mary's College (Autonomous)
Thoothukudi - 628 001.

S.P. 16/4/2021
EXAMINER
(S. PAULINE JENIFER)

DECLARATION

I do hereby declare that this work has been originally carried out by me under the guidance and supervision of guide **Dr. Mrs. G. Flora, M.Sc., M.Phil., Ph.D.,** Assistant professor of Botany, **St. Mary's College (Autonomous),** Thoothukudi and this work has not been submitted elsewhere for the award of any other degree.

G. Aslin Bernisla
Signature of the Candidate

Place: Thoothukudi

Date: 15.4.21

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We offer our praise and thanks to God almighty for his grace and choicest blessings, enabling me to complete this project. We are very happy to place on record our heartfelt gratitude to our guide **Dr. Mrs. G. Flora, M.Sc., M.Phil., Ph.D.,** Assistant professor of Botany, St. Mary's College (Autonomous), Thoothukudi for her meticulous guidance, wise counsel, untiring attention to finish this project successfully.

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CONTENT

INTRODUCTION

INTRODUCTION

Due to rapid industrialization and urbanization, our environment is undergoing great damage and a large amount of hazardous and unwanted chemical, gases or substance are released, and so now it is our need to learn about the secrets that are present in the Nature and is products which leads to the growth of advancements in the synthesis processes of nanoparticles. Nanotechnology applications are highly suitable for biological molecules, because of their exclusive properties. The biological molecules undergo highly controlled assembly for making them suitable for the metal nanoparticle synthesis which was found to be reliable and eco-friendly. The synthesis of metal and semiconductor nanoparticles is a vast area of research due to its potential applications which was implemented in the development of novel technologies. The field of nanotechnology is one of the upcoming areas of research in the modern field of material science. Nanoparticle show completely new or improved properties, such as size, distribution and morphology of the particles etc. Novel applications of nanoparticles and nanomaterials are emerging rapidly on various fields. Most of the chemical methods used for the synthesis of nanoparticles are too expensive and also involve the use of toxic, hazardous chemicals that are responsible for various biological risks. This enhances the growing need to develop environmental friendly processes through green synthesis and other biological approaches. Sometimes the synthesis the using various plants and their extracts can be advantageous over other biological synthesis processes which involve the very complex procedure of maintaining microbial cultures. Many such experiments have already been started such as the synthesis of various metal nanoparticles using fungi like *Fusarium oxysporum* (Nelson *et al*., 2005), *penicillium sp.* (Hemanth *et al*., 2010) and using bacteria such as *Bacillus subtilis* (Natarajan *et al*., 2010). But, synthesis of nanoparticles using plant extracts is the most adopted method of green, eco-

friendly production of nanoparticles and also has a special advantage that the plants are widely distributed, easily available, much safer to handle and act as a source of several metabolites. Their has also been several experiments performed on the synthesis of copper, cobalt and manganese oxide nanoparticles using medicinal plants such as *Oryza sativa, Helianthus annus, Saccharum officinarum, Sorghum Kobus, Medicago sativa* (Alfalfa), *Cinamomum camphora* and *Geranium sp.* In the recent days, copper, cobalt and manganese oxide nanoparticles have been synthesized from the naturally occurring sources and their products like green tea (*Camellia sinensis*), neem (*Azadirachta indica*), leguminous shrub (*Sesbania drummondii*), various leaf broth, nature rubber, starch, *Aloe vera* plant extract, lemongrass leaves extracts, etc. (Vijayaragavan *et al*., 2012). In the present study, use report synthesis approach of copper, cobalt and manganese oxide nanoparticles using plant extracts of *Acalypha indica, Clitoria ternatea, Leucas aspera* and *Pedalium murex.*

SCOPE AND OBJECTIVE

In materials science, “green” synthesis has gained extensive attention as a reliable, sustainable, and eco-friendly protocol for synthesizing a wide range of materials/nanomaterials including metal/metal oxides nanomaterials, hybrid materials, and bioinspired materials. As such, green synthesis is regarded as an important tool to reduce the destructive effects associated with the traditional methods of synthesis for nanoparticles commonly utilized in laboratory and industry. In this review, we summarized the fundamental processes and mechanisms of “green” synthesis approaches, especially for metal and metal oxide [e.g., copper oxide (CuO), Cobalt oxide (CoO) and Manganese oxide (MnO)] nanoparticles using natural extracts. Importantly, we explored the role of biological components, essential phytochemicals (e.g., flavonoids, alkaloids, terpenoids, amides, and aldehydes) as reducing agents and solvent systems.

Objectives

- Biosynthesis of Copper oxide, Cobalt oxide and Manganese oxide nanoparticles using the different extract of *Acalypha indica, Clitoria ternatea, Lucas aspera* and *Pedalium murex*
- Characterization of nanoparticles Copper oxide, Cobalt oxide and Manganese oxide nanoparticles using the different extract of *Acalypha indica, Clitoria ternatea, Lucas aspera* and *Pedalium murex* by UV Spectroscopy

Literature Review

The world ‘‘nano’’ is used to indicate one billionth of a meter or 10^{-9}. nanoparticles are clusters of atoms in the siege range of 1-100km ‘‘Nano’’ is a Greek word synonymous to dwarf meaning extremely small nanotechnology is a field that is mushrooming making an impact in all spheres of human life nanotechnology represent and economic alternative for chemical and physical method of nanoparticles formation. Nanoparticles (NP) attract greater attention due to their various application in different fields including ‘‘nanomedicine’’ the term nanotechnology was coined by professor Norio Taniguchi of Tokyo science university in the year 1974. Nanoparticles can be broadly grouped into two namely organic nanoparticles which include carbon nanoparticles (fullerness) while, some of the inorganic nanoparticles include magnefic nanoparticles node metal nanoparticles (like gold and silver) and semiconductor nanoparticles (like titanium oxide and zinc oxide) there is growing interest in inorganic nanoparticles i.e of noble metal nanoparticles as they provide superior material properties with function versatility metallic nanoparticles are most promising and remarkable biomedical agents silver, aluminum gold, zinc, carbon, titanium, palladium, iron, fullerenes and copper have been used for the synthesis of nanoparticles.

Nanoparticles:

In nanotechnology, nanoparticles synthesized either biologically or chemically must be characterized in order to understand their intrinsic properties such as monodispersity, aqueous stability, the net charge, absorption to biomolecules, aggregation and flocculation in various media, Copper oxide, Cobalt oxide, Manganese acetate nanoparticles have a great bactericidal effect on a serval range of microorganisms, its bactericidal effect depends on the size and shape of the particle. Recently there are, reports that algae are being used as a

bio factory for synthesis of metallic nanoparticles.

Copper sulphate nanoparticles have wide range of applications in nano-scale devices and technologies due to its chemical and inertness and resistance to surface oxidation. Copper sulphate play the vital role in nanobiotechnology as biomedicine because of surface bioconjugation with biomolecular probes and remarkable plasmon-resonant optical properties.

Characterization of nanoparticles

In nanotechnology, nanoparticles synthesized either biologically or chemically must be characterized in order to understand their intrinsic properties such as size, monodispersity, aqueous stability, the net charge, adsorption to biomolecules, aggregation and flocculation in various media. This provides vital information in terms of application of these nanoparticles. For instance, it provides answers to known whether a particular nanoparticle can be used in a biological application, or else to improve their synthetic processes, and/or chemical functionalization. A variety of characterization techniques are currently available some which precede the advent of nanoscience and technology and mostly drawn from material science.

The development of new and integrated methods suited to probe nanomaterials is however, a continuous process (Pool and Owens, 2003). The common techniques used in the characterization of nanoparticles are Dps = DNA binding proteins from starved calls. sHsp = small heat proteins, ultraviolet-visible (UV) spectroscopy, Fourier transform infrared spectroscopy (FTIR), inductively coupled atomic/optical emission spectroscopy (ICP/AES/OES), fluorescence spectroscopy (FS), X-ray photoelectron spectroscopy (XPS), scanning/transmission electron microscopy (SEM/TEM), dynamic light scattering (DLS), atomic force microscopy (AFM) and dispersion and analysis of X-rays (EDAX).

Production if nanoparticles from extract

Melda Altikatoglu *et al*., (2016) that the synthesis of copper oxide nanoparticles (CuO NPs) using *Lucas aspera* plant extract at room temperature. This method is completely a green method, free from toxic and harmful solvent. The CuO NPs were synthesized by mixing copper sulphate dehydrate (CuSO4.5H2O) and aqueous *Lucas aspera* leaf extract. The biosynthesized copper oxide nano-particles were characterized by UV-vis spectroscopy, Fourier-trans form infrared spectroscopy (FT-IR). The existence of the CuO NPs was revealed by UV-vis spectroscopy. The FTIR spectra of control (leaf extract) and synthesized CuO NPs identified the functional groups of the active components.

Mithun Kumar Ghosh *et al.,* (2020) synthesised copper nanoparticles (CuNPs) using a leaf extract from *Clitoria ternatea* (CT) has been documented in their research work. After six months, the *Clitoria ternatea* -CuNPs were found to be stable without any evidence of agglomeration. The *Clitoria ternates* -CuNPs were characterized by FT-IR and UV-vis spectrophotometry. The average particle and crystal sizes of the *Clitoria ternatea* -CuNPs were found to be 10 ± 1 and 12 ± 1 nm, respectively. The SPR peaks were found at 266 and 337 nm, measured using electronic spectroscopy.

Pooja Rawat *et al.,* (2020) prepared copper and copper sulfide nanoparticles using leaf extract of *Acalypha indica*. The effect of heating on the nanoparticles has also been studied. Nanoparticles have been characterized by using UV-visible absorption. Improvement in crystallinity of the nanoparticles was observed on heating in the temperature range 180-500 °C. The crystallite size of the copper nanoparticles heated at around 500 °C was found to be about 28 nm whereas that of copper sulfide nanoparticles heated at around 250 °C was found to be approximately 6 nm.

Kashif Ahmed *et al*., (2016) reported in their work total reducing strength or phenolic compounds in leaf extract of *Lucas aspera, Acalipha indica, clitoria ternata* and *Pedalium murex* were determined and then Cobalt nanoparticles (Co NPs) were synthesized by using aqueous, ethanal, and chloroform extract of leaves as reducing agent because of its higher values of total phenolic compounds in comparison. Characterization of the green synthesized Co NPs was performed by UV-visible absorption techniques. The size of Co NPs was estimated in the range of 20-60 nm. The usage of plant extract for the preparation of Co NPs makes the process cost effective, non-toxic and green method.

Hong *et al*. (2002) studied the highly dispersed copper oxide (CuO) nanoparticles with an average size of 6 nm have been successfully prepared by a novel quick-precipitation method. The as-prepared CuO nanoparticles were characterized by UV–Visible absorption spectroscopy. Results of this study scoured as-prepared CuO nanoparticles have high dispersion and narrow size distribution. The influence of reaction conditions on morphology of CuO nanocrystals was discussed. Spherical, ellipsoidal and needle-shaped CuO nanocrystals can be obtained simply by varying the reaction temperature and controlling the addition of NaOH.

Wang *et al*. (2002) synthesised CuO nanoparticles with an average size of 4 nm have been successfully prepared by microwave irradiation, using copper (II) acetate and sodium hydroxide as the starting materials and ethanol as the solvent. The CuO nanoparticles are characterized by using techniques such as UV–Visible absorption spectroscopy. The as-prepared CuO nanoparticles have regular shape, narrow size distribution and high purity. The band gap is estimated to be 2.43 eV according to the results of the optical measurements of the CuO nanoparticles.

Shun *et al*. (2005) synthesized new type of Cuo nanoparticles in liquid ammonia in the presence of sodium metal. First, Cu nanoparticles were obtained by reducing copper nitrate with the alkali metal in liquid ammonia, then, CuO nanoparticles were formed in the ambient conditions. The morphology and structure of as-prepared CuO nanoparticles were characterized by UV- Visible absorption spectroscopy, and the reason of aggregation of CuO nanoparticles was supposed.

Sankar *et al.* (2018) synthesized copper oxide nanoparticles (CuO Nps) using plant extract as fuel by solution combustion. The UV–visible absorption spectrum of CuO Nps indicates the blue shift with increase of concentration of plant extract.

Udayabhanu *et al*. (2015) studied a facile method for the green synthesis of copper oxide nanoparticles (CuO Nps) by a solution combustion method using water extract. The Nps were characterized by UV–visible studies. Photocatalytic activity studies of CuO Nps reveal that they act as very good catalyst for the effective degradation of methylene blue (MB) in the presence of UV and Sun light. Also, the degradation of MB was found to be pH dependent. The Nps found to inhibit the activity of 1,1-Diphenyl-2-picrylhydrazyl (DPPH) free radicals effectively. CuO Nps exhibit significant bactericidal activity against *Klebsiella aerogenes*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Staphylococcus aureus*. The study reveals a simple, ecofriendly and robust method for the synthesis of multifunctional CuO nanoparticle employing underutilized medicinal plants.

Muhammad Hafeez *et al.* (2020) synthesized Cobalt oxide nanoparticles (Co_3O_4-Nps) using leaf extract of Populus liliata, they have many applications and now a days the green methods of synthesis of these NPs are preferred over other methods because of associated benefits. The synthesized NPs were analysed by different techniques such as Fourier

transform spectroscopy (FTIR), x-ray diffraction (XRD), transmission electron microscopy(TEM) and scanning electron microscopy (SEM).

Ismat Bibi *et al*. (2017) fabricated cobalt oxide nanoparticles using *Punica granatum* peel extract from cobalt nitrate hexahydrate at low temperature. The synthesized cobalt-oxide NPs were characterized using X-ray powder diffraction, scanning electron microscopy, energy-dispersive X-ray, atomic force microscopy, fourier transform infrared spectroscopy and UV-visible techniques. The cobalt-oxide NPs were in highly uniform shape and size was in the size of 40–80 nm. Photo-catalytic activity (PCA) of the synthesized NPs was evaluated by degrading Remazol Brilliant Orange 3R (RBO 3R) dye and a degradation of 78.45% was achieved (dye conc. 150 mg/L) using 0.5 g cobalt-oxide NPs for 50 min irradiation time. In view of eco-benign and cost-effective nature, the present investigation revealed that *P. granatum* could be used for the synthesis of cobalt-oxide NPs for photo-catalytic applications.

In the recent years, plant and microbial extract-based nanoparticles (NPs) have become a sophisticated technology serving as an alternative strategy for the purpose of developing materials functionalized by structural diversity and enhanced energy efficiencies. Cobalt oxide nanoparticles (GCoO-NPs) have wide applications in several sectors due to their high resistance to corrosion as well as oxidation, eco-friendly nature, cost effectiveness and nontoxic potential. Plant based particles are credible alternatives as they reduce the burden of complicated and laborious protocols of physiochemical reliance. In this study, GCoO-NPs were synthesized using the grape Jumbo Muscadine (*Vitis rotundifolia*) using co-precipitation. The synthesized GCoO-NPs were characterized by UV–Vis spectrophotometer, Fourier transform infrared spectroscopy (FTIR), Powder X-ray diffraction (PXRD) and

Scanning electron microscopy (SEM) (Melvin S.Samuel *et al*., 2020)

Matinise *et al.*, (2018) research work involved the development of a better, inexpensive, reliable, easily and accurate way for the fabrication of Cobalt (II, III) oxide (Co_3O_4) nanoparticles through a green synthetic method using *Moringa Oleifera* extract. The electrochemical activity, crystalline structure, morphology, isothermal behaviour and optical properties of Co_3O_4 nanoparticles were studied using various characterization techniques. The X-ray diffraction (XRD) and Energy Dispersive X-ray Spectroscopy (EDS) analysis confirmed the formation of Co_3O_4 nanoparticles. The pseudo-capacitor behaviour of spinel Co_3O_4 nanoparticles on Nickel foam electrode was investigated by cyclic voltammetry (CV), galvanostatic charge–discharge (GCD) and electrochemical impedance spectroscopy (EIS) in 3M KOH solution. The CV curve revealed a pairs of redox peaks, indicating the pseudo-capacitive characteristics of the Ni/Co_3O_4 electrode. EIS results showed a small semicircle and Warburg impedance, indicating that the electrochemical process on the surface electrode is kinetically and diffusion controlled. The charge-discharge results indicating that the specific capacitance Ni/Co_3O_4 electrode is approximately 1060 F/g at a discharge current density of at 2 A/g.

Shakeel Ahmad Khan *et al*. (2020) synthesized MnO nanoparticles (AI-MnO NAPs) using biological molecules of *Abutilon indicum* leaf extract. Further, they were evaluated for antibacterial and cytotoxicity activity against different pathogenic microbes (*Escherichia coli*, *Bordetella bronchiseptica*, *Staphylococcus aureus*, and *Bacillus subtilis*) and HeLa cancerous cells. Synthesized NAPs were also investigated for photocatalytic dye degradation potential against methylene blue (MB), and adsorption activity against Cr(VI) was also determined. Results from Scanning electron microscope (SEM), X-ray powder diffraction

(XRD), Energy-dispersive X-ray (EDX), and Fourier-transform infrared spectroscopy (FTIR) confirmed the successful synthesis of NAPs with spherical morphology and crystalline nature. Biological activity results demonstrated that synthesized AI-MnO NAPs exhibited significant antibacterial and cytotoxicity propensities against pathogenic microbes and cancerous cells, respectively, compared with plant extract. Moreover, synthesized AI-MnO NAPs demonstrated the comparable biological activities results to standard drugs. These excellent biological activities results are attributed to the existence of the plant's biological molecules on their surfaces and small particle size (synergetic effect). Synthesized NAPs displayed better MB-photocatalyzing properties under sunlight than an ultraviolet lamp. The Cr(VI) adsorption result showed that synthesized NAPs efficiently adsorbed more Cr(VI) at higher acidic pH than at basic pH. Hence, the current findings suggest that *Abutilon indicum* is a valuable source for tailoring the potential of NAPs toward various enhanced biological, photocatalytic, and adsorption activities. Consequently, the plant's biological molecule-mediated synthesized AI-MnO NAPs could be excellent contenders for future therapeutic applications.

Maryam Usman Ahmed *et al*. (2020) synthesized manganese oxide nanoparticles from *Cassia tora* aqueous leave extract in order to investigate the toxicological effect of the biologically synthesized manganese oxide nanoparticles (MnO_2 NPs).

Changzhong Chen *et al*. (2013) synthesized manganese oxides nanomaterials, including MnO and MnO_2, have attracted great interest as anode materials in lithium-ion batteries (LIBs) for their high theoretical capacity, environmental benignity, low cost, and special properties. Up to now, manganese oxides nanostructures with excellent properties and various morphologies have been successfully synthesized. Herein, we provide an in-depth

discussion of recent development of the synthesis of manganese oxides nanomaterials and their application in the field of LIBs.

Sneha Bhatnagar *et al.* (2014) synthesized nanoparticles from living plant extracts can be used for conversion of metal ions into nano form. These nanoparticles are capable in controlling plant growth. If nanoparticles can enhance the growth of plants, it will be a great boon to the agricultural domain of developing countries that are particularly facing great challenges in order to improve the agricultural sector. Manganese is one of nine essential micronutrients for plant growth. Synthesis of Ag nanoparticles has been reported by aqueous extract of *Lawsonia innermis* but not much work has been done on the synthesis of manganese nanoparticles. For nanoparticle synthesis, 10% aqueous extract of plant is mixed with 10-3M MnSO4 solution and incubation of this reaction mixture was done at room temperature for 48 hours. Formation of nanoparticles was confirmed visually by the reaction mixture colour which turned to brown and the characterization of nanoparticles was done by UV-Vis Spectroscopy and particle size analysis. Results suggested that biosynthesized nanoparticle treatment significantly enhance all the growth parameters i.e., fresh weight, dry weight, root length and shoot length of Cicer arietinum as compared to other treatment.

Jayandran *et al.* (2015) nanoparticles reported a simple, convenient and low cost method for the synthesis of manganese nanoparticles by reducing manganese acetate with the help of easily available natural products viz., lemon extract as reducing agent and turmeric curcumin as a stabilizing agent. The curcumin was isolated from turmeric by using solvent extraction method and used for manganese nanoparticle stabilization. The characterization of curcumin and manganese nanoparticles was done by using UV- Vis and FT-IR spectroscopic techniques. The morphology of manganese nanoparticles was confirmed by SEM and TEM

techniques.

Vahid Hoseinpour *et al*. (2018) explained simple, efficient and eco-friendly procedure for the green synthesis of manganese dioxide nanoparticles (MnO2 NPs) by *Yucca gloriosa* leaf extract is described. The MnO2 NPs were synthesized using *Y. gloriosa* leaf extract and *curcumin* as reducing and stabilizing agents, respectively. Fourier-transform infrared spectra revealed the involvement of the plant extract in the formation of MnO2 NPs. The ultraviolet–visible absorption spectra of the synthesized MnO2 NPs exhibited absorption peaks at 410 nm, which were attributed to the band gap of the MnO2 NPs. Crystal phase identification of the MnO2 NPs was characterized by X-ray diffraction analysis and the formation of crystalline MnO2 NPs has been confirmed. Also, the X-ray diffraction pattern displayed that the average size of MnO2 NPs was about 80 nm. Furthermore, field emission scanning electron microscopy analysis showed that the synthesized MnO2 NPs have a spherical shape. MnO2 NPs have photocatalytic activities for the dye degradation in the visible light region. The photocatalytic activities for the dye degradation of MnO2 NPs were evaluated using Acid Orange as an organic contaminant.

Mahsa Souri *et al.* (2018) reported a simple, efficient, and eco-friendly procedure for the green synthesis of manganese dioxide nanoparticles (MnO2 NPs) by *Yucca gloriosa* leaf extract is described. The effect of three different factors such as pH of the metallic solution, time, and extract ratio was studied. Optimizing the factors was done by Response Surface Methodology (RSM). Considering the results, the ratio of the extract to the metallic solution and the time was the most important factors for the synthesis of MnO2 NPs. The optimal condition was claimed to be time = 120 min, pH 6, and extract ratio = 90%. Then, the MnO2 NPs re-synthesized using Y. gloriosa leaf extract and stabilized using turmeric extract.

Crystal phase identification of the MnO2 NPs was characterized by XRD analysis and the formation of crystalline MnO2 has been confirmed. In addition, XRD study confirms the attendance of MnO2 NPs with around size of 32 nm. Furthermore, FESEM and TEM analyses showed that the synthesized MnO2 NPs have the spherical shape.

Vineet Kumar *et al.* (2017) synthesized Manganese oxide (MnO) NPs are widely used in contaminant sensing, drug delivery, data storage, catalysis and biomedical imaging. Green synthesis of NPs is important due to increased concern of environmental pollution. Green chemistry based synthesis of NPs is preferred due to its ecofriendly nature. In this study, MnO NPs 0f different sizes were synthesized in aqueous medium using clove, i.e., *Syzygium aromaticum* extract (CE) as reducing and stabilizing agents. These NPs were used for the electrochemical sensing of p-nitrophenol (PNP). The synthesis of MnO NPs was over in 30 min. MnO NPs of different sizes were obtained by varying metal ion concentration, metal ion volume ratio, CE concentration, CE volume ratio, and incubation temperature. Selectively, ~4 nm MnO NPs were used for electrochemical sensing of paranitrophenol. The MnO NPs modified gold electrodes detected PNP with good sensitivity, 0.16 μA μM^{-1} cm^2. The limit of PNP detection was 15.65 μM. The MnO NPs prepared using CE based green chemistry approach is useful for PNP sensing. These NPs can also be useful for various in vivo applications in which the NPs come in human contact.

Mahmoud Nasrollahzadeh *et al.* (2016) reported Copper oxide (CuO) nanoparticles (NPs) were synthesized by biological method using aqueous extract of *Thymus vulgaris* L. leaves as a reducing and capping agent. The progress of the reaction was monitored using UV–visible spectroscopy. The advantages of this procedure are simple operation, use of cheap, natural, nontoxic and benign precursors, absence of toxic reagents and mild and

environmentally friendly conditions. The green synthesized CuO NPs was characterized by transmission electron microscopy (TEM), energy-dispersive X-ray spectroscopy (EDS), fourier-transform infrared (FT-IR) spectroscopy, X-ray diffraction analysis (XRD), thermogravimetric analysis (TGA) and differential thermal analysis (DTA). More importantly, the green synthesized CuO NPs was found to be an excellent heterogeneous catalyst for ligand-free *N*-arylation of indoles and amines. The *N*-arylated products were obtained in good to excellent yield and the catalyst can be recovered and reused for further catalytic reactions with almost no loss in activity.

Jitendra Kumar Sharma *et al.* (2015) synthesized copper oxide (CuO) nanoparticles (NPs) were employed as electrocatalytic materials for the fabrication of counter electrode in dye sensitized solar cells (DSSCs). Uniform CuO NPs were synthesized by the leaves extract of *Calotropis gigantea* plant in aqueous medium through green synthesis. The synthesized CuO NPs were extensively characterized in terms of morphology, crystalline nature, structural, electrochemical and photovoltaic properties using various experimental tools. The synthesized CuO NPs possessed a well crystalline nature which was perfectly matched to monoclinic structure of bulk CuO. For DSSC application, a thin film of synthesized CuO NPs was prepared by the paste of CuO NPs and coated onto FTO glass using glass rod. The cyclovoltametry measurement revealed that CuO NPs based thin film showed reasonably good surface for the reduction of triiodide ions in redox electrolyte, suggesting its good electrocatalytic activity toward the iodide ions. Moderately high solar to electrical energy conversion efficiency of ~3.4% along with high short circuit current density (J_{SC}) of ~8.13 mA/cm^2, open circuit voltage (V_{OC}) of ~0.676 V and fill factor (FF) of 0.62 was recorded in the DSSC fabricated with synthesized CuO NPs based counter electrode.

Fruit peels were discarded as inevitable wastes in the production of fruit juice which wasted our resources and caused pollution problems. Iron nanoparticles were synthesized using *Citrus maxima* peel extracts to reduce Fe(III) in aqueous solution. The nanoparticles were characterized by TEM, EDS, XPS, FTIR, DLS and Zeta potential methods. Based on the characterization results, irregular iron nanoparticles with diameters of 10–100 nm were synthesized successfully. Moreover, the nanoparticles were mainly composed of Fe^0 nanoparticles which were coated with various biomolecules from the extracts as capping or stabilizing agents (Yufen Wei *et al.,* 2016)

MATERIALS AND METHOD

Classification	
Class	Dicotyledons
Order	Euphorbiales
Family	Euphorbiaceae

Botanical Name : *Acalypha indica* **Plate 1**

Description

An erect annual herb that can be easily distinguished by the cup-shaped involucre that surrounds the small flowers in the catkin-like inflorescence. It can grow up to 1.2 m (3.9 ft) tall in favorable circumstances, but is usually smaller. The leaves are broad ovate, 1.2 cm–6.5 cm × 1 cm–4 cm (0.47 in–2.56 in × 0.39 in–1.57 in). The leaf base is rounded to shortly attenuate. The leaf margin is basally 5-nerved and is crenate-serrate with an acute or obtuse apex. The petiole is 1.5–5.5 cm (0.59–2.17 in) long. The flower spikes are axillary, 2.5–6 cm (0.98–2.36 in) long, monoecious, with a rachis terminating in a triradiate hood. The tiny male flowers are white-green, located on the upper part of the flower spikes, and are ebracteate, minute, and clustered with vermiculiform anthers. The pollens are roughly round and approximately 10–12 microns in diameter.

The green female flowers are located lower on the spikes, and are subtended by 3–7 mm (0.12–0.28 in) long suborbicular-cuneiform, many-nerved, toothed bracts that are foliaceous. The ovary is hispid, 3-lobed. Styles are 3, each 2-fid. Capsules are hispid, 3-

valved and concealed by a bract. The stem is striate (longitudinally ribbed) and pubescent. The fruit is 1.5–2 mm (0.059–0.079 in), 3-lobed, tuberculate and pubescent.

Classification	
Class	Dicotyledons
Order	Fabales
Family	Fabaceae

Botanical Name: *Clitoria ternatea* **Plate 2**

Description

It is a perennial herbaceous plant, with elliptic, obtuse leaves. It grows as a vine or creeper, doing well in moist, neutral soil. The most striking feature about this plant is the color of its flowers, a vivid deep blue; solitary, with light yellow markings. They are about 4 cm (1.6 in) long by 3 cm (1.2 in) wide. Some varieties yield white flowers. The fruits are 5–7 cm (2.0–2.8 in) long, flat pods with six to ten seeds in each pod. They are edible when tender. It is grown as an ornamental plant and as a revegetation species (e.g., in coal mines in Australia), requiring little care when cultivated. As a legume, its roots form a symbiotic association with soil bacteria known as rhizobia, which transform atmospheric N_2 into a plant-usable form (a process called nitrogen fixing), therefore, this plant is also used to improve soil quality through the decomposition of nitrogen rich plant material.

Classification	
Class	Dicotyledons
Order	Lamiales
Family	Lamiaceae

Botanical name: *Leucas aspera* **Plate 3**

Description

Common *Leucas* in an erect and diffusely branched annual herb. Flower are borne in distant spherical whorls, in uppermost leaf axils, 1-4, about 2.5 cm in diameter, about 16-20 flowered. Flowers are 8-10 mm white; upper lip short, densely bearded; lower lip clearly longer then upper and projecting forward. Sepal-cup is 7-9 mm, scarcely elongating in fruit, pale green, scarcely curved, with a prominently oblique mouth. Sepal teeth are 8-10, irregular in size, triangular, 2-3 mm long short spinulose tips. Bracts narrow linear, about ½ length of calyces, long-fringed with hairs at margins. Stems are15-30 cm with spreading short hairs. Leaves linear lanceshaped, weakly rounded toothed to subentire at margin, wedge-shaped above and below; up to 5 ×1.5 cm with adpressed hairs on upper surface and a denser indumentum of short soreading hairs below especially on nerves; leaf-stalk subabsent to about 5 mm on lower leaves. Common *Leucas* is found in India, Himalayas, and broadly in subtropical Asia.

Classification	
Class	Dicotyledons
Order	Lamiales
Family	Pedaliaceae

Botanical name: *Pedalium murex* **Plate 4**

Description

A succulent herb, grows upto 38 cm in height, very well branched, foetid smelling and also has rough slime secreting glands. Leaves: simple, opposite, somewhat fleshy, broadly ovate-oblong, coarsely crenate-serrate or sublobate, glabrous above and minute scales on the lower side. Flowers: bright yellow, axillary and solitary. Fruits: blunty 4-angled with stout, sharp, conical horizontal spines from the angles.

Collection of plant and powder preparation

The *Acalipha indica, Clitoria ternatea and Lucas aspera, Pedalium murex* were collect from Alankaratathu, Thoothukudi, Tamil Nadu. Then the plants were washed and dryed. Plants (100 mg) were dissolved in 100 ml of distilled water, ethanol, Chloroform and the extract formed were filtered through a muslin cloth. Then the filtrate were treated with equal volumes of water, chilled ethanol, chloroform and the resultant precipitate will be used for further experiments.

Biosynthesis of copper oxide nanoparticles

In a typical reaction mixture, 100 ml of aqueous 1mM copper sulphate dehydrate CuSO4.5H2O was treated with 10 ml aqueous leaf extract and stirred magnetically at room temperature for about 4 hrs. The formation of the particles can be seen within 2 hrs. The solution was aged for 15 hrs. Nanoparticles prepared were centrifuged and washed with double distilled water twice.

Biosynthesis of cobalt oxide nanoparticles

The cobalt nanoparticles were prepared in a 250 mL conical flask in which 50 mL centi molar solution of cobalt nitrate was mixed with 10 mL of the plant extract along with vigorous shaking on a hot plate till the appearance of dark brown color.

Biosynthesis of Manganese oxide nanoparticles

The method according to Paul *et al*. 5 was adopted in the synthesis of manganese oxide nanoparticles. 5 mL of plant extracts was added to 50 mL of aqueous solution of 0.2 M potassium permanganate (KMnO4) while, heating and stirring at 70EC and pH 7 for 60 min.

The KMnO4 solution changed from colorless to brown with formation of precipitate. The precipitate was centrifuged at 3000 rpm for 15 min and washed with distilled water 3 times.

Characterization of CuO NPs, Co NPs, MnO_2 NPs nanoparticles

UV-visible spectroscopy analysis

The bio reduction of copper oxide ion in solution was monitored using UV – spectrometer in 350 – 550 nm wave length range. It was observed that upon addition of the plant extracts into the flask containing the aqueous, ethanol, chloroform extracts of plants and copper oxide solution, the color of the medium change to brown with in 5 minutes. This indicated the formation of copper oxide nanoparticles.

The bio reduction of cobalt oxide ion in solution was monitored using UV – spectrometer in 350 – 550 nm wave length range. It was observed that upon addition of using of the plant extracts into the flask containing the aqueous cobalt oxide solution, the color of the medium change to brown with change to brown with in 5 minutes. This indicated the formation of cobalt oxide nanoparticles.

The bio reduction of manganese oxide ion in solution was monitored using UV – spectrometer in 350 – 550 nm wave length range. It was observed that upon addition of using of the plant extracts into the flask containing the aqueous manganese oxide solution, the color of the medium change to brown with change to brown with in 5 minutes. This indicated the formation of manganese oxide nanoparticles.

Result and Discussion

UV – visible spectroscopy is a practical technique that characterizes any optical activity eventuated from noble metal NPs, for instance Cu, Co and Mn.

These NPs reparent LSPR bands in the visible region, related to excitation of the conduction electrons of metal NPs after their unique interaction with the electromagnetic field of light (Jain *et al*., 2008).

The formation of Cu, Co_2 and Mn nanoparticles during the reduction process in UV dicated by change in the color of reaction solution from colorless to dark brown which can be visually observed (Plate 12) metal nanoparticle have electrons, which yield a surface plasmon resonance (SPR) absorption band, due to the mutual vibration of electrons of metal nanoparticles in resonance with light wave. The appearances of the peaks show the characterization of surface plasmon resonance of metal nanoparticles.

The UV visible absorption spectrum of copper oxide nanoparticles synthesis by treating 1mm aqueous copper sulphate solution with long of *Acalypha indica, Clitoria ternate, leucas aspera* and *Pedalium murex* stem extracts are shown in Fig 5-8. The copper nanoparticles prepared by *Acalypha indica* stem extracts (Distilled water, chloroform, ethanol) have displayed sharp peak at 400nm which is assigned to the absorption of CuONPs. This spectrum confirms the formation of CuO nanoparticles.

UV – visible spectroscopy is a very useful technique for analysing nanoparticle formation and the stability of metal nanoparticle in aqueous solution (Muniyappan. L. Nagaragan N.S., 2014). The UV – visible absorption spectrum of cobalt oxide nanoparticles

synthesized by *Acalypha indica, Clitoria ternate, Leucas aspera* and *pedalium murex* leaf and stem extract are shown in the Fig 9 – 16. The sharp peak is observed in 380nm in *Clitoria ternate* leaf ethanol extract, 430nm in *Acalypha indica* leaf distilled extract, 470nm in *Leucas aspera* leaf ethanol extract, 420nm in *Pedalium murex* leaf ethanol extract these spectrum confirms the formation of cobalt oxide nanoparticles.

The formation of the reduced Manganese nanoparticles colloidal solution were monited by using UV – visible spectrophotometer. The absorption spectra of the supernatants were taken between 350 to 550 nm. UV- visible spectrum of the biosynthesis of manganese nanoparticles using distilled water, ethanol and chloroform *Acalypha indica, Clitoria ternate, leucas aspera* and *Pedalium murex* leaf and stem extracts are shown in Fig. 17. UV – visible spectrum of the biosynthesis of manganese nanoparticles using the different extract of plant showed a peak 410nm, 490nm, 390nm, 450nm, 510nm, 540nm, 430nm, 390nm, 490nm, corresponding to the plasmon absorbance of manganese nanoparticles for the tested *Acalypha indica* leaf ethanal, distilled water, *Clitoria ternate* leaf ethanol, chloroform, *Leucas aspera* leaf chloroform, ethanal *Pedalium murex* leaf ethanol and chloroform extracts respectincly. Thesis is a relation between the particle size and the plasmon peak, as the particles become larger when the plasmon peak, shifts to longer wavelengths values for synthesized nanoparticles size and plasmon maxima that were reported with Solomon *et al*. (2007) indicated that UV spectra were in lower wavelengths (384-414), with 10 to 4nm particle size. Rosemary and Pradeep (2003) revealed that UV spectra at 438nm wavelength gave particles size of 60-80nm, obtained results indicated that lower wavelength of UV. Spectra at 383nm recoded particle size at 1-25nm. The UV-visible absorbance spectrum is highly dependent on nanoparticle geometry. Our result show a broad absorption peak of between 350-550 nm

which is due to the surface plasmon resonance (SPR) of electrons present at the surface of metal nanoparticles. Hence this result signifies the formation Cu, Co and Mn nanoparticles which are supported by the result presented by Eman *et al.* and Suresh *et al.* the maximum absorption peak of metal nanoparticles appeared at 530nm (Fig 13). Jt clearly renealed that formation of nanoparticles. (Suresh *et al.,* 2014 and Suranurar *et al.,* 2016).

Plate 5: Copper oxide nanoparticles synthesized by treating 1m of aqueous copper sulphate solution with 10 mg of *Acalypha indica* leaf extract (Water ethanol, chloroform)

Plate 6: Copper oxide nanoparticles synthesis by treating 1m of aqueous copper sulphate solution with 10 mg of *Lucas aspera* leaf extract (Water ethanol, chloroform)

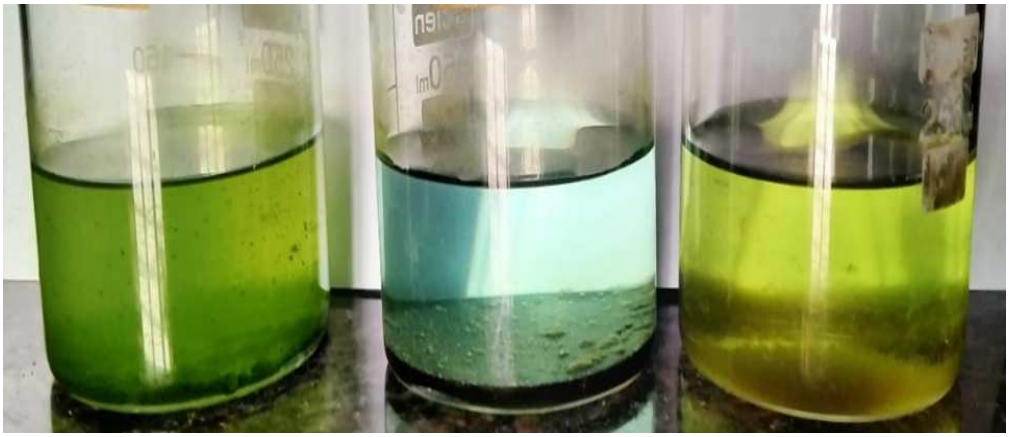
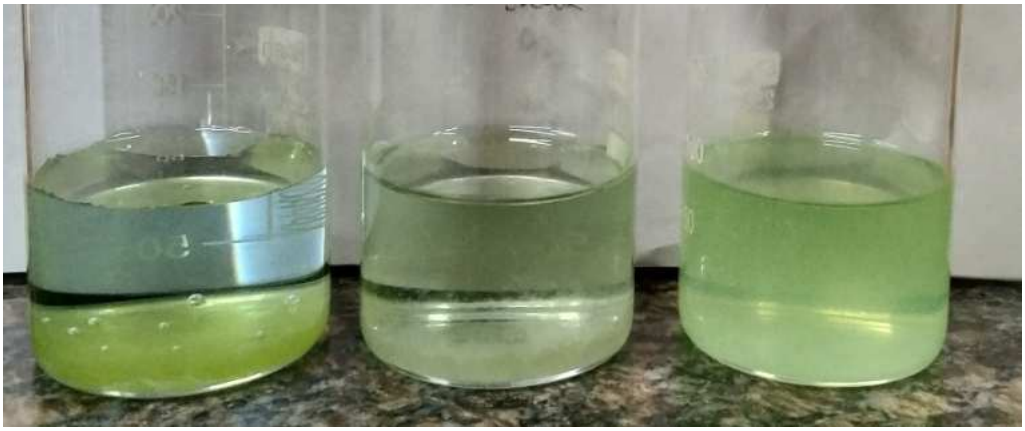


Plate 7: Copper oxide nanoparticles synthesis by treating 1m of aqueous copper sulphate solution with 10 mg of *Clitoria ternatea* leaf extract (Water ethanol, chloroform)

Plate 8: Copper oxide nanoparticles synthesis by treating 1m of aqueous copper sulphate solution with 10 mg of *Pedalium murex* leaf extract (Water ethanol, chloroform)



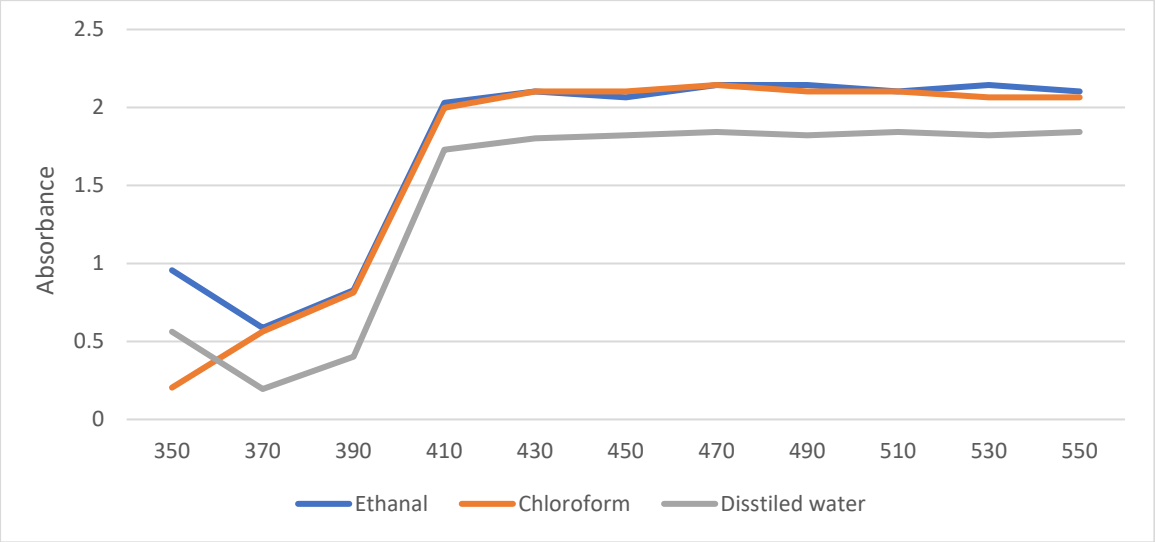


Figure 1: UV-visible spectral analysis of copper oxide nanoparticles synthesis by treating 1 m aqueous copper sulphate solution with 10 mg of *Acalypha indica* leaf extract (Distilled water, Chloroform, ethanol)

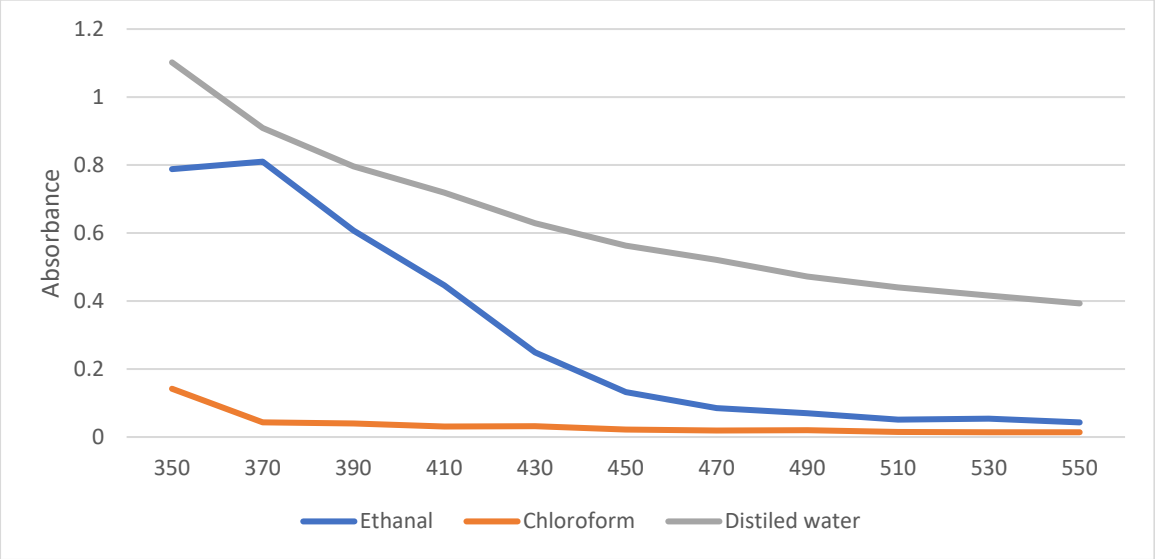


Figure 2: UV-visible spectral analysis of copper oxide nanoparticles synthesis by treating 1m aqueous copper sulphate solution with 10 mg of *Clitoria ternatea* leaf extract (Distilled water, Chloroform, ethanol)

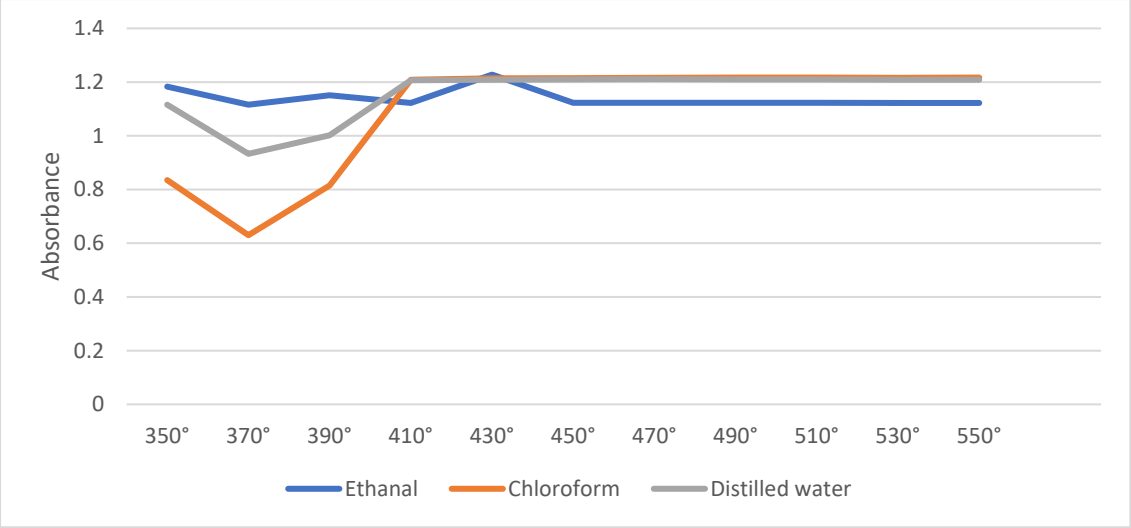


Figure 3: UV-visible spectral analysis of copper oxide nanoparticles synthesis by treating 1 m aqueous copper sulphate solution with 10 mg of *Leucas aspera* leaf extract (Distilled water, Chloroform, ethanol)

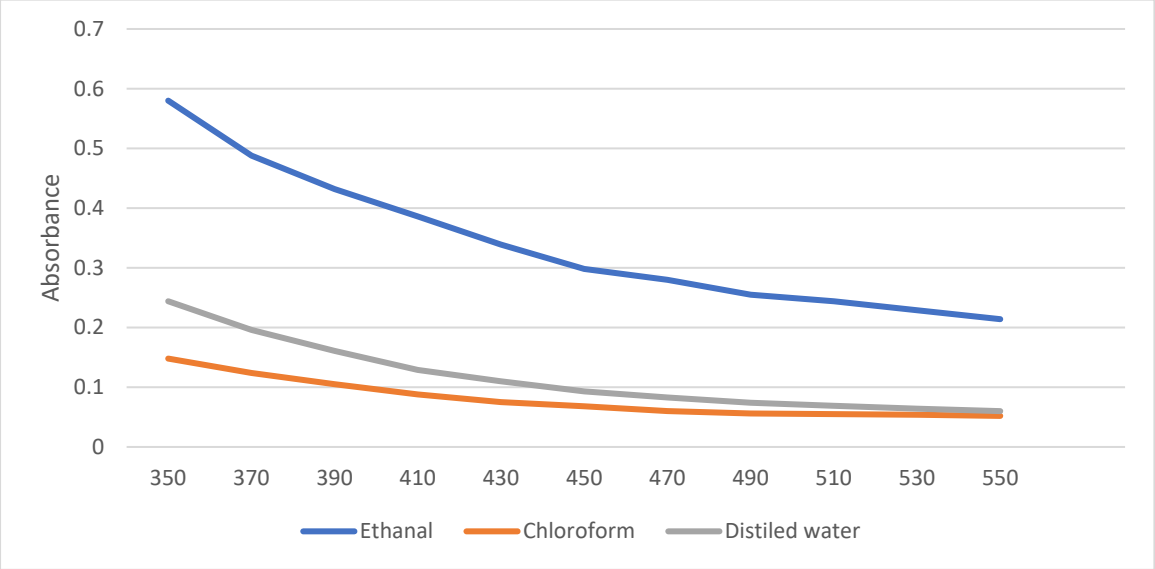


Figure 4: UV-visible spectral analysis of copper oxide nanoparticles synthesis by treating 1 m aqueous copper sulphate solution with 10 mg of *Pedalium murex* leaf extract (Distilled water, Chloroform, ethanol)

Plate 9: Copper oxide nanoparticles synthesis by treating 1m of aqueous copper sulphate solution with 10 mg of *Acalypha indica* stem extract (Water ethanol, chloroform)

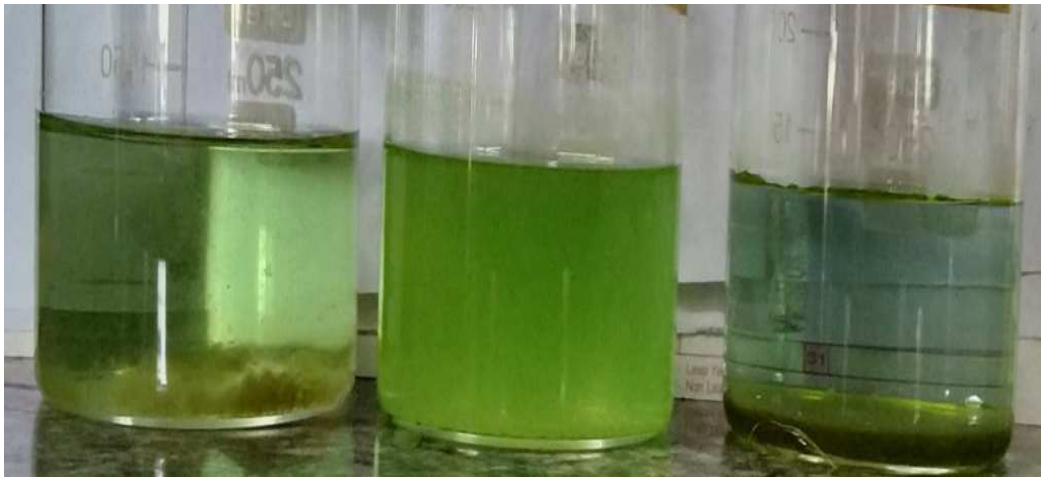


Plate 10: Copper oxide nanoparticles synthesis by treating 1m of aqueous copper sulphate solution with 10 mg of *Clitoria ternatea* stem extract (Water ethanol, chloroform)

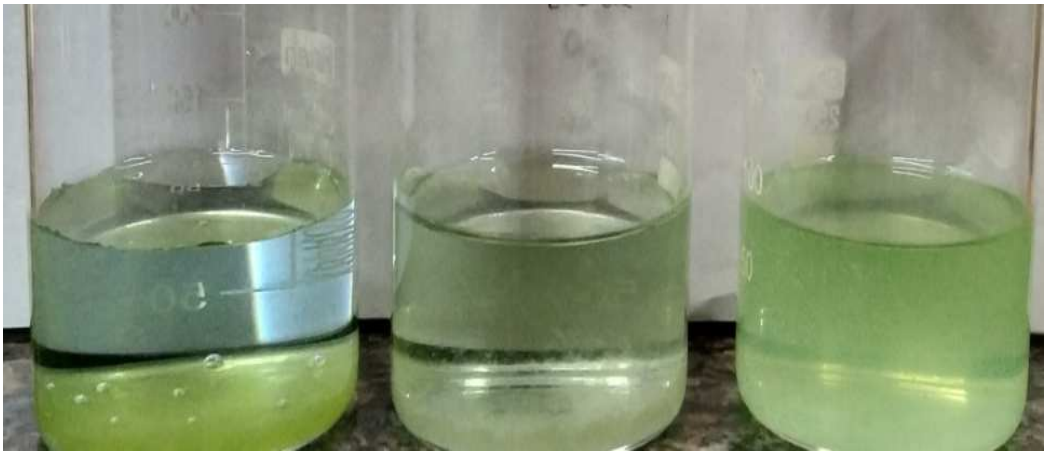


Plate 11: Copper oxide nanoparticles synthesis by treating 1m of aqueous copper sulphate solution with 10 mg of *Lucas aspera* stem extract (Water ethanol, chloroform)

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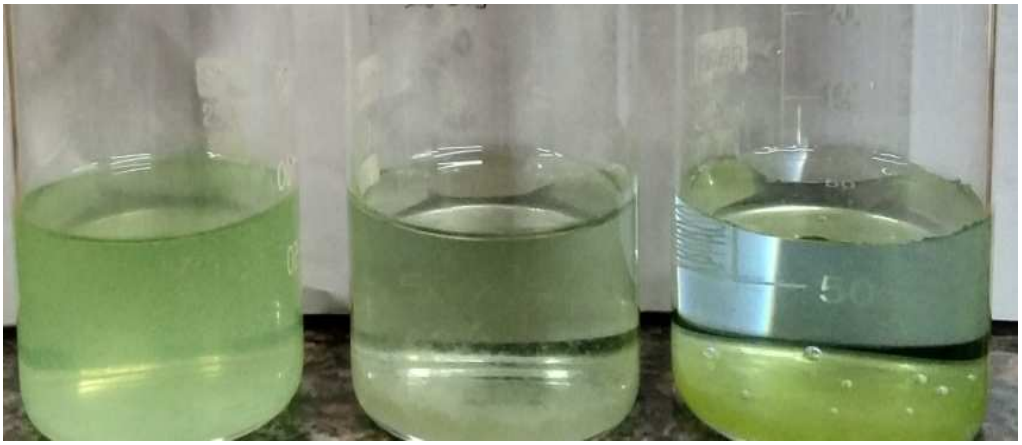
Plate 12: Copper oxide nanoparticles synthesis by treating 1m of aqueous copper sulphate solution with 10 mg of *Pedalium murex* stem extract (Water ethanol, chloroform).



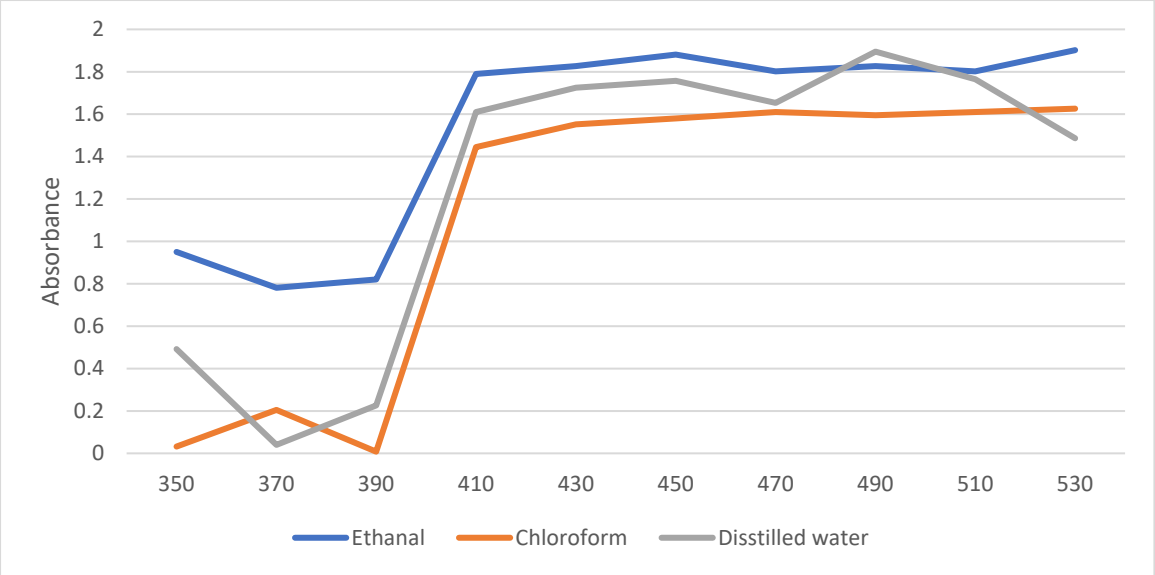


Figure 5: UV-visible spectral analysis of copper oxide nanoparticles synthesis by treating 1m aqueous copper sulphate solution with 10 mg of *Acalypha indica* stem extract (Distilled water, Chloroform, ethanol)

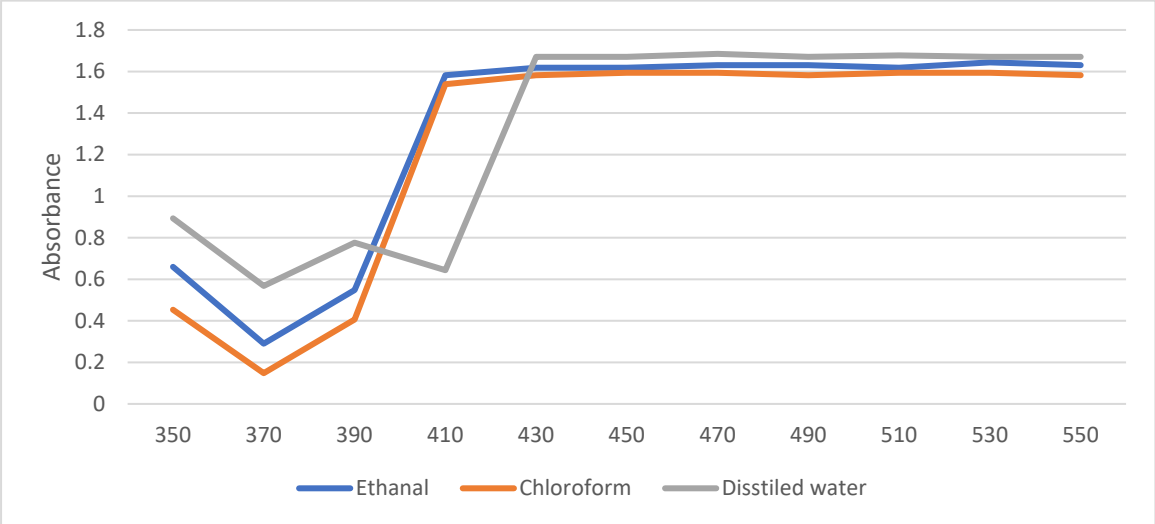


Figure 6: UV-visible spectral analysis of copper oxide nanoparticles synthesis by treating 1m aqueous copper sulphate solution with 10 mg of *Clitoria ternate* stem extract (Distilled water, Chloroform, ethanol)

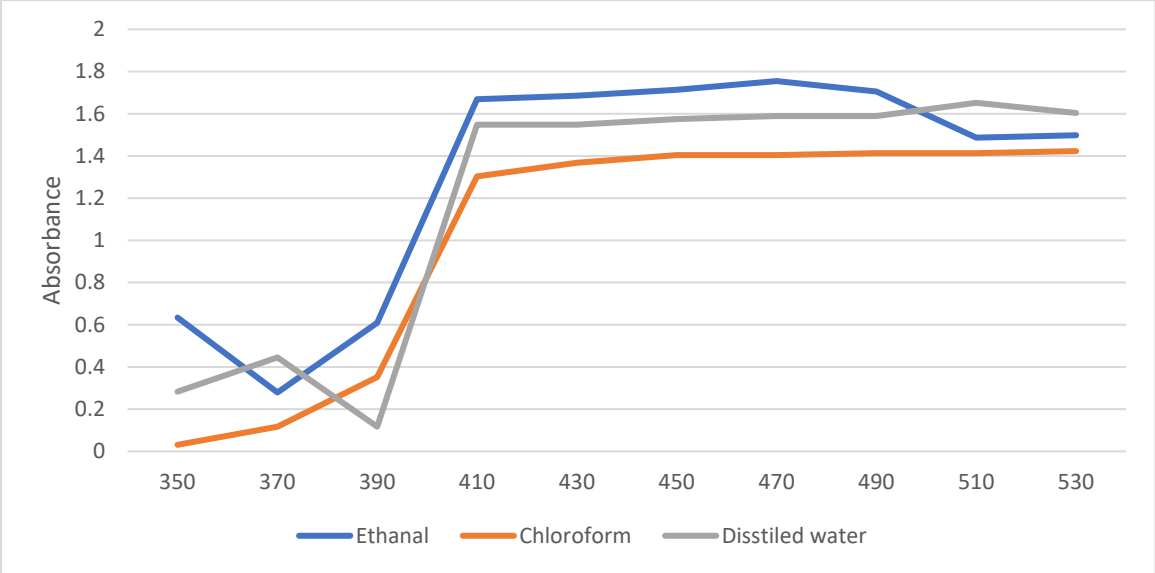


Figure 7: UV-visible spectral analysis of copper oxide nanoparticles synthesis by treating 1m aqueous copper sulphate solution with 10 mg of *Leucas aspera* stem extract (Distilled water, Chloroform, ethanol)

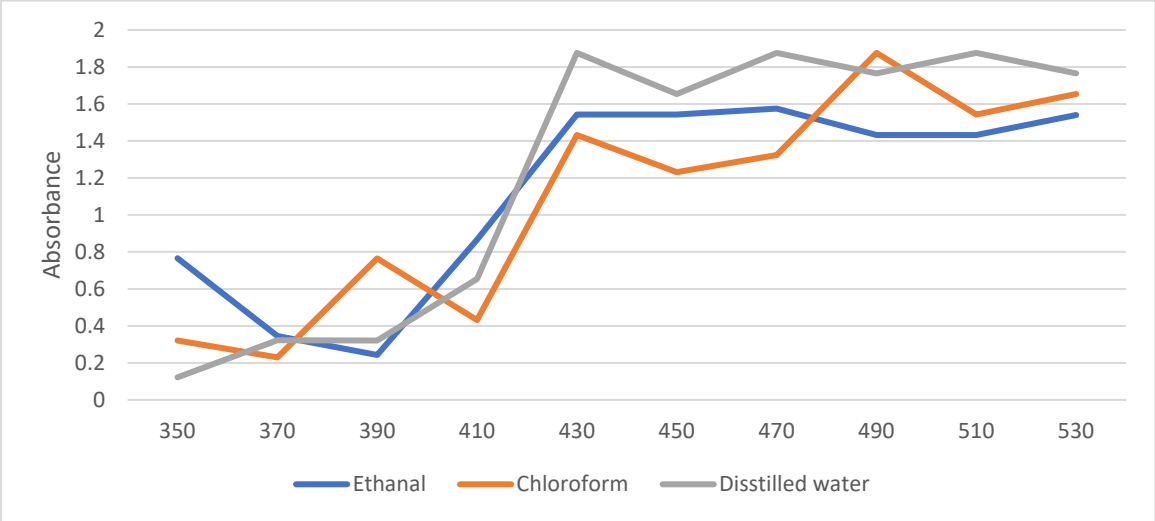


Figure 8: UV-visible spectral analysis of copper oxide nanoparticles synthesis by treating 1m aqueous copper sulphate solution with 10 mg of *Pedalium murex* stem extract (Distilled water, Chloroform, ethanol)

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Plate 13: Cobalt oxide nanoparticles synthesis by treating 1m of aqueous cobalt nitrate solution with 10 mg of *Acalypha indica* leaf extract (Water ethanol, chloroform)

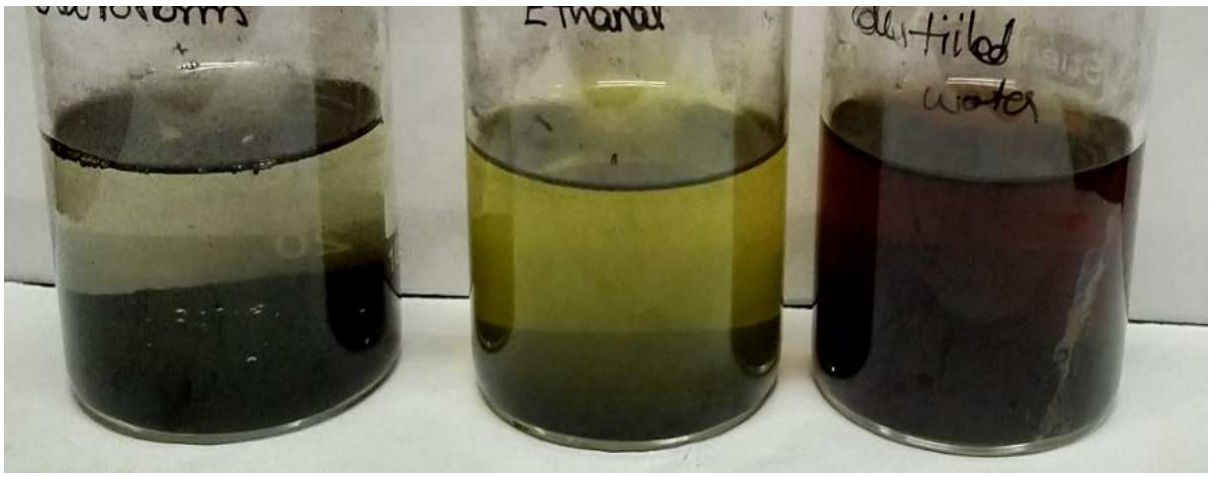


Plate 14: Cobalt oxide nanoparticles synthesis by treating 1m of aqueous cobalt nitrate solution with 10 mg of *Clitoria ternatea* leaf extract (Water ethanol, chloroform)

Plate 15: Cobalt oxide nanoparticles synthesis by treating 1m of aqueous cobalt nitrate solution with 10 mg of *Leucas aspera* leaf extract (Water ethanol, chloroform)

Plate 16: Cobalt oxide nanoparticles synthesis by treating 1m of aqueous cobalt nitrate solution with 10 mg of *Pedalium murex* leaf extract (Water ethanol, chloroform)

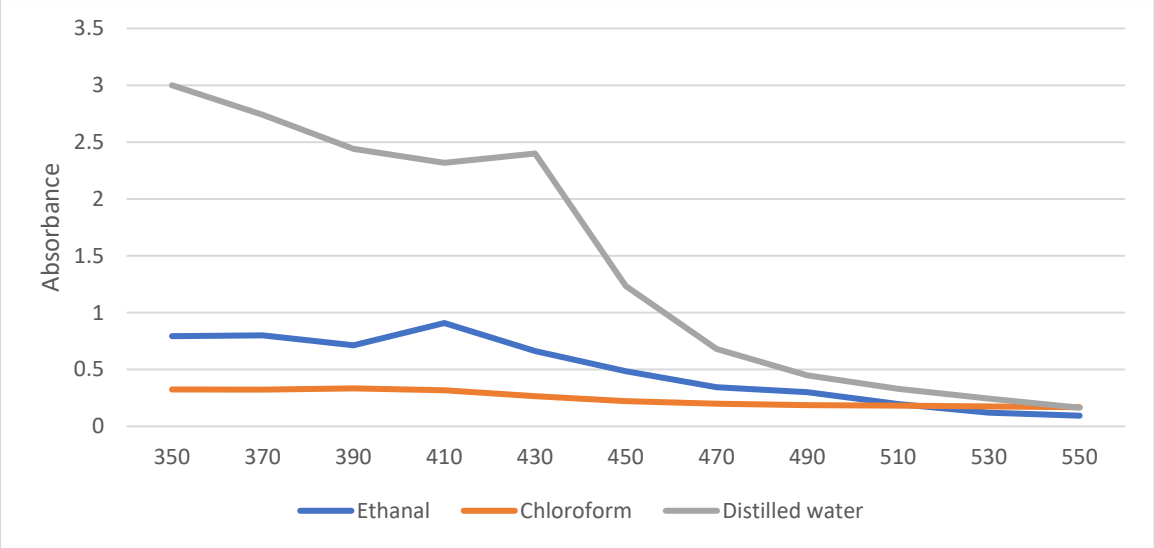


Figure 9: UV-visible spectral analysis of cobalt oxide nanoparticles synthesis by treating 1 m aqueous cobalt nitrate solution with 10 mg of *Acalypha indica* leaf extract (Distilled water, Chloroform, ethanal)

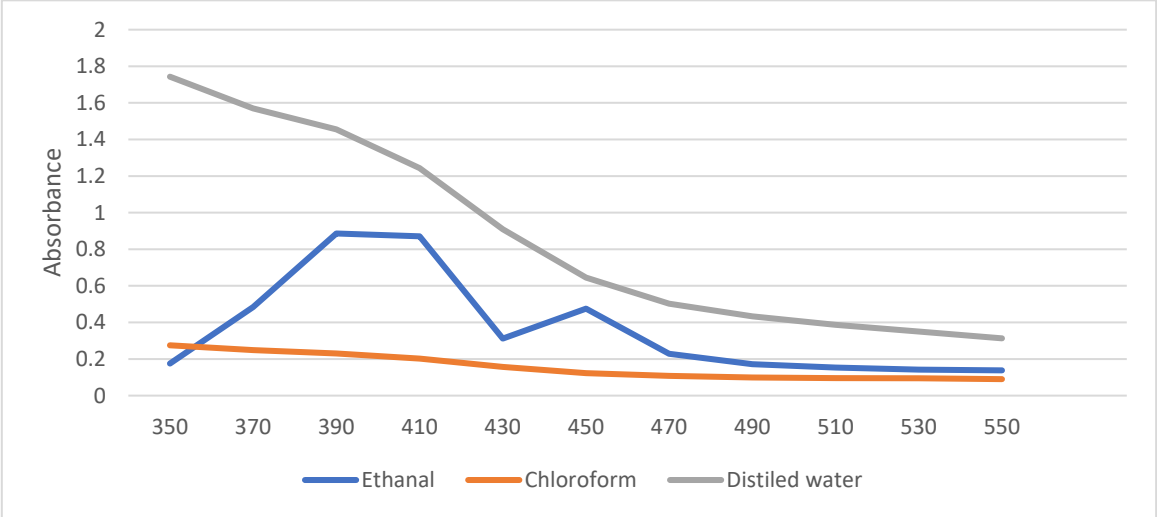


Figure 10: UV-visible spectral analysis of cobalt oxide nanoparticles synthesis by treating 1m aqueous cobalt nitrate solution with 10 mg of *Clitoria ternatea* leaf extract (Distilled water, Chloroform, ethanol)

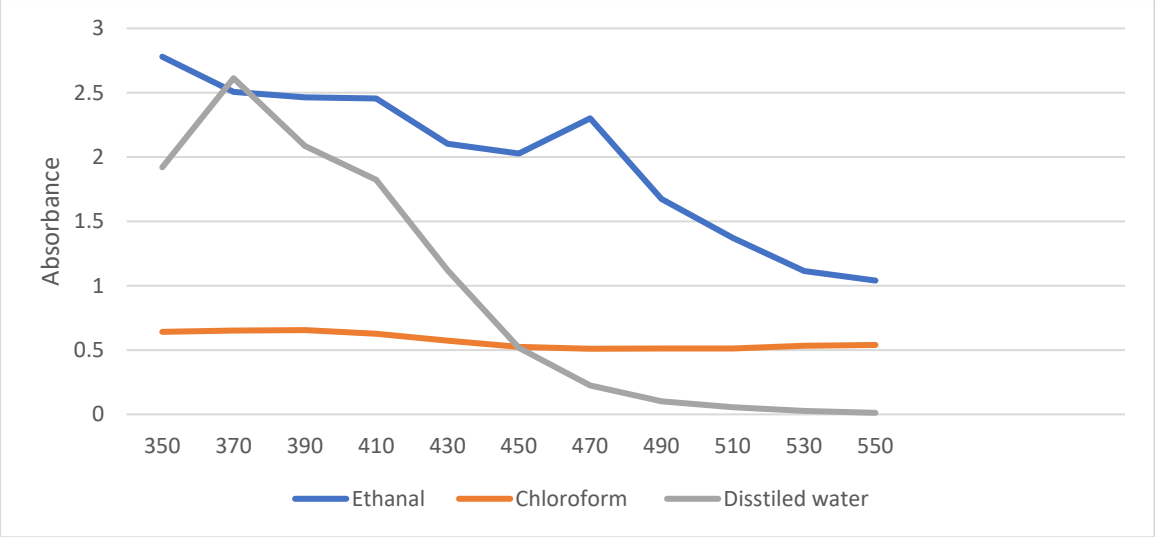


Figure 11: UV-visible spectral analysis of cobalt oxide nanoparticles synthesis by treating 1m aqueous cobalt nitrate solution with 10 mg of *Leucas aspera* leaf extract (Distilled water, Chloroform, ethanol)

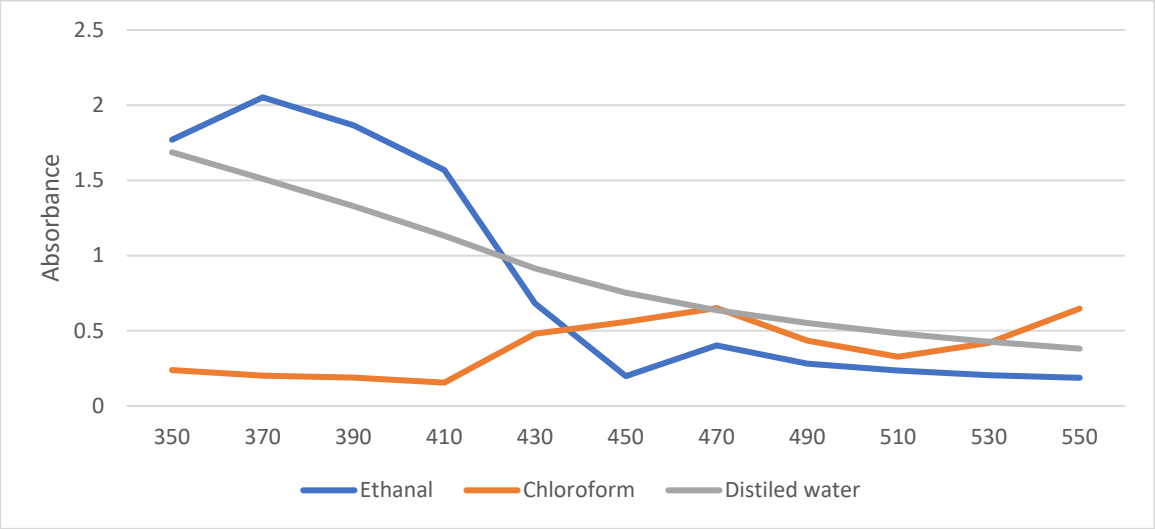


Figure 12: UV-visible spectral analysis of cobalt oxide nanoparticles synthesis by treating 1m aqueous cobalt nitrate solution with 10 mg of *Pedalium murex* leaf extract (Distilled water, Chloroform, ethanol)

Plate 17: Cobalt oxide nanoparticles synthesis by treating 1m of aqueous cobalt nitrate solution with 10 mg of *Acalypha indica* stem extract (Water ethanol, chloroform)

Plate 18: Cobalt oxide nanoparticles synthesis by treating 1m of aqueous cobalt nitrate solution with 10 mg of *Clitoria ternatea* stem extract (Water ethanol, chloroform)

Plate 19: Cobalt oxide nanoparticles synthesis by treating 1 m of aqueous cobalt nitrate solution with 10 mg of *Leucas aspera* stem extract (Water ethanol, chloroform)

Plate 20: Cobalt oxide nanoparticles synthesis by treating 1m of aqueous cobalt nitrate solution with 10 mg of *Pedalium murex* stem extract (Water ethanol, chloroform).

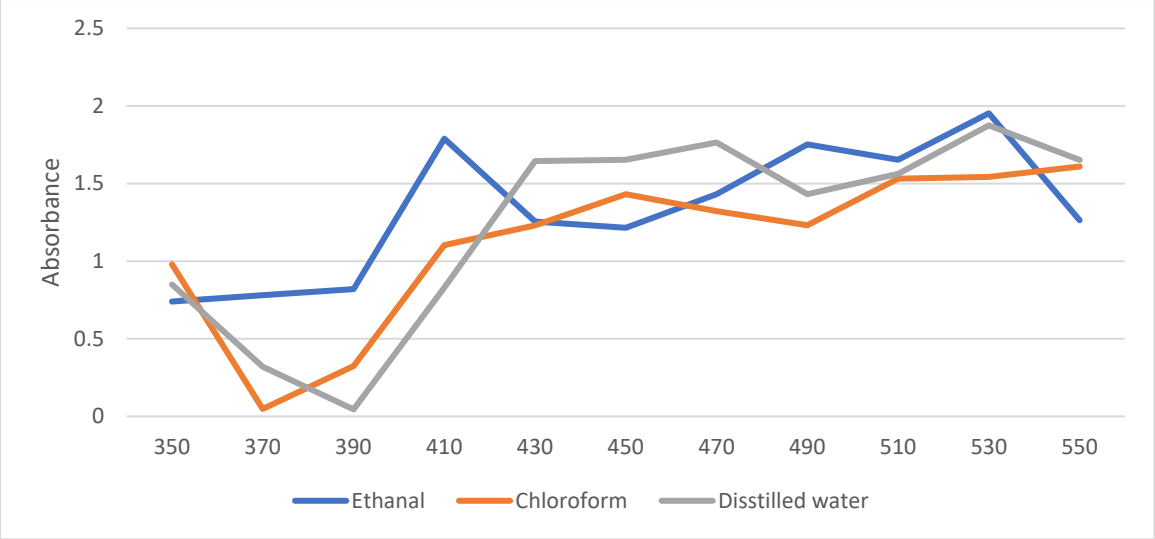


Figure 13: UV-visible spectral analysis of cobalt oxide nanoparticles synthesis by treating 1m aqueous cobalt nitrate solution with 10 mg of *Acalypha indica* stem extract (Distilled water, chloroform, ethanol).

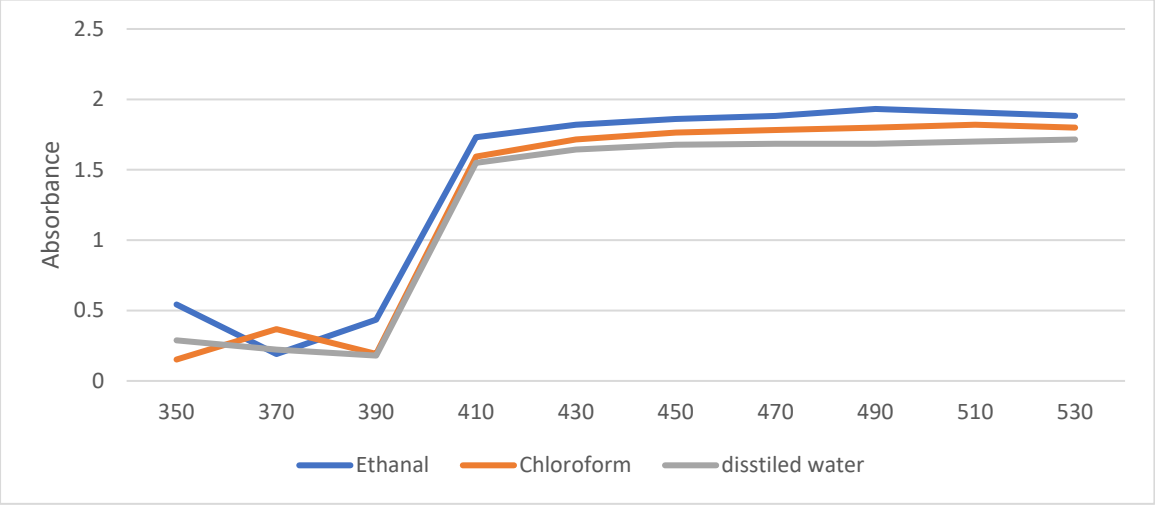


Figure 14: UV-visible spectral analysis of cobalt oxide nanoparticles synthesis by treating 1m aqueous cobalt nitrate solution with 10 mg of *Clitoria ternatea* stem extrac (Distilled water, chloroform, ethanol)

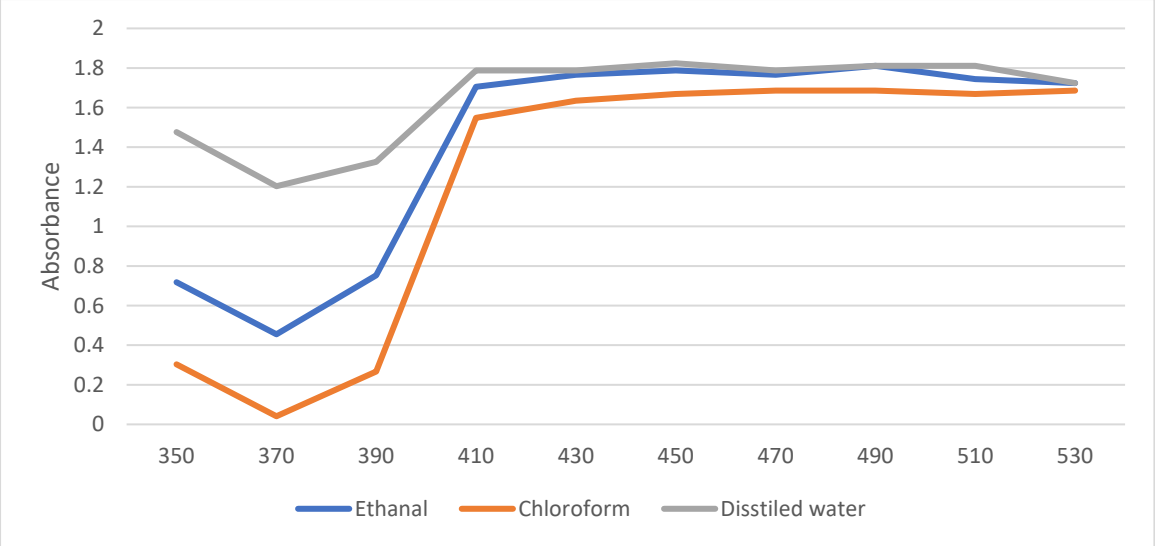


Figure 15: UV-visible spectral analysis of cobalt oxide nanoparticles synthesis by treating 1m aqueous cobalt nitrate solution with 10 mg of *Leucas aspera* stem extract (Distilled water, Chloroform, ethanol)

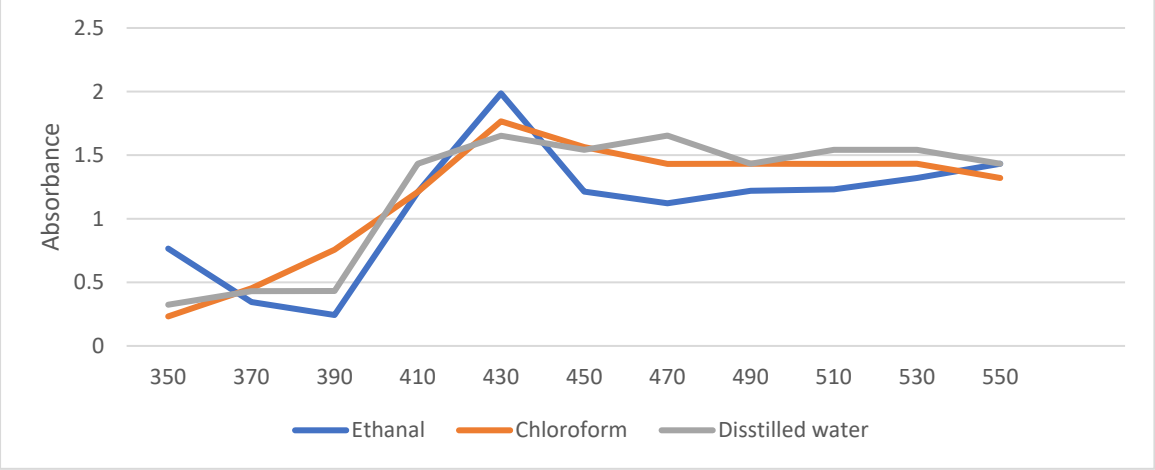


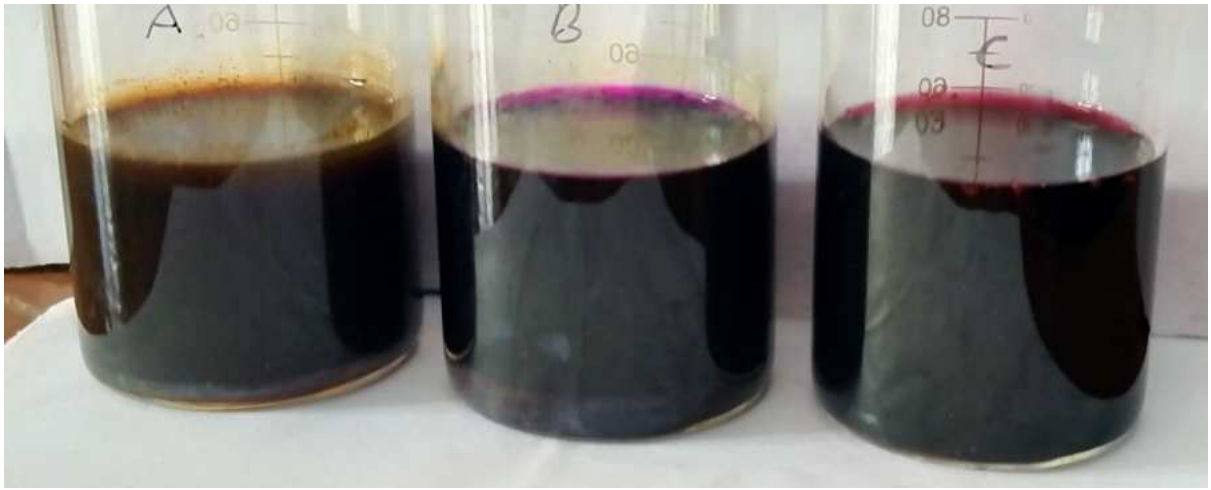
Figure 16: UV-visible spectral analysis of cobalt oxide nanoparticles synthesis by treating 1m aqueous cobalt nitrate solution with 10 mg of *Clitoria ternatea* stem extract (Distilled water, Chloroform, ethanol).

Plate 21: Manganese oxide nanoparticles synthesis by treating 0.2 m of aqueous potassium permanganate solution with 10 mg of *Acalypha indica* leaf extract (Water, ethanol, chloroform)

Plate 22: Manganese oxide nanoparticles synthesis by treating 0.2 m of aqueous potassium permanganate solution with 10 mg of *Clitoria ternatea* leaf extract (Water ethanol, chloroform).

Plate 23: Manganese oxide nanoparticles synthesis by treating 0.2 m of aqueous potassium permanganate solution with 10 mg of *Leucas aspera* leaf extract (Water ethanol, chloroform).

Plate 24: Manganese oxide nanoparticles synthesis by treating 0.2 m of aqueous potassium permanganate solution with 10 mg of *Pedalium murex* leaf extract (Water ethanol, chloroform).



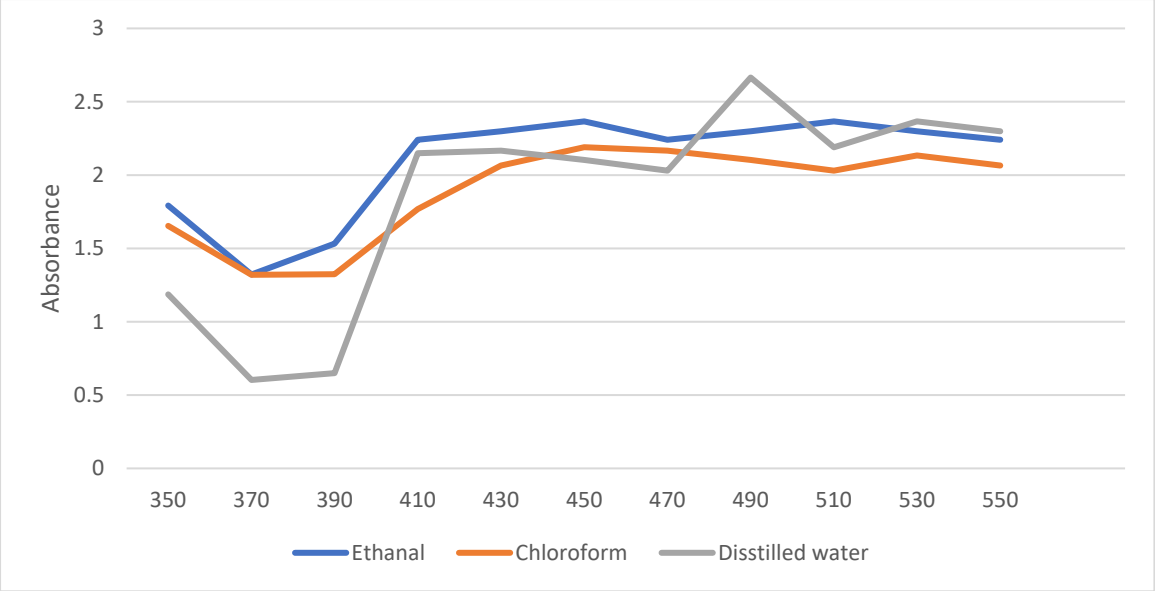


Figure 17: Manganese oxide nanoparticles synthesis by treating 0.2 m of aqueous potassium permanganate solution with 10 mg of *Acalypha indica* leaf extract (Water ethanol, chloroform).

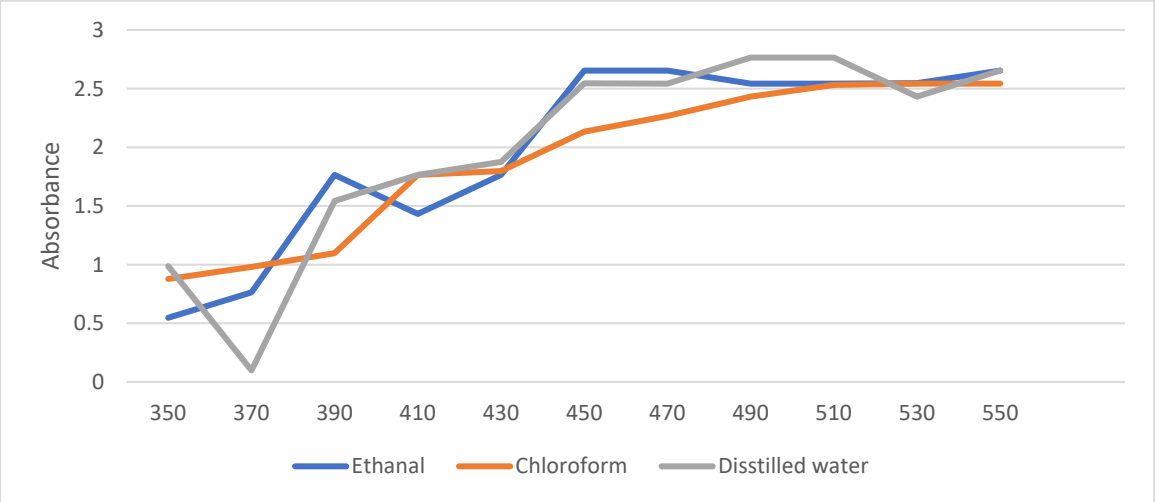


Figure 18: manganese oxide nanoparticles synthesis by treating 0.2 m of aqueous potassium permanganate solution with 10 mg of *Clitoria ternatea* leaf extract (Water ethanol, chloroform)

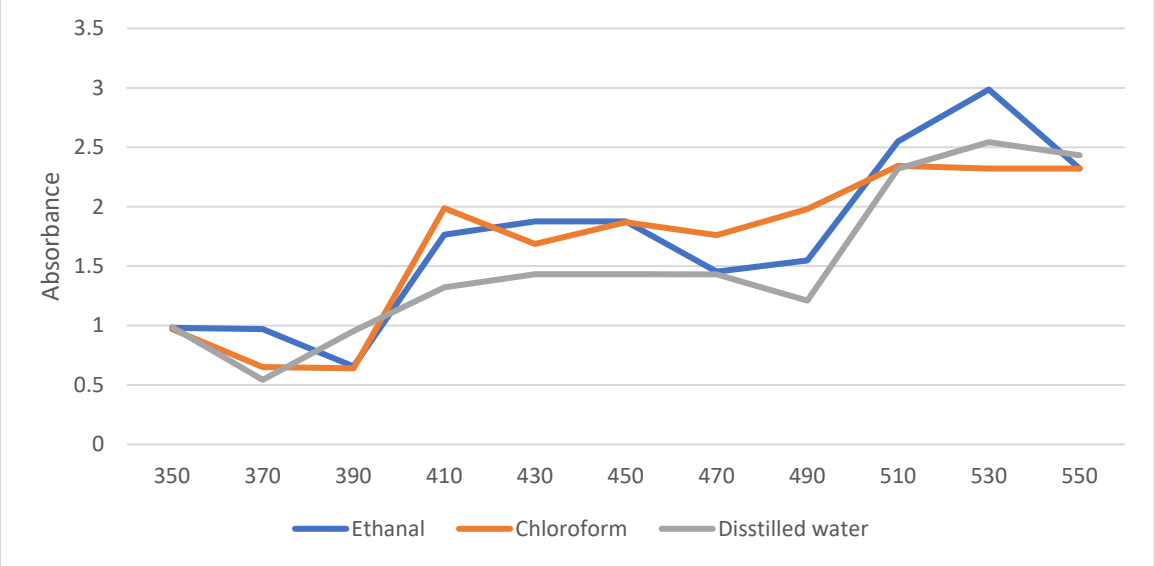


Figure 19: Manganese oxide nanoparticles synthesis by treating 0.2 m of aqueous potassium permanganate solution with 10 mg of *Leucas aspera* leaf extract (Water ethanol, chloroform)

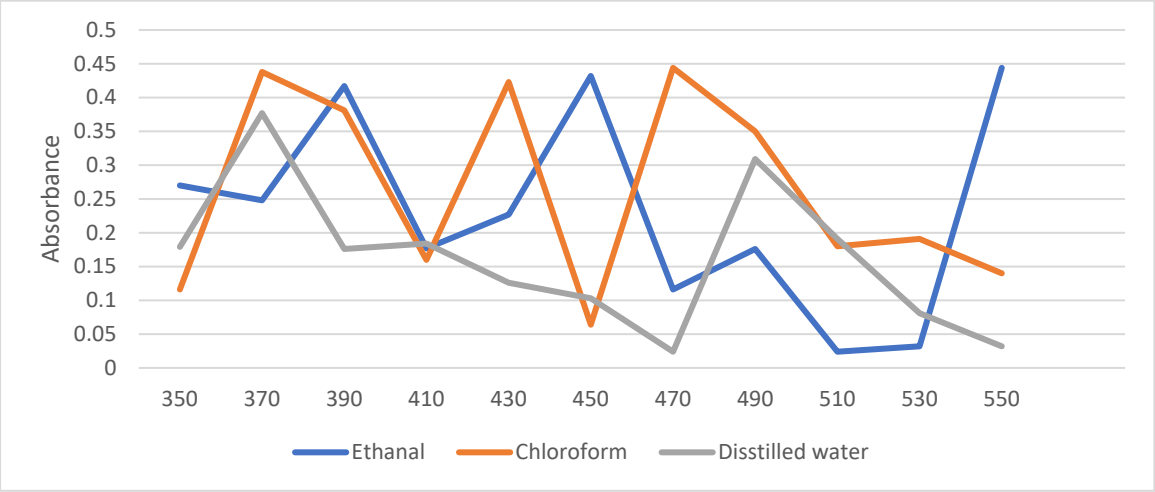


Figure 20: Manganese oxide nanoparticles synthesis by treating 0.2 m of aqueous potassium permanganate solution with 10 mg of *Pedalium murex* leaf extract (Water ethanol, chloroform)

Summary and Conclusion

Metal nanoparticles (MNPs) have been widely used in a range of recent scientific and technological applications. They can be produced by conventional chemical synthesis or green synthesis methods. Green synthesis consists of a myriad of promising approaches for the production of MNPs with desired properties. Plants represent the most explored group of living organisms for the green synthesis of MNPs, and to date, hundreds of species have been used. However, several factors that should be taken into account when performing green synthesis of MNPs remain underestimated or unexplored. The present work depict biosynthesis of Copper oxide, Cobalt oxide and Manganese oxide nanoparticles using the different extract (distilled water, ethanol and chloroform) of *Acalypha indica, Clitoria ternatea, Lucas aspera* and *Pedalium murex.* Plants provide a better platform for nanoparticle synthesis as they are free from toxic chemicals and provide natural capping agents. The reduction of Cu, Co and Mn using the plant leaf and stem extracts were viewed by the colour change in the reaction solutions. Characterization of nanoparticles was done by using Ultraviolet–Visible Spectroscopy. The UV–visible absorption spectra of the Cu, Co and Mn nanoparticles with different extracts of *Acalypha indica, Clitoria ternatea, Lucas aspera* and *Pedalium murex* were recorded. Manganese metal nanoparticles have free electrons, which yield a surface plasmon resonance (SPR) absorption band, due to the mutual vibration of electrons of metal nanoparticles in resonance with light wave. The appearances of the peaks show the characteristics of surface plasmon resonance of Cu, Co and Mn nanoparticles. The UV–Vis spectral studies confirmed the surface plasmon resonance of green-synthesized metal nanoparticles.

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A STUDY ON STHALAVRIKSHAS IN TEMPLES OF THOOTHUKUDI DISTRICT, TAMIL NADU

A dissertation submitted to

ST. MARY'S COLLEGE (AUTONOMOUS), THOOTHUKUDI.

Affiliated to

MANONMANIAM SUNDARANAR UNIVERSITY, THIRUNELVELI.

in partial fulfillment of the requirements for the Degree of

MASTER OF SCIENCE IN BOTANY

By

S. CHITHRA

Reg.No. 19APBO06

DEPARTMENT OF BOTANY

ST. MARY'S COLLEGE (Autonomous)

THOOTHUKUDI -628001

2020- 2021

CERTIFICATE

This is to certify that this dissertation entitled, **"A Study on Sthalavrikshas in Temples of Thoothukudi District, Tamil Nadu"** submitted by **S. Chithra Reg. No. 19APBO06** to ST. MARY'S COLLEGE (Autonomous), THOOTHUKUDI in partial fulfillment for the award of the degree of **"Master of Science in Botany"** is done by her under my supervision. It is further certified that this dissertation or any part of this has not been submitted elsewhere for any other degree.

S.D, M.L.d.
GUIDE

M. Glory
HOD

HOD of Botany
St. Mary's College (Autonomous)
Thoothukudi- 628 001.

16.04.21
EXAMINER

Lucia Rose
PRINCIPAL
St. Mary's College (Autonomous)
Thoothukudi - 628 001.

DECLARATION

I do here by declare that this dissertation entitled, **"A Study on Sthalavrikshas in Temples of Thoothukudi District, Tamil Nadu"** submitted by me in partial fulfillment for the award of the degree of **'Master of Science in Botany'**, in the result of my original and independent work carried out under the guidance of **Dr. E. Daffodil D Almeida, M. Sc., SET, Ph. D.,** Assistant Professor, Department of Botany, St. Mary's College (Autonomous), Thoothukudi and it has not been submitted elsewhere for the award of any other degree.

S. Chithra

Station: Thoothukudi **(S. Chithra)**

Date : 15.04.2021

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CONTENT

INTRODUCTION

INTRODUCTION

Various ethnic teams of India have preserved and guarded many forest patches and even individual trees or animals thanks to their ancient belief and respect for nature (Tripathi, 2001). In Tamil Nadu state, India this customary apply follows with religious faiths and culture. One such religious worship is understood as Sthalavriksha in temples. Sthalaviruksam is a practice of growing and maintaining trees in Hindu temples of Tamil Nadu, India. Sthalavriksha refers to the plant of which 'Sthala' means Place and 'Vriksha' means tree. From ancient periods, trees were thought-about as sacred and loved in Indian mythology and by folklore (Prabakaran and Sabari Lakshmi, 2017). The tree is treated as holy and prayers are performed frequently. Devotees create needs by tying a thread or writing their needs in an exceedingly piece of paper and fastening on the tree. Sacred groves with all sorts of vegetation assure conservation of floras (Manimozhi, 2019).

The Sthalavrikshas have played a vital role in the well-being of humanity. Sthalavriksha or temple tree is a single plant worshipped as equal as the prime deity in the temples. In both Hinduism and Buddhism, temple tree worship holds a bigger significance. The Sthalavrikshas are symbolic of a single genetic resource and play an important role in the conservation of biodiversity. The process of conserving economically, ecologically and medicinally important plants by declaring them as sacred also protected the genetic value of several plant species (Kaliyamoorthy, 2019). Thus the preservation of Sthalavrikshas may also help in

the conservation of local floral wealth. It is the natural tree found in temple site before construction of the temple. After the construction of temples, these plants are treated as Sthalavriksha or temple trees (sacred plants). Due to traditional beliefs, both the devotees and temple authorities serve as protectors of the Sthalavriksha in temples (Gunasekaran and Balasubramanian, 2012).

The worshipper who comes to the temple attains a healthy spiritual enlightment. Sthalavriksha is a single plant mostly in the form of a tree or in some places occurring as a herb, shrub, grass or climber. In Hinduism, especially in Shaivam, there are three important aspects of the temple grounds, Moorthy (a Deity), Sthalam, (a Shrine and Sthalavriksha) and a Theertham, (Sacred tank or water body). These are the three prime elements to learn about the antiquity of a temple. The worship of these three elements will yield wisdom even without a guru or teacher (Thambiran, 1963). In Tamil Nadu there are 25,000 ancient temples and these heritage sites play a vital role in conserving traditional arts, temple architectures, Tamil culture and also Sthalavrikshas. Sthalavrikshas are an integral part of temple worship (Gunasekaran and Balasubramanian, 2012).

References from Indian literary works, data from history, folk tradition, traditional knowledge, evidences from epigraphical records and copper plates are the standing proof for the above practice. Hindu Religious and Charitable Endowments is one of the state department that controls about 36425 temples in Tamil Nadu. Lord Shiva temples are around 1008 and among these, Pancha Bootha Sthalangal are five, Pancha Sabai Sthalangal are five, Navagraha Sthalangal are 9 and Paadal Petra Sthalangal are approximately 275. A survey by international union for conservation of nature and natural Resources (IUCN) has

estimated that out of 18,000 to 20,000 species of flowering plants in India, about 1000 species falls under conservation category (Manimozhi, 2019). According to Jain (1987) out of about 15,000 species of Indian flowering plants, nearly 2500 species are under various degrees of threat. However, religion and religious values of Hindu have a major role in persuading constant efforts in protecting and managing the plants in temples and their vanity for more than 3000 years. The main idea behind the establishment of Sthalavriksam, Nandavanas and dedication of sacred groves are ensured in planting, maintaining, protecting and conserving plant species for posterity. All these became evidence for the people's knowledge on the holistic importance of plants in purifying the temple's atmosphere and devotees' health (Prabakaran and Sabari Lakshmi, 2017).

The plant, primarily worshipped are Peepal (*Ficus religiosa*), Neem (*Azardirachta indica*), Bael (*Aegle marmelos*), etc. There are some temples which have more than one Sthalavriksham simultaneously, like *Ficus religiosa* L. and *Azardirachta indica* Adr. Juss. etc. Some of the important temple festivals are associated with the sthalavriksham of the temples. Sthalavriksha mostly occurs in tree habit, in main or big temples of Tamilnadu. In some temples, it occurs in herb, shrub, grass or climber forms. Sacred trees are therefore handled as any other sacred space, and it is thus not surprising that many of the customs and ceremonies mentioned in sacred places, in general, are also observed at the sites of sacred trees. This habit shows characteristically the importance of medicinal plants in Indian System of Medicine. Medicinal parts of the Sacred Trees (Sthalavrikshas) are practiced in different forms. It is presented in the form of

paste, juice, dried powder and made into tablets and juices mixed with sugar and honey to cure various diseases (Amirthalingam, 1998).

Traditionally Sthalavriksham plant has been used for the treatment of various diseases. These medicinal plants are also used as food, flavonoid, medicine or perfume and also in certain spiritual activities. Medicinal plants are essential natural resource which constitutes one of the potential sources of new products and bioactive compounds for drug development (Jayakumar, 2017).

Plants have the ability to synthesize a wide variety of chemical compounds that are used to perform important biological functions, and to defend against attack from predators such as insects, fungi and herbivorous mammals. The use of plants as medicines predates written human history. Ethnobotany is recognized as an effective way to discover future medicines. (Fabricant and Farnsworth March 2001). All plants produce chemical compounds as part of their normal metabolic activities. These phytochemicals are divided into primary metabolites such as sugars and fats, which are found in all plants; and secondary metabolites compounds which are found in a smaller range of plants, serving a more specific function, (Meskin and Mark 2002). In 2001, researchers identified 122 compounds used in modern medicine which were derived from "ethnomedical" plant sources; 80% of these have had an ethnomedical use identical or related to the current use of the active ingredients of the plant. Many of the medicines currently available to physicians have a long history of use as herbal remedies, including aspirin, digitalis, quinine, and opium, (Jayakumar, 2013a; Jayakumar, 2016a & b).

The wood of the sacred trees like vilvam, banyan, vanni, purasu and pipal is never employed as fuel, as it is believed to invite the anger of gods. But it is employed in other ways, in sacrificial rites and ceremonies (Sudhakar 2016).

'Sthalavriksham' to be preserved through cloning:

The Hindu Religious and Charitable Endowments Department has asked the executive officers of temples and departmental inspectors to adopt cloning to preserve Sthalavriksham or tree unique to each temple.

Describing every Sthalavriksham as a unique germplasm, a recent communication of the department urges the officials to take the help of experts from the Agriculture Department or the Tamil Nadu Agricultural University (TNAU) for the task. The communication, issued by Additional Chief Secretary, also asks the executive officers to ensure that enough clones are kept in carefully quarantined, separate places in nandavanam or flower garden. Even in private temples, the HR&CE inspectors should take steps to preserve Sthalavriksham. (http://www.thehindu.com/The Hindu /Home> News> National> Tamil Nadu/ by T Ramakrishnan / Chennai – December 03rd, 2014)

SCOPE AND OBJECTIVES

SCOPE AND OBJECTIVES

The plant kingdom in a locality is the basic evidence of that location even the place surpasses several eras. Among them, some trees are very special to spirituality. A tree in a temple is called Sthalaviruksha that is a useful one religiously, medically and also helpful to devotees to give its shade. The Sthala vriksha gives leaves and flowers. Each temple will have different virukshas (trees) according to the temple puranas and every viruksha is having a story. Traditionally, the Indian population has been worshipping rivers, lakes, mountains, trees and other natural resources. People of India have been living very closely with the Nature and thriving well on the natural resources.

In a Hindu temple, there will be three important factors namely Sthala, Theertham and Murthy. Sthala refers to the Temple, Theertham, to the Temple tank and Murthy, the deity worshipped. The reason for this is, the place, the water resource, and the trees will have to be venerated along with the deities as all are important for peaceful living of all beings. A temple may also be associated with trees - sthala vriksham.

In Hinduism, It is belief that Gods residing in trees. For example, the peepal tree represent the three supreme gods- Vishnu, Brahma, Shiva. The roots represent Brahma, the trunk represent Vishnu and the leaves of the tree represent Shiva. Peepal tree has its uses in Ayurvedic medicines as well and is known to treat many ailments.

Sthalaviruksha's main motivation is to use the flowers, leaf for temple poojas. Bilva leaf for Shiva pooja and similarly every tree in the sthala will be used for pooja along with flowers. The significance of growing Sthalaviruksha is useful for immediate medical purposes. Ladies who fear pimples, its paste is a good medicine. Even the sting of scorpion is cured when its ashes are applied on the place of bite. As it is an anti-microbial agent, it purifies the environment. In particular, medicinal uses of sthalavirishas were referred to, based on secondary sources only.

Traditionally known as “Pearl City” on account of the prevailing Pearl fish in the past in the area, Thoothukudi has a fascinating History. There are many famous temples in and around Thoothukudi. So, it is an important place for Hindu pilgrims. conducted in field was scanty. The study and survey of sthalavriksha of Thoothukudi was not carried out before and the present work is the first of its kind. Hence the present survey on ‘Sthalavirikshas of various temples in Thoothukudi, Tamil Nadu was designated to gather data. The survey has been undertaken with the following objectives:

- To document different Sthalavriksha species and their associated deities in the temples.
- To study the biological description of sthalavriksham
- To study the medicinal value of sthalavriksham
- To know the conservation status of sthalavriksham

AREA OF STUDY

AREA OF STUDY

Thoothukudi, is a port city, a municipal corporation and an industrial city of Tamil Nadu. Traditionally known as “Pearl City” on account of the prevailing pearl fish in the past in the area, Thoothukudi has a fascinating History. Forming part of the Pandian kingdom between 7th and 9th Century A.D. The city lies in the Coromandel Coast of Bay of Bengal. Thoothukudi is the capital and headquarters of Thoothukudi district. Thoothukudi and Rameswaram shores in the Gulf of Mannar are noted as the first Marine Biosphere Reserve of India, and have around 36,000 species of flora and fauna. This protected area is called Gulf of Mannar Marine National Park.

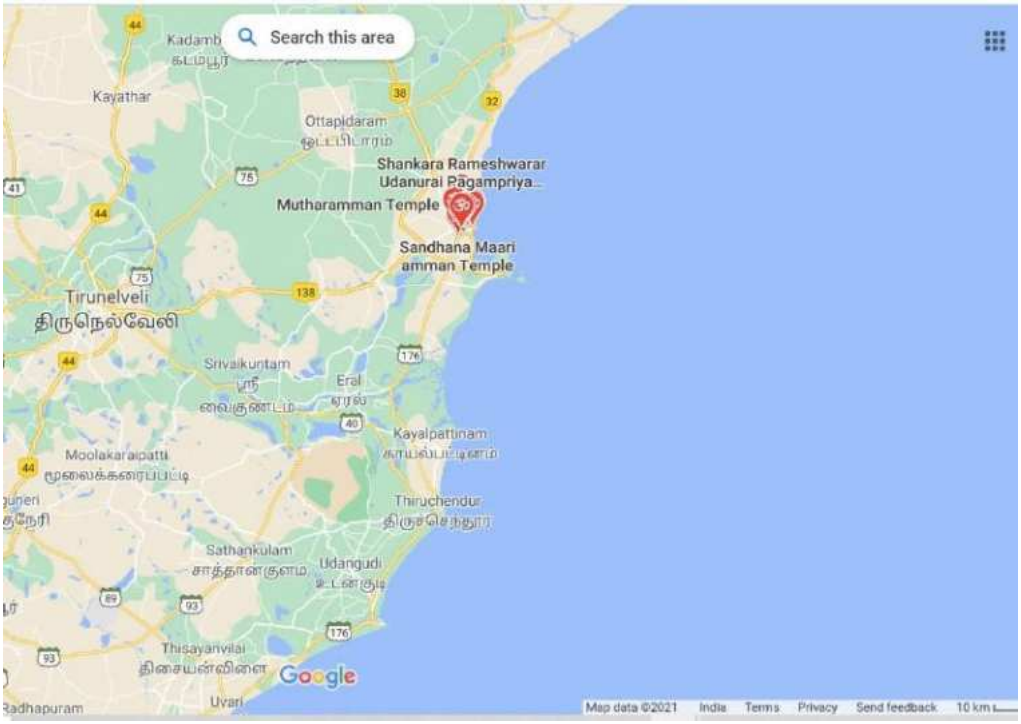


Figure 1: Study Area- Thoothukudi District (Google Map)

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Sthalavrikshams in temples indicate the importance given for plants and trees in those days. Every temple has one plant or tree as Sthalavriksham. While these are protected in some temples, they are not seen in some (Jayakumar, 2019). Most of these temples have their own Sthalavrikshas (temple trees) and Nanthavanam (flower garden). Sacred plants provide food, shelter and nesting substratum for several species of birds and squirrels. All souls of certain species are completely protected (Kumar and Aruna, 2018).

Today, such groves occur in many parts of India, both in tribal tracts and outside of it. Due to traditional beliefs, both the devotees and temple authorities serve as protectors of the Sthalavriksha in temples and if a tree dies because of old age, it is usually replaced by a sapling from the same species. Hence, the tree occurs constantly in a temple for several centuries (Gunasekaran and Balasubramanian, 2012). Sacred groves are a group of trees or a patch of vegetation protected by the local people through religious and cultural practices evolved to minimize destruction. The sacred trees are very good ex- situ conservation where a single plant is conserved and worshipped Such sacred trees are rather medicinal plants, or a representative element of respective ecological region or a source of edible parts of a plant (Israel *et al*., 1997).

Occurrence of sacred groves at several spaces in India, clearly defines the community's attachment to nature. Sacred plants play a really significant role in ecology. Due to their ecological value and effective properties, sacred plants continue to be employed in the religious and social festivals of the Hindus

(Vinoth kumar and Aruna 2018). In contrast to 'sthala viruskshas' certain patches of forest, ponds, and stretches of river are protected by the local community by giving them a sacred status. Sacred groves are usually dedicated to a deity, very often a local deity (Israel *et al*. 1997).

Sthalavrikshas are valued for their botanical, medicinal, environmental, religious and mythical importance. The Sthalavrikshas of Tamil Nadu constitute a part of genetic resources for the conservation of species diversity and forms an important biological heritage of our nation (Sasikala, 2014).

Study of Sthalaviruksham:

Mohantry *et al*. (1997) surveyed the role of temples and holy places in nurturing the surrounding flora and its habitat. They recorded eightysix kinds of plants in temple yards and gardens of Orissa, where they are cultivated and preserved for different temple rituals.

Narasimhan *et al*. (1997) conducted a survey of sacred groves in Tamil Nadu. They surveyed 383 temple sthalas in Tamil Nadu of which 275 represent 'Siva sthalas' and 109 the 'Vaishnava sthalas' endangered and endemic species were recorded.

Ethnomedicinal uses of Sthalavriskshas survey was conducted by Gunasekaran and Balasubramanian (2012) in the temple of Tamilnadu at 1165 ancient temples of the state and revealed the occurance of 112 plant species. At the time of study, several ethnomedicinal uses of 101 Sthalavriksha species were

recorded by both direct observations and referred to by devotees, priests and Nattuvaidyas in the temples.

Twenty temples of Tiruchirappalli were visited during the period of June 2008 to May 2009 and sixteen Sacred Trees were recorded along with the name of the place, temple, deity, binomial and common name. The botanical features, ecological, economic and medicinal potentials were enumerated based on the literature surveyed (Umavathi and Parvathi, 2012).

Nandkishor (2013) recorded plants from sacred groves and their medicinal uses from Amravati District (Maharashtra).

Tholkappiyavathi *et al.* (2013) surveyed the Sthalavrikshas of Nagapattinam. According to their records, 16 temples have Sthalavrikshas while 20 such element exists in remaining temples. 9 species of Sthalavrikshas have been recorded in these 16 temples.

Taxonomy and ethnobotany of Sthalavrikshas survey was conducted by Greeshma *et al*. (2014). They surveyed 120 temples and out of 120 temples 68 temples only have the sthalavrikshas. They recorded 16 plant species. Among the 16 species 15 have reported medicinal values.

Nair *et al.* (2014) described the taxonomy and ethnobotany of Sthalavrikshas in Palakkad, the largest district of Kerala. He surveyed 68 out of 120 temples within the study region reported presence of Sthalavrikshas and pre-designed questionnaire w to collect data on individual Sthalavrikshas. Information pertaining to beliefs, rituals, and culture associated with sthalavriksha worship were prepared by interviewing temple priests, temple staff and local public.

Historic details of respective temples were compiled from temple manuscripts. Ethnobotanical importance sthalavrikshas were captured by consulting local traditional healers and practicing doctors of Indian System of Medicine (Ayurveda) and available elders.Sivali

Gautam and Rajan (2014) highlighted the importance of traditional knowledge, culture and tradition practices in achieving sustainable development. They reviewed the description of eco-centric approach of different religions, community, ethnic groups and sects of India. They described sacred groves, environmental laws and some of the most significant environmental movements of India.

Sivalingam *et al.* (2016) have undertaken a survey at six big temples in Cuddalore district, Tamil Nadu, India. They recorded six plant species and their conservation status of, their religious value, social and economic values. They were used to treat 30 diseases and 12 major ailment categories. Leaves were the most frequently used plant part. Based on IUCN red data, the identified sacred plants include one least concerned species, one vulnerable species and two endangered and threatened species.

Prabakaran and Lakshmi (2017) surveyed the Sthalavrikshas of 106 temples in Salem, Namakkal and Karur districts of Tamil Nadu, India. In this study 81 temples of Sthalavrikshas were found and a total of 18 plant species were recorded. They also recorded temple gardens of 27 temples and there were 41 species were recorded. Few Sthalavrikshas were found as fossilized forms and this was unique to the study.

The survey of Sthalavriksha of temples was conducted in Erode district of Tamil Nadu, India from the month November 2017 to March 2018 (Periyasamy and Saranya, 2018). A total number of 52 temples surveyed which includes 28 Shiva temples, 8 Perumal temples, 7 Amman temples, 7 Murugan temples and two other deity temples. Out of 52 temples surveyed, Sthalavrikshas found in 47 temples. A total of 25 plant species of Sthalavriksha belonging to 14 families, belong to 25 genera were recorded. All the recorded 25 Sthalavrikshas species belong to angiosperms and are dicotyledons. Among 14 families, Caesalpiniaceae is the most dominant family represented by 3 species followed by Rutaceae and Moraceae represented 2 species each. Among the 25 species, Aegle marmelos was the most frequently recorded in temples followed by Prosopis spicigera, *Ficus religiosa* and *Azadirachta indica*. The medicinal uses of the Sthalavriksha have gathered from the available literature, priests and temple authorities. All 18 plant species of Sthalavrikshas have medicinal values. Different parts of the Sthalavriksha are used for medicinal purpose. The entire plant (2 species), bark (9 species), fruits (4 species), leaves (8 species), flower (3 species), gum and seed (2 species) and root (1 species) are used for medicinal purpose.

A study of Sthalavrikshas was conducted by Kumar and Aruna (2018) in the temples of Madurai district. The study revealed the presence of Sthalavrikshas in 65 temples out of 100 temples studied. Totally 31 species of Sthalavrikshas recorded in these 65 temples. The recorded plant species belong to 20 families. Among that family of Moraceae and Fabaceae dominated together with 4 species followed by Rutaceae, Rubiaceae, Anacardiaceae and Mimosaceae family consequently represented with 2 plant species.

Periyasamy *el al*. (2018) conducted a survey of Sthalavriksha of temples in Erode district of Tamil Nadu, India from the month November 2017 to March 2018. They surveyed 52 temples surveyed which includes 28 Shiva temples, 8 Perumal temples, 7 Amman temples, 7 Murugan temples and two other deity temples. Out of 52 temples surveyed, Sthalavrikshas found in 47 temples. Total of 25 plant species of Sthalavriksha belonging to 14 families, belong to 25 genera were recorded. All the recorded 25 Sthalavrikshas species belong to angiosperms and are dicotyledons. They recorded medicinal uses of the Sthalavriksha have gathered from the available literature, priests and temple authorities.

Jeyakumar (2019) documented the sthalavrikshas of 5 Shiva temples in and around Mayiladuthurai, Southern India, 5 plant species were recorded. Plant species were arranged by binomial name, vernacular name, family name with age of trees, ICBN norms and medicinal uses. The phenology of Sthalavrikshas was documented by interview and direct observation of plants.

Manimozhi (2019) conducted a survey on sthalavirutcham and conservation of medicinal plants in the temples of Tamil Nadu, Southern India. The survey was conducted in about 19 temples of Lord Shiva located in Tamil Nadu, Southern India including Pancha Bootha Sthalangal, Pancha Sabai Sthalangal, Navagraha Sthalangal and documented to ensure awareness on the traditionally important plants.

MATERIALS AND METHODS

MATERIALS AND METHODS

The survey of Sthalavirukshas was conducted in the temples of Thoothukudi District. Temples were frequently visited and surveyed for the Sthalavrikshas from the month of December 2020 to March 2021. Sthalavrikshas were photographed (Plate 1-19) and characters were noted for the identification purpose. Taxonomic identification was done using Gamble's Flora of presidency of Madras (1997). Binomials of the plants with family, their local names, parts used and medicinal uses were recorded from the available literature (Joshi, 2008). The conservation status of the Sthalavrikshas was recorded based on IUCN red data list (IUCN redlist.org.2014).

TAXONOMIC DESCRIPTION

Botanical Name: ***Aegle marmelos*** **Corr.**

Vernacular Name: Vilvam

Family: Rutaceae

Description:

Aromatic tree, grows up to 18 meters tall and bears long thorns, leaves usually 3-foliolate, sometimes 5-foliolate; leaflets ovate-lanceolate, lateral sessile, terminal long-petioled, Flowers: borne in few-flowered, axillary panicles, greenish-white, sweet-scented. Fruits: large, 8-15 celled, pulp orange, sweet, seeds numerous in aromatic pulp, oblong, compressed and mucilaginous.

Botanical Name: ***Azadirachta indica*** **A. Juss.**

Vernacular Name: Vembu

Family: Meliaceae

Description:

Trees, Leaves alternate, imparipinnate, leaflets sub opposite, serrate, very unequal at base, flowers hermaphrodite, in auxillary panicles. Calyx 5-lobed. Petals 5, imbricate, free. Disk 0.Staminal tube a little shorter than the petals, cylindric, widening above, 9-10 lobed at the apex, the lobed at the apex, the lobes truncate, again slightly toothed, Ovary 3-celled; ovules 2 in each cell, Fruit a 1-seeded drupe.

Botanical Name: ***Borassus flabellifer*** **Linn**

Vernacular Name: Panai

Family: Arecaceae

Description:

Tree and can reach a height of 30 metres, trunk is grey, robust and ringed with leaf scars, leaves are fan-shaped and 3 m long, Petals 3, obovate-spathulate, to 2 mm. Stamens 6; anthers to 1 mm; pistillodes small, bristly. Female flowers large, globose. Perianth fleshy, accrescent. Sepals reniform, imbricate. Petals smaller, convolute. Ovary globose, entire, 4-celled; ovules basal; stigmas 3, sessile, recurved; staminodes 6-9. Drupes to 13 cm across, globose, yellow when ripe, with 1-3 compressed pyrenes. male flowers are less than 1 cm long and form semi-circular clusters, which are hidden beneath scale-like bracts within the catkin-like inflorescences, the female flowers are golfball-sized and solitary, sitting upon the surface of the inflorescence axis, fleshy fruits, each containing 1-3 seeds, fruits are black to brown with sweet, fibrous pulp and each seed is enclosed within a woody endocarp.

Botanical Name: ***Tamarindus indica*** **Linn**

Vernacular Name: Puli

Family: Fabaceae

Description:

Tree. Leaves abruptly pinnate; leaflets many, small, opposite; stiples0.Flowers in racemes at the ends of branches; calyx-tube turbinate, lobes 4 lanceolate, membranous, imbricate. Petals 3, stamens 3, ovary stipitate, stylefiliform; stigma capitates. Pod linear-oblong, incurved, thick, seeds obovate-orbicular, compressed, testa hard, albumen 0. Usually oblong; ovules 1-2, basal and erect fruit a ring of one - seeded berries.

Botanical Name: ***Ocimum tenuiflorum*** **Linn**

Vernacular Name: Tulsi

Family: Lamiaceae

Description:

Herbs, strongly aromatic from oil glands. Leaves opposite, toothed, petioled; floral leaves small, bract like, usually caduceus. Flowers small, in whorls of 6-10 on the axis of elongate spikes, which are some times panicled; pedicled; pedicles with recurved tips; bracts small, caduceus. Calyx ovoid, deflexed in fruit and then usually enlarged and hardened, 2 liped; upper broad, flat decurrent, lower lip with 4 mucronate teeth, the 2 middle ones usually the longest. Corolla 2-liped; tube short, upper lip subequally 4-lobed. Stamens 4 didynamous, declinate, exserted; filaments out. Disk entire or 3-4 lobed. Ovary 4-partite; style slender, bitfid at apex. Fruit of 4 dry, smooth, nutlets, often mucilaginous when wetted.

Botanical Name: ***Ficus benghalensis*** **Linn**

Vernacular Name: Aala

Family: Moraceae

Description:

Trees. Leaves alternate, stipules sheathing the bud, caduceus and leaving annular scars, flowers minute inserted on the inner walls of a fleshy receptacle, stamens usually 1, erect in bud. Style excentric; ovulesolitary, pendulous neuter perianth usually 3-fid. Fruit an fleshy; Albumen scanty; embryo curved, cotyledons equal.

Botanical Name: ***Ficus religiosa*** **Linn**

Vernacular Name: Arasu

Family: Moraceae

Description:

Deciduous tree, 20 m tall, irregularly-shaped, with wide-spreading branches, bark is grey with brownish specks, leaves alternate, spirally arranged and broadly ovate, glossy, coriaceous (leathery), stipulate, base-cordate. Petioles is slender, Galls on leaves, margin entire, undulate, glabrous, flowers axillary sessile, unisexual, male flowers ostiolar, sessile, in one ring, tepals 2, ovate-lanceolate, free, reddish; stamen 1, anther oblong, parallel; female flowers sessile; tepals 3-4, linear-lanceolate, free stigma rounded inflorescence a syconia, sessile ovary superior, ovoid-oblong.

Botanical Name: ***Calophyllum inophyllum*** **Linn**

Vernacular Name: Punnai

Family: Calophyllaceae

Description:

Large tree, usually grows 12-20 m in height, tall, often leaning, with broad, spreading crowns, opposite leaves are elliptical, dark green, shiny, rounded, leaf veins run parallel to each other and perpendicular to the midrib, bears clusters of fragrant white flowers, 4-8 oblong petals, fruit ball-shaped, which turns yellow and then brown and wrinkled when the fruit is ripe, covers the thin pulp, the shell, a corky inner layer, and a single seed kernel, large brown.

Botanical Name: ***Ipomaea pes-caprae*****n Sweet**

Vernacular Name: Kadambam

Family: Convolvulaceae

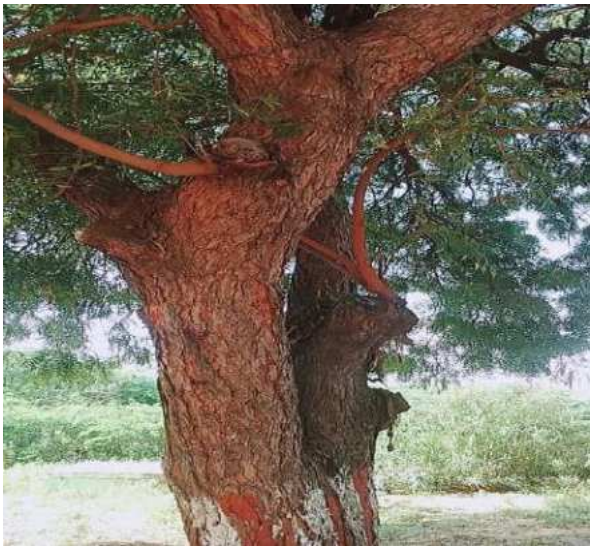
Description:

Prostrate perennial slender, creeping vine, with a succulent stem, leaves are thick, notched at the apex, creating two equal lobes, fleshy leathery leaves, ovate, obovate, elliptic, orbicular or transversely elliptic to kidney-shaped, stem is flexible, branches freely and roots at the nodes, flowers are very showy, pink to lavender purple funnels about 2 in long, stamens and style are surrounded entirely by the petals, filaments are hairy at the base and the ovary is smooth, fruit is a spherical, 4-locular capsule, single dark brown seed.

Sthalavrikshas of Various Temples in Thoothukudi

Plate 1

Maragatha Vinayagar
***Ficus religiosa* Linn**
***Azadirachta indica* A. Juss**



Arulmigu Sri Ulaganda Eeswari Amman Kovil
***Azadirachta indica* A. Juss**

Karpaga Vinayagar
***Ficus religiosa* Linn**

Arulmigu Sri Muniyasamy Thirukkovil
***Ficus religiosa* Linn**

Plate 2

Arulmigu Sri Santhanamariamman Kovil
***Azadirachta indica* A Juss**

Sri Sakthi Vinayagar Alayam
***Ficus religiosa* Linn**

Sri Mariamman Kovil
***Azadirachta indica* A Juss**

Sri Pathrakaliamman Kovil
***Ficus religiosa* Linn**

Plate 3

Arulmigu Sri Bharatha Sithi Vinayagar Alayam
***Ficus religiosa* Linn**

Sri Varatha Sithi Vinayagar Alayam

***Ficus religiosa* Linn**

Sri Mela Kali Amman Kovil
***Azadirachta indica* A Juss**

Sri Anantha Leengeshwarar kovil
***Azadirachta indica* A Juss**
***Ficus religiosa* Linn**

Plate 4

Sri Gnana Ganapathy Thirukkovil
***Ficus religiosa* Linn**

Sri Esakkiamman Kovil
***Azadirachta indica* A Juss**

Sri Sundara Vinayagar
***Ficus religiosa* Linn**

Vinayagar Alayam
***Ficus religiosa* Linn**

Plate 5

Sri Vinai Theerkum Vinayagar Alayam
***Ficus religiosa* Linn**

Sri Sakthi Vinayagar Thirukovil
***Ficus religiosa* Linn**

Sri Kailasanathar temple
***Aegle marmelos* Corr**

Kankanda Theivam Sri Pathirakaliamman
***Ficus benghalensis* Linn**

Plate 6

Arulmigu Sri Selva Vinayagar Alayam
***Ficus religiosa* Linn**

Sri Sithi Vinayagar Thirukovil
***Ficus religiosa* Linn**

Sri Santhivinayagar Thirukovil
***Ficus religiosa* Linn**

Arulmigu Sivanaintha perumal Thirukovil
***Ficus religiosa* Linn**

Plate 7

Sri Durga Alayam
***Azadirachta indica* A Juss**

Sri Selva Vinayagar Alayam
***Ficus religiosa* Linn**

Sri Selva Vinayagar Kovil
***Ficus religiosa* Linn**

Arulmigu Sri Mangalajala Kanapathi Alayam
***Ficus religiosa* Linn**
***Azadirachta indica* A Juss**

Plate 8

Arulmigu Sri Kaliamman Thirukovil
***Azadirachta indica* A Juss**

Sri Sri Radhakarishnaperumal Alayam
***Ocimum tenuiflorum* Linn**

Arulmigu Sri Muktheeshwarar Thirukkovil
***Ficus religiosa* Linn**

Arulmigu Sri Petchi Ambaal Thirukkovil
***Ficus religiosa* Linn**

Plate 9

Sri Bala Vinayagar
***Ficus religiosa* Linn**

Sri Chithi Vinayagar
***Ficus religiosa* Linn**

Arulmigu Sri Pathirakaliamman Thirukkovil
***Azadirachta indica* A Juss**

Arultharum Annai Sri Santhana Mariamman Kovil
***Azadirachta indica* A Juss**

Plate 10

Sri Karpaga Vinayagar Alayam
***Ficus religiosa* Linn**

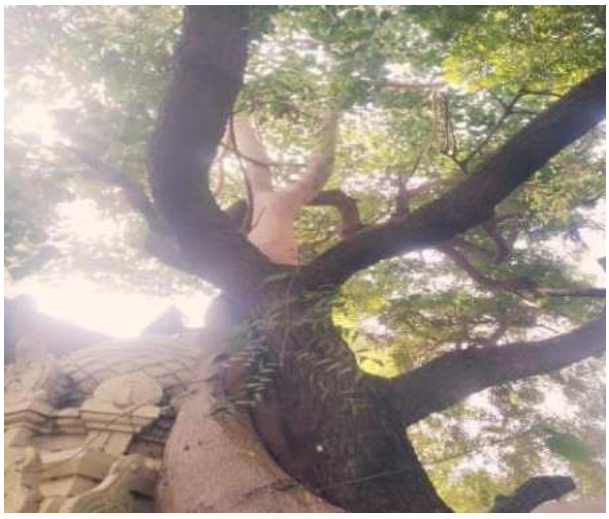
Om Sakthi Amman Kovil
***Azadirachta indica* A Juss**

Arulmigu Sri pathirakaliamman Kovil
***Ficus benghalensis* Linn**

Sri Sithi Vinayar Alayam
***Ficus religiosa* Linn**
***Azadirachta indica* A Juss**

Plate 11

Arulmigu Sri Pathirakali Amman Thirukovil
***Azadirachta indica* A Juss**



Arulmigu Sri Pathirakali Amman Kovil
***Azadirachta indica* A Juss**
***Ficus religiosa* Linn**

Sri Santhana Mariamman Kovil
***Azadirachta indica* A Juss**

Arulmigu Sri Karpaga Vinayagar Alayam
***Aegle marmelos* Corr**

Plate 12

Sri Sundara Pandiya Vinayagar
***Ficus religiosa* L**

Arulmigu Sri Perumal Kovil
***Ocimum tenuiflorum* Linn**

Arulmigu Sri Kaliamman Thirukovil
Aza*dirachta indica* A Juss

Sri Utchi Vinayagar Kovil
***Ficus religiosa* Linn**

Plate 13

Arulmigu Sri Peraathu Selvi Ambaal Thirukkovil
***Ficus religiosa* Linn**

Sri Athisakthi Vinayagar Thirukkovil
***Ficus religiosa* Linn**

Sri Santhana Mariamman Thirukkovil
***Tamarindus indica* Linn**

Sri Kanni Vinayagar Kovil
***Ficus religiosa* Linn**

Plate 14

Sri Amirtha Vinayagar alayam
***Ficus religiosa* Linn**

Sri Pon Vinayagar
***Ficus religiosa* Linn**

Sri Vinayagar Alayam
***Ficus religiosa* Linn**

Sri Bala Vinayagar Alayam
***Ficus religiosa* Linn**
***Azadirachta indica* A Juss**

Plate 15

Sri Selva vinayagar Thirukovil
***Ficus religiosa* Linn**

Vaikundapathi Perumal Kovil
***Ocimum tenuiflorum* Linn**

Arulmigu Sri Nagakanni Amman Thirukkovil
***Calophyllum inophyllum* Linn**

Punnai Sri Srinivasa Perumal Kovil
***Borassus flabellifer* Linn**

Plate 16

Arulmigu Suyambulinga Swamy Thirukkovil
***Ipomaea pes-caprae* Sweet**

Sri Vinayagar Thirukkovil
***Ficus religios*a Linn**
***Azadirachta indica* A Juss**

Arulmigu Sivakkozhuntheeswarar Thirukkovil
***Aegle marmelos* Corr**

Thiruchendur Arulmigu Subramaniya Swamy Temple
***Ficus religiosa* Linn**

Plate 17

Sri Varasithi Vinayagar Thirukkovil
***Azadirachta indica* A Juss**

Sri Sundara Pandiya Vinayagar Kovil
***Ficus religiosa* Linn**

Shankara Rameshwarar Udanurai Pagampriya Kovil
***Aegle marmelos* Corr**

Arultharum Mutharamman Thirukkovil
***Ficus religuosa* Linn**

Plate 18

Arulmigu Vembadi Esakkiamman Thirukkovil
***Azadirachta indica* A Juss**

Arulmigu Sri Sakthi Sundara Vinayagar Thirukkovil
***Ficus religiosa* Linn**

Sri Ganapathy Alayam
***Ficus religiosa* Linn**

Sri samayapuram Kaliamman Alayam
***Azadirachta indica* A Juss**

Plate 19

Arulmigu Sri Pathirakaliamman Thirukovil
***Azadirachta indica* A Juss**

RESULTS AND DISCUSSION

RESULT AND DISCUSSIONS

Sthalavriksha worship in temples is a famous religious practice in India. Sthalavrikshas worshipped in plants are a means of conservation of plants. Plants in the temple gardens are cultivated and maintained and this is also a means of conservation of plants. This investigation is the first attempt to survey the Sthalavrikshas of 92 temples in Thoothukudi District, Tamilnadu, India.

Table 1 explains that, out of the surveyed 92 temples, there were only 73 temples in which Sthalavriksha were present (Plate 1-19). The temples found among them 36 were Vinayagar temples, 24 were Amman temples, 7 were Lord Siva temples, 4 were Perumal temples, 1 were Lord Murugan temple and 1 were Muniyasamy temple. Binomial name of Sthalaviriksham, their local name and family were also noted in Table 1. Total of 9 plant species belongs to 8 genera and 8 families were recorded (Table 2). Figure 2 indicates the percentage of distribution of Sthalavirukshas. Most of these plants belong to Dicotyledons of angiosperms and one species belongs to Monocotyledons (*Borassus flabellifer*, L). The frequently occurring species was *Ficus religiosa* recorded in 42 temples followed by *Azadirachta indica* in 20 temples.

The Sthalavrikshas also well noted for their biocultural aspects. From the survey, it was noted that the devotees tie bangles, cradles, paper slips containing their demand and mantras and even clothes on these Sthalavrikshas for good health, to possess child, for getting married and to achieve desired boon. In some temple the Sthalavrikshas are worshipped as God by devotees by offering flowers,

Table 1: Sthalavriksham of various Temples in Thoothukudi District

S.No	Temple name	Sthalaviriksham	Local name	Family
1	Arulmigu Sri Sakthi Sundara Vinayagar Thirukkovil	*Ficus religiosa* Linn	Arasu	Moraceae
2	Arulmigu Vembadi Esakkiamman Thirukkovil	*Azadirachta indica* A Juss	Vembu	Meliaceae
3	Arultharum Mutharamman Thirukkovil	*Ficus religiosa* Linn	Arasu	Moraceae
4	Shankara Rameshwarar Udanurai Pagampriya Kovil	*Aegle marmelos* Corr	Vilvam	Rutaceae
5	Sri Sundara Pandiya Vinayagar Kovil	*Ficus religiosa* Linn	Arasu	Moraceae
6	Sri samayapuram Kaliamman Alayam	*Azadirachta indica* A Juss	Vembu	Meliaceae
7	Sri Varasithi Vinayagar Thirukkovil	*Ficus religiosa* Linn	Arasu	Moraceae
8	Thiruchendur Arulmigu Subramaniya Swamy Temple	*Ficus religiosa* Linn	Arasu	Moraceae
9	Arulmigu Sivakkozhuntheeswarar Thirukkovil	*Aegle marmelos* Corr	Vilvam	Rutaceae
10	Sri Vinayagar Thirukkovil, Uvari	*Ficus religiosa* Linn *Azadirachta indica* A Juss	Arasu Vembu	Moraceae Meliaceae
11	Arulmigu Suyambulinga Swamy Thirukkovil	*Ipomaea pes-caprae* Sweet	Kadamba m	Convolvulaceae
12	Punnai Sri Srinivasa Perumal Kovil	*Borassus flabellifer* Linn	Palm	Arecaceae

13	Arulmigu Sri Nagakanni Amman Thirukkovil	*Calophyllum inophyllum L*inn	Punnai	Calophyllacea e
14	Vaikundapathi Perumal Kovil	*Ocimum tenuiflorum* Linn	Tulsi	Lamiaceae
15	Arulmigu Sri selva vinayagar thirukkovil	*Ficus religiosa* Linn	Arasu	Moraceae
16	Sri Bala Vinayagar Alayam	*Ficus religiosa* Linn *Azadirachta indica* A Juss	Arasu Vembu	Moraceae Meliaceae
17	Sri Vinayagar Alayam	*Ficus religiosa* Linn	Arasu	Moraceae
18	Sri Pon Vinayagar	*Ficus religiosa* Linn	Arasu	Moraceae
19	Sri Amirtha Vinayagar alayam	*Ficus religiosa* Linn	Arasu	Moraceae
20	Sri Kanni Vinayagar Kovil	*Ficus religiosa* Linn	Arasu	Moraceae
21	Sri Santhana Mariamman Thirukkovil	*Tamarindus indica* Linn	Puli	Fabaceae
22	Sri Athisakthi Vinayagar Thirukkovil	*Ficus religiosa* Linn	Arasu	Moraceae
23	Arulmigu Sri Peraathu Selvi Ambaal Thirukkovil	*Ficus religiosa* Linn	Arasu	Moraceae
24	Sri Utchi Vinayagar Kovil	*Ficus religiosa* Linn *Azadirachta indica* A Juss	Arasu Vembu	Moraceae Meliaceae
25	Arulmigu Sri Kaliamman Thirukkovil	*Azadirachta indica* A Juss	Vembu	Meliaceae
26	Arulmigu Sri Perumal Kovil	*Ocimum tenuiflorum* Linn	Tulsi	Lamiaceae
27	Sri Sundara Pandiya Vinayagar	*Ficus religiosa* Linn	Arasu	Moraceae
28	Arulmigu Sri Karpaga Vinayagar Alayam	*Aegle marmelos* Corr	Vilvam	Rutaceae

29	Sri Santhana Mariamman Kovil	*Azadirachta indica* A Juss	Vembu	Meliaceae
30	Arulmigu Sri Pathirakali Amman Thirukovil	*Azadirachta indica* A Juss	Vembu	Meliaceae
31	Arulmigu Sri Pathirakali Amman Thirukovil	*Azadirachta indica* A Juss	Vembu	Meliaceae
32	Sri Sithi Vinayar Alayam	*Ficus religiosa* Linn	Arasu	Moraceae
33	Arulmigu Sri pathirakaliamman Kovil	*Ficus benghalensis* Linn	Aala	Moraceae
34	Om Sakthi Amman Kovil	*Azadirachta indica* A Juss	Vembu	Meliaceae
35	Sri Karpaga Vinayagar Alayam	*Ficus religiosa* Linn	Arasu	Moraceae
36	Arultharum Annai Sri Santhanamariamman Kovil	*Azadirachta indica* A Juss	Vembu	Meliaceae
37	Arulmigu Sri Pathirakaliamman Thirukovil	*Azadirachta indica* A Juss	Vembu	Meliaceae
38	Sri Sithi Vinayagar	*Ficus religiosa* Linn	Arasu	Moraceae
39	Sri Bala Vinayagar	*Ficus religiosa* Linn	Arasu	Moraceae
40	Arulmigu Sri Petchi Ambaal Thirukovil	*Ficus religiosa* Linn	Arasu	Moraceae
41	Arulmigu Sri Muktheeswarar Thirukovil	*Ficus religiosa* Linn	Arasu	Moraceae
42	Sri Sri Rathakrishna Perumal Alayam	*Ocimum tenuiflorum* Linn	Tulsi	Lamiaceae
43	Arulmigu Sri Kaliamman Thirukovil	*Azadirachta indica* A Juss	Vembu	Meliaceae
44	Arulmigu Sri Mangalajala	*Ficus religiosa* Linn	Arasu	Moraceae

	Ganapathy Alayam	*Azadirachta indica* A Juss	Vembu	Meliaceae
45	Sri Selva Vinayagar Kovil	*Ficus religiosa* Linn	Arasu	Moraceae
46	Sri Selva Vinayagar Alayam	*Ficus religiosa* Linn	Arasu	Moraceae
47	Sri Durga Alayam	*Azadirachta indica* A Juss	Vembu	Meliaceae
48	Arulmigu Sivanaintha perumal Thirukovil	*Ficus religiosa* Linn	Arasu	Moraceae
49	Sri Santhivinayagar Thirukovil	*Ficus religiosa* Linn	Arasu	Moraceae
50	Sri Sithi Vinayagar Thirukovil	*Ficus religiosa* Linn	Arasu	Moraceae
51	Arulmigu Sri Selva Vinayagar Alayam	*Ficus religiosa* Linn	Arasu	Moraceae
52	Kankanda Theivam Sri Pathirakali Amman	*Ficus benghalensis* Linn	Aala	Moraceae
53	Arulmigu sri Kailasanathar Thirukovil	*Ficus religiosa* Linn	Arasu	Moraceae
54	Sri Sakthi Vinayagar Thirukovil	*Ficus religiosa* Linn	Arasu	Moraceae
55	Sri Vinai Theerkum Vinayagar Alayam	*Ficus religiosa* Linn	Arasu	Moraceae
56	Vinayagar Alayam	*Ficus religiosa* Linn	Arasu	Moraceae
57	Sri Sundara Vinayagar	*Ficus religiosa* Linn	Arasu	Moraceae
58	Sri Esakkiamman Kovil	*Azadirachta indica* A Juss	Vembu	Meliaceae
59	Sri Gnana Ganapathy Thirukkovil	*Ficus religiosa* Linn	Arasu	Moraceae

60	Sri Anantha Leengeshwarar kovil	*Azadirachta indica* A Juss *Ficus religiosa* Linn	Vembu Arasu	Meliaceae Moraceae
61	Sri Mela Kali Amman Kovil	*Azadirachta indica* A Juss	Vembu	Meliaceae
62	Sri Varatha Sithi Vinayagar Alayam	*Ficus religiosa* Linn	Arasu	Moraceae
63	Arulmigu Sri Bharatha Sithi Vinayagar Alayam	*Ficus religiosa* Linn	Arasu	Moraceae
64	Sri Pathrakaliamman Kovil	*Azadirachta indica* A Juss	Vembu	Meliaceae
65	Sri Mariamman Kovil	*Azadirachta indica* A Juss	Vembu	Meliaceae
66	Sri Sakthi Vinayagar Alayam	*Ficus religiosa* Linn	Arasu	Moraceae
67	Arulmigu Sri Santhanamariamman Kovil	*Azadirachta indica* A Juss	Vembu	Meliaceae
68	Arulmigu Sri Muniyasamy Thirukkovil	*Ficus religiosa* Linn	Arasu	Moraceae
69	Karpaga Vinayagar	*Ficus religiosa* Linn	Arasu	Moraceae
70	Arulmigu Sri Pathirakaliamman Thirukovil	*Azadirachta indica* A Juss	Vembu	Meliaceae
71	Arulmigu Sri Ulaganda Eeswari Amman Kovil	*Azadirachta indica* A Juss	Vembu	Meliaceae
72	Maragatha Vinayagar	*Ficus religiosa* Linn Azadirachta indica A Juss	Arasu Vembu	Moraceae Meliaceae
73	Sri Ganapathy Alayam	*Ficus religiosa* Linn	Arasu	Moraceae

Table 2: Distribution of Sthalavrikshas in different classes of Angiosperms

S.No	Class	Families		Genera		Species	
		No	%	No	%	No	%
1	Dicotyledons	8	88.89	7	87.5	8	88.89
2	Monocotyledons	1	11.11	1	12.5	1	11.11

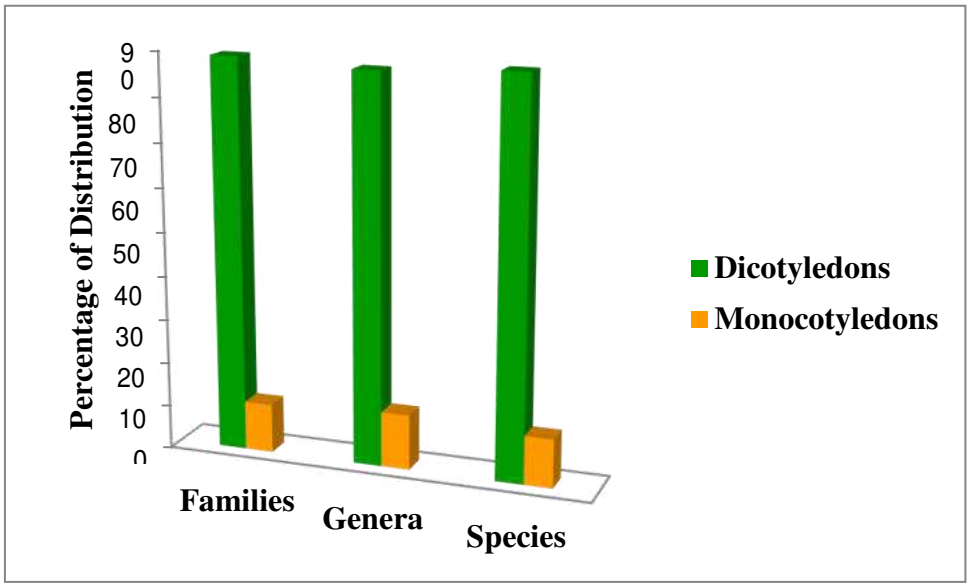


Figure 2: Percentage of Distribution of Sthalavrikshas in different classes of Angiosperms

lighting of camphors and place turmeric and kumkum over the trunk. Similarly, women often collect the withered plant materials of Sthalavriksha based on local beliefs, for instance to tie the plant parts at their doorsteps to ward off evil spirits (Gunasekaran *et al*. 2012). Those days our ancestors advised married women who wished to have a child to go around the peepal tree and carry out their prayers. Scientific researchers have revealed that peepal is the only tree that produces oxygen in abundant quantity day and night. Those days' peepal trees were planted along with neem tree. The magnetic field created by both the trees along with pure oxygen influenced the body organs and stimulated FSH/LH hormones which enhance the reproductive process (Prabakaran and Lakshmi, 2014). All these became evidence for the people's knowledge on importance of plants in purifying the temple's atmosphere (Prabakaran *et al. 2017*).

Conservation Status of the Plants

The plant species *Borassus flabellifer* comes under endangered category whereas *Aegle marmelos* comes under the near threatened category, all other species except *Ocimum tenuiflorum* are of least concerned status (Table 3)

Generic Diversity:

Table 4 shows generic diversity of 8 families studied. Dicot families such as Moraceae Lamiaceae, Rutaceae, Meliaceae, Convolvulaceae, Calophyllaceae, Fabaceae represents one genus. Among the 8 families only one belongs to monocotyledon (Arecaceae) that has one genus.

Table 3: IUCN category of Sthalavrikshas

S.No	Sthalavriksham	IUCN Category
1	*Aegle marmelos*	Near threatened
2	*Azadirachta indica*	Least concern
3	*Borassus flabellifer*	Endangered
4	*Calophyllum inophyllum*	Least Concern
5	*Ficus benghalensis*	Least concern
6	*Ficus religiosa*	Common
7	*Ipomaea pes-caprae*	Least concern
8	*Ocimum tenuiflorum*	Not Assessed
9	*Tamarindus indica*	Least concern

Table 4: Generic diversity in different families of dicotyledons and monocotyledons

Dicotyledons	Monocotyledons	No. of genera
Moraceae		1
Lamiaceae		1
Rutaceae		1
Meliaceae		1
Convolvulaceae		1
Calophyllaceae		1
Fabaceae		1
	Arecaceae	1

Species Diversity:

Species diversity of this study is recorded in Table 5. 9 species belonging to 8 families have been identified and studied. Moraceae has two species, other families of dicot such as Lamiaceae, Rutaceae, Meliaceae, Convolvulaceae, Calophyllaceae, Fabaceae represents one species. Among the 8 families only one belongs to monocotyledon (Arecaceae) has only one species.

Diversity of Habitat Form:

Table 6 depicts different habit of species in different families. Most of the habit is represented by tree. Among 9 species, tree contribute (77.78%), Herb (11.11%), Climber (11.11%). There are no shrubs in both monocotyledons and dicotyledons. (Figure-3)

Medicinal Taxa:

Medicinal taxa among Sthalavrikshas, their useful parts, medicinal properties and their medicinal uses are given in the Table 7. All the surveyed plants have the medicinal values. *Aegle marmelos* is the most utilized Sthalavriksha followed by *Azadirachta indica*, *Ocimum tenuiflorum*. The sacred plants cure various diseases, taken from various forms, such as juice, decoction, powder, paste, used to cure for various diseases and ailments like Diarrhea, fever, cough, cold, etc.

The devotees and local traditional medical practitioners use several Sthalavriksha plants for treating various ailments. Normally, the priests or the Vaidyas prescribe medicines with devotion and devotees consume the medicines

Table 5: Species diversity in different families of dicotyledons and monocotyledons

Dicotyledons	Monocotyledons	No. of species
Moraceae		2
Lamiaceae		1
Rutaceae		1
Meliaceae		1
Convolvulaceae		1
Calophyllaceae		1
Fabaceae		1
	Arecaceae	1

Table 6: Habit forms of Sthalavrikshas

S.No	Botanical Name	Family	Habit
1	*Aegle marmelos*	Rutaceae	Tree
2	*Azadirachta indica*	Meliaceae	Tree
3	*Borassus flabellifer*	Arecaceae	Tree
4	*Calophyllum inophyllum*	Calophyllaceae	Tree
5	*Ficus benghalensis*	Moraceae	Tree
6	*Ficus religiosa* Linn	Moraceae	Tree
7	*Ipomaea pes-caprae*	Convolvulaceae	Climber
8	*Ocimum tenuiflorum*	Lamiaceae	Herb
9	*Tamarindus indica*	Fabaceae	Tree

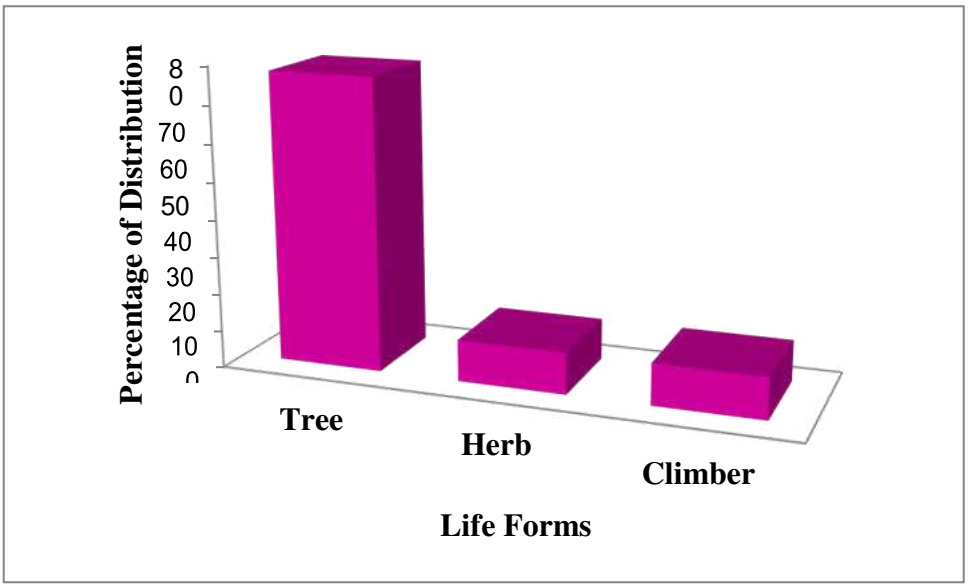


Figure 3: Distribution of taxa in different habit forms

Table 7: Sthalavrikshas of temples and their medicinal significance

Botanical name	Useful parts	Medicinal properties	Medicinal uses
Aegle marmelos	Fruit, root, bark, seeds, leaves, flowers	Antibilious, antiparasitical, antipyretic, aphrodisiac, aromatic, alternative, astringent, digestive stimulant, febrifuge, hemostatic, laxative, nutritive, stomachic, stimulant, tonic. Fruits: cooling and laxative.	Diabetes, dyspepsia, chronic diarrhea, heart diseases, dysentery, against peptic ulcers and respiratory disorders, arrest secretion or blooding, cure diahorrea and dysentery, ear problems.
Azadirachta indica	Leaves, Flower, Seed Oil, Bark	Anthelmintic, antiseptic, antifungal, antidiabetic, antibacterial, antiviral, contraceptive, sedative, mosquito repellent, anti-desertification properties and good carbon dioxide sink.	Skin diseases, eczema, fever, wound, ulcer, burning sensation, tumor, worms, cough, diabetes, inflammation and rheumatoid arthritis. Leaf juice used as blood purifier. Used in cosmetics and bio-pesticide.
Tamarindus indica	Fruit, leaves, seed-kernel, stem-bark.	Laxative, Anthelminthic, antimicrobial, antiseptic, antiviral, astringent.	Seed-kernel: used as milk purgative and stimulant, used as liver tonic, gastropathy, alcoholic intoxication, dipsia, stoamachic, constipating, leaves juice useful in bleeding piles and dysuria, swellings, fever, scalding of urine, gastropathy, helminthiasis, wounds.
Ocimum tenuiflorum	leaves, stem, flower, root, seeds	Oleanolic acid, ursolic acid, rosmarinic acid, eugenol, carvacrol,	treatment of bronchitis, asthma, malaria, diarrhea, dysentery, skin

		linalool, and β-caryophyllene	diseases, arthritis, painful eye diseases, chronic fever, insect bite etc.
Ficus religiosa	Bark,Leaf,Fruit,root	Antibacterial, antidiabetic, antiulcer	Piles, Diarrhea, Cut Wounds, Ulcer, dysentery, impotency and various blood-related problems
Ficus religiosa	Bark,Leaf,Fruit,root	Antibacterial, antidiabetic, antiulcer	Piles, Diarrhea, Cut Wounds, Ulcer, dysentery, impotency and various blood-related problems
Borassus flabellifer	Leaves, fruits	Antihelminthic and diuretic.	Antipyretic effects, anti-inflammatory activity
Ipomaea pes-caprae	Leaves, roots, seeds,	alkaloids, flavonoids and sterols, pentasaccharide resin glycosides, betulinic acid, α-amyrin acetate, β-amyrin acetate	treat skin affections, ulcers, boils, swellings, stings and wounds, stomach pain, fever.
Calophyllum inophyllum	Bark, leaves, flower, seeds, roots	anticancer, anti-HIV, antiviral, antitumor, anti-inflammatory, antimicrobial, antineoplastic, antiplatelet, antipsychotics, antioxidant, antiaging	The bark is used to treat orchitis, The resin is used to treat wounds and insect bites, flowers are used as a heart tonic, leaf infusion is used to treat sore eyes, dysentery, root decoction is used to treat ulcers, boils and ophthalmia
Ficus benghalensis	Bark, root-fibers, leaves, seeds, milky juice (i.e. latex).	Astringent, aphrodisiac, antiinflammatory	ulcers, erysipelas, vomiting, vaginal complains, fever, inflammations, leprosy, treatment of biliousness, dysentery, inflammation of liver

with great belief. Most of the plants are said to contain medicinal properties (Anon, 1988-89). Plants in the temple gardens are cultivated and maintained and this is also a means of conservation of plants. The role of people in the conservation of plant has been an age-old practice since historic period (Prabakaran *et al.,* 2017).

Edible Taxa:

Table 8 shows edible taxa among Sthalavrikshas and their edible parts. Among 9 taxa, 7 species are edible. Of the seven species, fruits of six species namely *Aegle marmelos, Borassus flabellifer, Calophyllum inophyllum, Ficus benghalensis, Ficus religiosa* and *Tamarindus indica* are edible and they are has high nutrition values. In 2 species include *Ipomaea pes-caprae* and *Tamarindus indica* leaves are edible.

Thus, the above results and discussion proved the relation of the human and the nature towards plant conservation. The traditional worshipping has protected many plants which have tremendous medicinal value and made them as sacred, so that with the fear of deity nobody eradicates it. So, we have to protect these sacred plants for us and our next generation for better survival. On the basis of this study, we have to follow our ancestor's belief in humanity and nature sustainability.

Table 8: Edible taxa among surveyed Sthalavrikshas

S.No	Botanical Name	Family	Edible parts
1	*Aegle marmelos*, Corr.	Rutaceae	Fruit
2	*Borassus flabellifer.* L	Arecaceae	Fruit
3	*Calophyllum inophyllum*, Linn	Calophyllaceae	Fruit
4	*Ficus benghalensis, L*	Moraceae	Fruit
5	*Ficus religiosa*, Linn	Moraceae	Fruit
6	*Ipomaea pes-caprae*, Sweet	Convolvulaceae	Leaves
7	*Tamarindus indica*, L.	Fabaceae	Fruit, leaves

SUMMARY AND CONCLUSION

SUMMARY AND CONCLUSION

Thoothukudi District, unitary of the ancient districts of Tamil Nadu famous for its religious culture, was investigated for the Sthalavrikshas in temples by taking frequent visits for four months. This study is the first survey of Sthalavrikshas of various temple and their medicinal uses in Thoothukudi District, Tamil Nadu. We recorded 73 temples of Sthalavrikshas out of 92 temples. We recorded 9 plant species belonging to 8 families. All the plants have the medicinal values. Medicines are obtained from the Sthalavrikshas and are used in different forms. Sthalavrikshas are valued for their botanical, medicinal, environmental, religious and mythical importance. According to IUCN red data list (2014), the plant species *Borassus flabellifer* comes under endangered category while *Aegle marmelos* comes under the near threatened category. So necessary measures have to be taken to preserve these plants.

Propagation of sthalavrikshas in temples contributes to the conservation of our floral diversity. Some trees are significant for their economic use of shipbuilding or in the timber industry, some for providing homes for various animals, birds, and others for their medicinal value. In the present study, it is concluded that the religious activities are having a close relationship with plants boost up the mental health of local people of Thoothukudi District and many of the sacred plants found in the household and temples were used for various religious cultural activities as well as for health care. These sacred plants are worshiped by the local people for getting the blessing of health and wealth by positive powers of nature. Hence the religious ceremonies, rites act as a protective

factor or device for the conservation of sacred plants. So, it is the duty of the present generation to preserve and promote these aesthetic treasures to conserve biodiversity and nature, which will surely play a part in the progression of human beings. These sacred trees preserved through millennia by our ancestors as potential bio resources should be respected and conserved for the future generation.

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A STUDY OF THE NACL STRESS ON THE GROWTH PARAMETERS AND ANATOMY OF SELECTED ECONOMICALLY IMPORTANT LEGUMES

A dissertation submitted to

ST.MARY'S COLLEGE (Autonomous), Thoothukudi

Affiliated to

MANONMANIAM SUNDARANAR UNIVERSITY, Thirunelveli

in partial fulfilment of the requirements for the degree of

MASTER OF SCIENCE IN BOTANY

By

JEEVA SATHYA PITHA D

Reg. No. 19APBO07

DEPARTMENT OF BOTANY

ST. MARY'S COLLEGE (Autonomous)

THOOTHUKUDI -628001

2020 - 2021

CERTIFICATE

It is certified that this short term project work entitled **"A STUDY OF THE NACL STRESS ON THE GROWTH PARAMETERS AND ANATOMY OF SELECTED ECONOMICALLY IMPORTANT LEGUMES"** submitted to **St. Mary's College (Autonomous)** affiliated to **Manonmaniam Sundaranar University** in partial fulfillment of the requirements for the degree of **Master of Science in Botany** and is a record of work done in the Department of Botany, St. Mary's College (Autonomous), Thoothukudi during the year 2020 – 2021 by **Jeeva Sathya Pitha D**, Reg. No. 19APBO07.

S. P. 15/4/2021

GUIDE
(S. PAULINE JENIFER)

M. Glory

HEAD OF THE DEPARTMENT

HOD of Botany
St. Mary's College (Autonomous)
Thoothukudi- 628 001

F. Day Wbo

EXAMINER

Lucia Rose

PRINCIPAL
St. Mary's College (Autonomous)
Thoothukudi - 628 001.

DECLARATION

I do hereby declare that this dissertation entitled **"A STUDY OF THE NACL STRESS ON THE GROWTH PARAMETERS AND ANATOMY OF SELECTED ECONOMICALLY IMPORTANT LEGUMES"** submitted by me in partial fulfillment for the award of the degree of **Master of Science in Botany**, is the result of my original and independent work carried out under the guidance of **Ms. S. Pauline Jenifer M.Sc., B.Ed., SET,** Assistant Professor of Botany, St. Mary's College (Autonomous), Thoothukudi and it has not been submitted elsewhere for the award of any other degree.

Station: **JEEVA SATHYA PITHA D**

Date:

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JEEVA SATHYA PITHA D

CONTENT

Introduction

INTRODUCTION

The environmental stress is a major area of scientific concern because it constraints plant as well as crop productivity. This situation has been further worsened by anthropogenic activities. Therefore, there is a much scientific saddle on researchers to enhance crop productivity under environmental stress in order to cope with the increasing food demands. The abiotic stresses such as salinity, drought, cold, and heat negatively influence the survival, biomass production and yield of staple food crops. According to an estimate of FAO, over 6% of the world's land is affected by salinity. Thus, salinity stress appears to be a major constraint to plant and crop productivity. Here, we review our understanding of salinity impact on various aspects of plant metabolism and its tolerance strategies in plants **(Parihar *et al.,* 2014).**

The extent of agricultural land that is affected by high salinity is increasing worldwide, due to both natural phenomena and agricultural practices such as irrigation systems **(Munns and Tester 2008).** Salinity poses two major threats to plant growth: osmotic stress and ionic stress **(Flower and Colmer 2008).**

Salt in plants includes any factors that could lead to the death of the plants. Salt stress as an over powering pressure of some adverse forces that prevent or decrease the normal system of functioning. It is an unstable phenomenon and may occur in any developmental stages of plant life. One of the cornerstones of civilization is sustainable agriculture to provide food for the population. Although, we achieved this centuries ago, there are still problems that need to be resolved. One problem is that as we use irrigation to water our crops we deposit salts in the soil that are carried by irrigation water but not absorbed by the plant in higher concentration **(Abdul Qados, 2011).**

Salt stress is a condition where excessive salts in soil solution cause inhibition of plant growth or plant death. On the world scale, no toxic substance restricts plant growth more than saline stress. Salt stress presents an increasing threat to plant agriculture **(Ahmad and Jhon, 2005).** Salt stress has been a serious threat for crop production in irrigated land, as expected salt stressed irrigated land is more than 20% **(Pitman and Lauchli, 2002),** and estimated stressed area will expand to 50% of irrigated land by 2050 **(Wang *et al.*, 2011).** Plant physiological physiognomies are extensively susceptible to the highly saline rhizosphere. High salt level affects seed germination, plant life, and cropproductivity. Among the monocot crops, rice is salt sensitive, and its productivity is severely affected by the accumulation of soluble salts in soils **(Ashraf, 2009).**

. The physiological responses of a plant to salinity are often complex and multi-faceted, which makes experiments difficult to design and interpret. Current plant physiology has advanced, given the development of so-called 'omics-driven' research. Physiological measurements have been revolutionized by new technologies, such as high-throughput phenotyping, bioinformatics and novel analytical methods that have enabled fields such as metabolomics to emerge. At a basic level, the response of plants to salinity can be described in two main phases: the shoot ion- independent response occurs first, within minutes to days, and is thought to be related to NaCl sensing and signalling **(Gilroy *et al.,* 2014).**

Plant responses to salinity have been divided into two main phases. An ion-independent growth reduction, which takes place within minutes to days, causes stomatal closure and inhibition of cell expansion mainly in the shoot **(Rajendran *et al.,* 2009).** A second phase takes place over days or even weeks and

pertains to the build-up of cytotoxic ion levels, which slows down metabolic processes, causes premature senescence, and ultimately cell death **(Roy *et al.,* 2014).**

Soil salinity has increased due to poor irrigation practices, the improper application of fertilizers, and industrial pollution **(Ouhibi *et al.,* 2014).** High salinity is commonly due to high concentrations of Na and Cl in the soil solution, resulting in hyperosmotic and hyperionic conditions, which impede plant absorption of water and nutrients from the soil **(Ismail *et al.,* 2014).** Most crops are glycophytes, in which growth and productivity are affected by salt stress **(Slama *et al.*, 2015).** Due to their sessile nature, plants have had to develop suitable mechanisms to adapt to highsalinity environments during their long evolutionary history. Salt stress induces ionic stress, osmotic stress, and secondary stresses, especially oxidative stress, in plants **(Yang and Guo, 2018).** Under salt stress, plants have to adjust their physiological and biochemical processes, involved in regulating ion and osmotic homeostasis, as well as stress damage control and repair (detoxification) **(Zhu, 2002).** Adaptive responses to salt stress can be grouped into three processes: osmotic stress, ionic stress, and detoxification responses.

During germination under saline conditions, high osmotic pressure of saline water is created due to capillary rise leading to more salts density at seed depth than at lower soil profile, which reduces time and rate of germination **(Mudgal e*t al.,* 2010).** In mungbean seedlings, high salt concentration causes increased H_2O_2 content in both roots and leaves, hence salts should be removed to ensure proper growth and development **(Saha *et al.,* 2010).** Both root and shoot lengths were reduced with increased NaCl concentration, but roots were more damaged, with an increase in number of lateral roots and increase in its thickness, compared to shoots **(Misra *et al.*, 1996).** Photosynthetic activity of mungbean is reduced due to reduced

function of electron transport and instability of pigment protein complex **(Promila and Kumar, 2000).** High salinity results in a decrease in total leaf area and stomatal opening. Proline and glycinebetaine levels in roots and shoots increased in mungbean (tolerant) cultivar 'T 44' subjected to NaCl stress at seedling stage. Increase was seen with a supply of 5 mM $CaCl_2$ to 200 mM NaCl. Calcium ions play a key role in osmoprotection and effects of Na^+ and Ca^{2+} are thus harmonizing the accretion of osmolytes **(Hu and Schmidhalter, 2005).**

Increased proline levels occurred when proline oxidase activity was low and high production of P-5-CR and y-glutamyl kinase in both roots and shoots. Thus, calcium facilitated osmolytes synthesis in NaCl-stressed mungbean seedlings. When three species of *Vigna (V. radiata, V. mungo*, and *V. unguiculata)* subjected to varied doses of NaCl (50, 75, 100, 125, and 150 mM), reduction in chlorophyll content, sugar, starchand peroxidase enzyme activity were observed in shoots and roots **(Arulbalachandran *et al.,* 2009).** Germination %, seedling growth rate, RWC and photosynthesis decreased with increasing NaCl levels in all species. The growth decrease was higher in mungbean than in black gram and cowpea. However, increase of compatible solutes was higher in cowpea than in black gram and mungbean, suggesting cowpea is more salt-tolerant than other two.

The effect of pre-soaking seed in 50, 100, and 1000 µM SA on growth parameters of two mungbean genotypes (NM 19-19 and NM 20-21) under salinity stress (50 and 100 mM NaCl) was studied **(Shakeel and Mansoor, 2012)** and found a reduced seedling length and fresh/dry weight of both genotypes. Pre-soaking treatments (100 µM) with SA reduced salinity-induced decline. However, pre- treatment with a high concentration (1000 µM) prior to salt treatment caused a significant reduction in mean seedling length. NM 19-19 respond better under salt

stress than NM 20-21. In a pot experiment (from Bangladesh), effect of salinity levels (e.g., 0, 0.1, 0.2, 0.3, and 0.4% of NaCl) on germination, growth and nodulation of mungbean varieties (BARI Mung 4, BARI Mung 5 and BARI Mung 6) was observed. Salinity affected germination and root elongation. Root growth was significantly reduced with higher salt and BARI Mung 4 showed better performance than other varieties. All showed similar performance in yield traits at higher NaCl levels. No effect on nodulation at a higher (0.4% NaCl) dose was seen in BARI Mung 5. However **(Naher and Alam, 2010),** reported nodules per plant decreased but not nodule size with increase in salinity.

The genus *Vigna* includes many wild and cultivated species with pantropical distribution. Among the pulse crops (*i.e.* annual leguminous food crops) *Vigna* is far the most important. For mankind *Vigna* species are important sources of high quality proteins and amino acids and like many other leguminous plants; they play a key role in crop rotation due to their ability to fix nitrogen. To support the awareness on this matter; the United Nations declared 2016 the International year of pulses. In India, horse gram has a wide geographic distribution extending over a range of environmental conditions. However, as other crops in India, horse gram is also subjected to environmental stresses, particularly salinity. Although much information is available on the agronomics aspects of horse gram, very little isknown about the effects of salinity on physiological and biochemical aspects of horse gram.

Scope and Objectives

SCOPE AND OBJECTIVES

Salinity stress is one of the main factors limiting legume productivity in many parts of the world. In this study, an attempt was made to examine the effect of salinization on three species of *Macrotyloma uniflorum* (Lam.) Verdc, *Vigna radiata* (L) Wilczek, and *Vigna unguiculata* (L) Walp. through glasshouse experiment. The main aim of this study was to compare salt (NaCl) stress tolerance potential of selected spp.

The objectives are

1. To grow the selected seeds in saline environment
2. To measure the growth parameters
3. To identify the anatomical changes through microscopic studies.

Review of Literature

REVIEW OF LITERATURE

Many species of higher plants, including most crops, are subjected to growth inhibition under high-sodium chloride conditions. The salt-induced inhibition of plant growth, so-called salt stress, is caused not only by osmotic effects on water uptake but also by variable effects on plant cell metabolism under salt stress. While the first component can bring about water deficit, the excess of a specific ion can cause toxicity and or induce nutritional disorders. Natural boundaries imposed by soil salinity also limit the caloric and the nutritional potential of agricultural production. These constraints are most acute in areas of the world where food distribution is problematic because of insufficient infrastructure or political instability **(Yokoi *et al.*, 2002).** Water and soil management practices have facilitated agricultural production on soils marginalized by salinity but additional gain by these approaches seems problematic. On the horizon, are crop improvement strategies that are based on the use of molecular marker techniques and biotechnology can be used in conjunction with traditional breeding efforts **(Hussain *et al*., 2010).**

Nearly one billion hectare of land is affected by salinity or sodicity out of 13 billion hectares of land on the earth. India, for instance has about 7 million hectare of cultivable land affected by salinity. In Tamil Nadu, area affected by salinity is one lakh hectares. The districts where the problem soils are most prevalent are Chengalpattu, Salem, Tanjavur, Trichy, North Arcot, Thirunelveli, Dharmapuri and Ramanathapuram **(Vadivel *et al.*, 2001).**

The morphological and physiological response of three different seeds *Macrotyloma uniflorum, Vigna radiate,* and *Vigna unguiculata* was studied. The effect of different salinity concentration (50, 100, 150 mM) on carbohydrate

content, plant growth, leaf area, and number of leaves. Salinitys tress significantly reduce the plant growth, leaf water potential and relative water content in all seeds. Whereas, the content of carbohydrates, water uptake capacity was increased **(Divya and Viswanatha, 2017).**

The effect of salt stress on four varieties of seeds *Macrotyloma uniflorum, ,Vigna radiata,* and *Vigna unguiculata* was investigated. Plants were cultured in hydroponic condition with Nacl treatments. The Nacl content in all varieties significantly increased in shoot **(Marjan and Nasser, 2014).**

To determinethe effect of salt stress on seedling growth of *Macrotyloma uniflorum, Vigna radiata,* and *Vigna unguiculata.*The salt stress was induced by putting the seeds of this crop in different solution of NaCl 50, 100, 150 mM and demonized sterile water was used like control. The salinity stress seedlings growth at salt concentration above 150 mM showed greater variation **(Emlynmena *et al.*, 2016).**

The effect of salinity on growth, modulation, acetylene reduction activity (ARA), nodule leghemoglobin content from varieties of seeds were studied. The depressed effect of saline stress (50,100,150 mM NaCl) on dry weight ARA of nodules was directly related to the salt induced decline in dry weight and N content in shoots. Salt treatment of plants decreased respiratory capacity only in pea plant. Under serve stress the reduction in leghemoglobin content may be involved in Salt induced inhibition of nitrogen fixation **(Delgado *et al*., 2002).**

To determine the physiological response to salt (NaCl) stress in selected seeds, the seed were germinated and grown for 15 days prior to salt treatment (daily 50,100,150 mM NaCl) for 21 days compared with the control, the NaCl treatment

significantly reduced plant height, leaf area, fresh weight, viability of plants and dry weight. Increase in leaf N, Z and Mn concentration were also observed in the NaCl treat plants. Reduction in plant height is a simple, easy, sensitive, non destruction measurements to evaluate salt tolerance in plants **(Sarah *et al*., 2010)**

The effect of NaCl concentration (50, 100, 150 mM) on growth, carbohydrate content, leghemoglobin content of seedlings was investigated. NaCl caused an increase in plant height with low and medium concentrations and a decrease with the highest concentration, in both measurement periods. No significant effect was observed in the number of leaves or leaf area with low concentration while a decrease was noticed for each, with two higher concentrations and in both measurement periods. Salinity increased both fresh and dry weights of the shoot in the two measurement periods **(Amira and Abdul, 2011).**

The effect of salt stress on growth and nitrogen fixation by *Macrotyloma uniflorum, vingna radiata*and *Vigna unguiculata* N_2 fixation activity and nodule respiration are inhibited sharply on exposure of plants to saline conditions. The decrease in nitrogen fixation has been describedto direct effect on nitrogenase activity or an indirect effect through decrease in leghemoglobin content, respiratory rate, malate concentration in nodules and photosynthate availability. Salinity increase oxygen diffusion resistance in the nodules and alter their ultrastructure. Decrease in nitrogen fixation in nodules under salinity is also accompaloopnied by parallel decrease in the activity of H_2O_2 scavenging enzyme like catalase, ascorbate peroxidase and the level of antioxidants like ascorbic acid (

Swaraj and Bishnoi , 1999).

The effect of 50 mM to 150mM NaCl does on growth and nitrogen fixation parameters, as well as carbohydrates content and carbon metabolism of *Macrotyloma uniflorum, Vigna radiate* and *Vigna unguiculata* nodules. The leghemoglobin content and nitrogen fixation rate were approximately 1and 2 times higher respectively in nodules of *Macrotyloma uniflorum* when compared with other plants. Plant growth parameters and nitrogenase activities decreased with NaCl treatments in all plants **(Miguel *et al*., 2008).**

The impact of salt stress under different salinity levels (50,100,150 mM NaCl) on four varieties of plants *Macrotyloma uniflorum, Vigna radiata,Vigna unguiculata* were conducted. The different level of salinity significantly affected the growth attributes by reducing root and shoot length for salinity below 100mM. Fresh weight and dry weight of root and shoot were reduced significantly with subsequent treatment. Regarding germination maximum germination were found in variety *Vigna radiata* in all the treatments and maximum inhibition were found to be in case of *Macrotyloma uniflorum* variety at 150 mM salinity level **(Datta *et al*.,2009).**

The effect of applied NaCl on growth and nitrogen fixation of *Macrotyloma uniflorum, Vigna radiata, Vigna unguiculata*plant were investigated. The experimental soil were salinized with NaCl at the rates of 50mM 100mM and 150mM Nacl. Growth of the plants were inhibited by salinity. Applied NaCl significantly decreased dry of plants. NaCl caused to increase Na and Cl concentrations of plants **(Turan *et al*., 2009).**

The effect of salinity stress were evaluated in the leaves and roots of three plants (*Macrotyloma uniflorum, Vigna radiata, Vigna unguiculata*). In overall, salinity negatively affected the growth of four plants with more pronounced effect on

Vigna radiata. The plant cultivars, salinity increased proline content. In all plants, Na content increased in plant organs with increasing Na in the media **(Zahra *et al*., 2017).**

Plants were exposed to salinity levels of 50Mm 100mM and 150mM for the duration of 3, 9 and 12 days with the objective to test their tolerance on the basis of plant water status, N2 fixation and mineral distribution. The water potential of leaves root and nodules became more with increasing salt stress. Relative water content of leaves, root and nodules decreased significantly, while a sharp rise in proline content was observed **(Nandwal *et al*., 1999).**

Macrotyloma uniflorum, Vigna radiate and *Vigna unguiculata* are important legumes forage crops. However, it's genetic improment for salt tolerance is challenging as *Macrotyloma uniflorum, Vigna radiata, Vigna unguiculata and alfalfa* response to salt is genetically and physiologicaly complex. The knowledge of morphological physiological and nitrogen fixation of all plants to salt stress, and to discuss the potential of applying morden plant technologies to enhance *Macrotyloma uniflorum* resistant breeding including genomic selection and N_2 content **(Bhattarai *et al*., 2020).**

An Experiment was conducted in order to evaluation the salinity stress effect on growth parameters and stem anatomical changes of varieties of grown under controlled conditions. Salinity stress were induced by adding NaCl concentration of 50, 100 and 150mM. Fifteen days after sowing plants were harvested and growth parameters and anatomical changes were evaluated. The salinity stress significantly decreased shoot and root weight either fresh weight or dry weight, in addition, total plant weight, plant height and leaf number were decreased due to salinity stress.

Salinity stress significantly increased cut in mass and trichome density on epidermal cells. On the other hand, cortex thickness was decreased because of salinity stress while xylem thickness had upward increase when soybean plants were grown under salinity stress especially high level of salinity **(Dolatabadian *et al*., 2011).**

The effect of salinity on some physio and morphological parameters in plants of five different legumes and experiment were performed under controlled saline conditions. Inresponse to salt stress, the physiological responses were measured. Salinity affected all of the parameters under study. The high salt concentrations caused a great reduction in growth parameters such as fresh and dry weights of shoots and roots **(Balal *et al*., 2011).**

The effects of salinity stress on stomatal aperture and density, xylem vessels, the activities of antioxidant enzymes, such as superoxide dismutase (SOD) and peroxidase and xylem embolism (PLC values) in *Macrotyloma uniflorum , Vigna radiata, Vigna unguiculata* and *alfalfa* the. The experiment were conducted at different concentrations of salt (50, 100, 150, and 200 mM NaCl) contained in the irrigation water used for 15 days. The POD activity increased with the increase in the severity of NaCl stress. Salinity stress affected water transport, which reduced native PLC value, whereas xylem vessel area was also decreased **(Rajput *et al*., 2015).**

The effect of salt stress on some plant varieties were studied. The experiment was carried out at three salinity stress levels (50 mM,100 mM and 150mM NaCl). Salt stress, high temperature and salinity induced osmotic stress severely limited the plant growth, morphology, physiology and yield characteristics during summer. Measured parameters were less affected in *Vigna unguiculata.* The growth parameters of plant height, plant length, leaf area, and shoot length **(Nirmalasehrawat *et al*., 2015).**

Growth parameters and photosynthetic pigments changes in horse gram were investigated under salinity of different concentrations (50,100,150mM). Growth parameters such as plant height, lea area, fresh and dry weight of the whole plants decreased in under salinity stressed condition **(Kanagaraj and Sathish, 2017).**

The effect of salt stress on some plant varieties were studied. The leaf and stem anatomical feature change of plants from adaptation to salinity stress. A reduction in the anatomical traits of stem and leaf diameter, wall thickness, diameter of the hollow pith cavity, total number of vascular bundles, number of large and small vascular bundles, bundle length and width, thickness of phloem tissue, and diameter of the metaxylem vessel of plants were found **(Rania *et al.,* 2020).**

The effect of salinity stress (0, 50, 100 and 150 mM NaCl) on *Macrotyloma uniflorum, Vigna unguiculata*and *Vigna radiate* by measuring physiological and morphometric traits related to growth. The measuring physiological traits showed significant differences compared to the control at 50 and 100 mM (relative water content, net assimilation rate, specific leaf weight, and specific leaf area), even at 150mM most parameter variations were significantly different **(Amador *et al.,* 2015).**

The morphological, physiological and biochemical responses, besides investigating the possible tolerance mechanism utilized by the *Vigna unguiculata*. The seeds during germination under conditions of the severe and moderate salt stress (50,100 and 150 mM NaCl, respectively).Salinity stress were significantly decreased shoot and root weight either fresh weight or dry weight, in addition, total plant weight, plant height leaf number were decreased due to salinity stress **(Lobato *et al*., 2009).**

Macrotyloma uniflorum, Vigna unguiculata, and Vigna radiata were tested for their salt tolerance at different degrees of salinity; 0, 50, 100 and 150 mM of NaCl, in the laboratory. . In the laboratory, Na^+, K^+, K/Na ratio, plant height, roots dry weights, stems and leaves were investigated. Na^+ was significantly increased with increasing NaCl concentrations in all plant organs (**Taffouo *et al*., 2009).**

The effect of saline stress on growth and anatomy of *Macrotyloma uniflorum, Vigna unguiculata and Vigna radiata* plant were investigated. The effect of different salinity concentration, (50,100,150mM) on carbohydrate content, plant height, number of leaves in all plants. Salt stress significantly enhanced leaf and root Na^+ and root Ca^{2+} contents in plants. **(Kanwal *et al*. 2013).**

The effects of seedlings of a salt-sensitive plant, mung bean (*Vigna radiata* (L.) *Macrotyloma uniflorum* and Vigna radiata , grown on 50 mM ,100 mM and 100 mM NaCl concentrations. The growth parameters were plant height, number of leaves, leaf area, leaf and stem fresh and dry weights in which all parameters significantly decreased in salt levels. Growth of the plants were inhibited by salinity. Applied NaCl significantly decreased dry of plants. NaCl caused to increase Na and Cl concentration of plants **(Ozlem *et al*., 2017).**

To determined the effect of salt stress on seedling growth of *Macrotyloma uniflorum, Vignaradiata, Vigna mungo* and *Vigna unguiculata.* Exposure to sodium chloride stress decreased the morphological traits. Seed priming improved the dry weight and length of plumule and radicle in plants under stress. Seed priming improved the dry weight and length of plumule and radicle in plants under stress. Chlorophyll a and b decreased by increasing of sodium chloride concentrations **(Farahmandfar *et al*., 2013).**

The effect of salt stress on morphological characteristics and Na^+, K^+ and Ca^+ ion contents in*Macrotyloma uniflorum ,Vigna unguiculata* and *Vigna radiata.* The experiment was carried out at three salinity stress levels (50 mM, 100m M and 150mM NaCl). The plant height, root length, shoot dry weight, root dry weight, and number of leaves per plant was reduced with the increase of salinity level. Increasing salinity level led to an increase in the amount of Na^+ in shoot dry matter, while the amount of Ca^{+2} and K^+ and Ca^{+2}/Na^+ and K^+/Na^+ratios were decreased **(Archangi *et al*., 2012).**

A study was conducted to determine the effect of salt stress (NaCl 50, 100, and 150mM) on growth of three plants namely *Macrotyloma uniflorum, Vigna unguiculata* and *Vigna radiata).* NaCl treatment induced drastic reduction in growth characteristics ofplants through decreasing the shoot and root lengths, number of lateral roots and number of leaves, total area of leaves as well as fresh and dry weights of shoot and root of plants. Salt stress increased sodium and chlorine contents and decreased potassium, calcium and magnesium levels in root and shoot of all plants **(Abdel and Mohammed 2007).**

Salinity affects many aspects of the metabolism of plants and to induce changes in their anatomy and morphology. These changes are often considered to be adaptations which increase the chances of the plant to endure the stress imposed by salinity; alternatively, they may be considered to be signs of damage and disruption of the normal equilibrium of life processes (Poljakoff-Mayber, 1975).

The effect of increasing NaCl concentrations (50,100 and 150mM) was studied of three different Varieties (*Macrotyloma uniflorum Vigna radiata and Vigna unguiculata).* The response of the plants to salinity stress was analyses by

estimating the levels of Photosynthetic enzymes activity. Photosynthetic rate, activities of RuBP carboxylase and sucrose phosphate synthase (SPS) decreased with increasing salinity. The growth parameters were plant height, number of leaves, leaf area, leaf and stem fresh and dry weights in which all parameters significantly decreased in salt levels **(Desingh and Kanagaraj 2020).**

The effects of soil salinity on growth and mineral nutrients in plants under saline conditions. The plants were treated with different concentrations of NaCl, 50, 100 and 150mM on 6 days after sowing. Salinity affected all the morphological parameters and decreased the growth performance. The mineral contents (nitrogen, phosphorus, calcium, magnesium, potassium, iron, manganese and zinc) were analysed from treated as well as the control plants. All the treatments altered the mineral contents when compared to the untreated control plants but a significant change was found in 50 mM NaCl concentration, in which the levels of some minerals increased. Tissue sodium uptake was determined for all the treated plants and was found to have increased to a significant level when compared to the untreated plants **(Jaleel *et al*., 2008).**

An experiment was conducted in order to evaluation the salinity stress effect on growth parameters and stem anatomical changes of varieties of grown under controlled conditions. The experimental treatments were including different concentrations of salinity (50, 100 and 150 mM). The seedling dry weight, seed vigor index, radicle length, radicle fresh and dry weight, shoot length, shoot fresh and dry weight with increasing salinity stress **(Kabiri and MEHDI Naghizadeh).**

The salinity stresses were studied on the anatomical alteration, changes of enzymatic antioxidant system and lipid peroxidation in *Macrotyloma uniflorum,*

Vigna unguiculata and *Vigna radiata.* Salt stress significantly decreased growth, relative water content (RWC), protein level, catalase (CAT) and polyphenol oxidase (PPO) activities, and increased proline content, MDA content, H_2O_2 level. Anatomical observations of stem showed that the epidermal cells diameter and thickness of cortex decreased by salinity whereas thickness of hypodermal layer, diameter of hypodermal cell, pith area and pith cell diameter increased by high salinity **(Haddadi *et al*., 2016).**

The effect of salt stress on some plant varieties were studied. The experiment was carried out at three salinity stress levels (50 mM,100mM and 150mM NaCl). Various concentrations of salt had a highly significant effect upon the survival% age, plant height, number of branches, shoot fresh and dry weight, root fresh and dry weight and root moisture contents. Number of leaves also varied significantly. However, leaf length and shoot moisture contents exhibited non-significant differences. Differences among the test species for all the parameters under consideration were also highly significant (**Muhammad and Hussain, 2010).**

The experimental were studied in Morpho-anatomical appraisal of some pulse crops under salinity stress. There were a significant variation in relative values (%) of plant height, root length (%) root dry matter (%) and shoot dry matter (%) of seven selected pulse crop varieties due to the salinity stress. The highest percentage of relative plant height (92), relative root length (98), relative root dry weight (89) and relative shoot dry weight (72.8) were observed in cowpea followed by grass pea and the lowest percentage of relative plant height (51), relative root length (56), relative root dry weight (54) and relative shoot dry weight (48) were observed in lentil. The stem anatomical features were found similar changes in xylem and phloem area. Among the pulse crop varieties, cowpea and grass pea were performed better whereas

lentil and black gram were found more susceptible species than the others according to their morphological and anatomical attributes **(Mahkhan *et al*., 2019).**

The effect of salinity stress on growth *Macrotyloma uniflorum, Vigna unguiculata* and *vigna radiata.* The germination, seedling growth, biomass accumulation, seedling survivability, salinity scores, root and shoot anatomy, sodium ion (Na^+), chloride ion (Cl^-) and potassium ion (K^+) concentrations, proline and antioxidant activities were measured to evaluate the performance of all the genotypes. The genotypic variation for salinity tolerance was observed among the genotypes screened under hydroponic and saline conditions. Plant concentrations of Na^+ and Cl^- at 150mM NaCl were found significantly correlated with germination, root and shoot length, fresh and dry weight of roots and shoots, seedling survivability, salinity scores and K^+ under controlled conditions. Root and shoot anatomy of tolerant line and wild accession plant showed restricted uptake of Na^+ and Cl^- due to thick layer of their epidermis and endodermis as compared to sensitive cultigen **(Dharmendrasingh *et al*., 2017).**

Salinity effect was evaluated on the basis of biomass yield reduction, physiological attributes, and stem-root anatomical changes. The salinity stress caused significant in all measured parameters and the highest salinity showed more detrimental effect compared to control as well as lower salinity levels. The fresh and dry matter production was found to increase in salinity. Salt stress decreased the stem diameter, epidermis cell size, cortex zone thickness, vascular bundle width, cambium thickness, xylem width, trachea diameter and phloem width in the seedlings non- pretreated with the growth regulators, in comparison with the control seedlings grown in distilled water medium **(Amirul *et al*., 2015).**

Effect of salt stress tolerance in *Macrotyloma uniflorum, Vigna unguiculata,* and *Vigna radiate* were studied. Salt stress reduces the ability of plants to take up water and this quickly causes reductions in growth rate. The initial reduction in shoot growth is probably due to salt effects. If excessive amounts of salt enter into the plant, salt will eventually rise to toxic levels and reduce the photosynthetic leaf area of the plant that cannot sustain growth. In order to understand the processes that give rise to tolerance of salt and to identify the salt stress proteins in the salt stress effect of on plant growth was studied NaCl different concentrations like 50mM, 100mM, 150mM **(Rani, 2011).**

The effects of salt stress on physiological parameters associated to salinity tolerance in (*Macrotyloma uniflorum, Vigna unguiculata,* and *Vigna radiata)* plants. The depressed effect of saline stress (50, 100, 150 Mm NaCl) on dry weight plant root was directly related to the salt induced decline in dry weight and N content in shoots. The leaves ofhigher water content, higher accumulation of proline, and lower accumulation of malondialdehyde (MDA) as well as less reduction of chlorophyll in salinity stress **(Akter *et al*., 2020).**

To determine the response of plants *Macrotyloma uniflorum, Vigna unguiculata, Vinga radiata* to saline conditions. The investigated elements were the effects on gas exchange parameters, water use efficiency (WUE) as well as leaf area and the contents of total chlorophylls and measure the plant parts. At the stage of three plants salt stress were applied for 6 days, and. treatments were tested. The growth experimental soil were salinized with NaCl at the rate of 50mM, 100mM and 150mM. The saline stress the adaptations are mainly morphological (by reducing leaf area), physiological (reduction in net CO_2 assimilation rate, stomatal

conductance and transpiration, and improvement of WUE) and biochemical responses (decrease of chlorophyll content) **(Bacha *et al.*, 2017).**

The effect of salt stress on physiological response of hydroponically grown *Macrotyloma uniflorum, Vigna unguiculata, Vinga radiate* were investigated. Salt stress was applied by using Na salt treatment and it plays an important role on all parameters studied in this experiment. Plants growth rate, predawn water potential, osmotic potential and cuticle permeability were significantly lower in treated plants than in control plants. The reduction of growth rate coincided with the reduction of water potential in plant tissue due to salt stress. The water potential of leaves and osmotic potential of leaves and root became more with increasing salinity stress **(Hossain and Nonami, 2012).**

. The effect of salinity on growth, protein content and antioxidant enzymes were studied in three plants (*Macrotyloma uniflorum, Vigna unguiculata, Vinga radiata).* Seedlings were NaCl stress (50, 100, 150 mM) for 6 days and caused a reduction in germination percentage, relative germination rate (RGR), relative salt injury rate (RSIR) and relative water content (RWC). All the plants under NaCl stress exhibited reduction in total protein content **(Prasanthikumari and Vishnuvardhan, 2015).**The effect of salt stress on the morphological physiological activity and anatomy of Cow pea plant were different salt stress levels (50mM ,100mM,150mM NaCl) at germination and early seedling growth stage of plant development. Data were analyzed for growth parameters such as plant height, fresh and dry weight, leaf water content (LWC), and length of radicle and plumule during germination period, and biochemical parameters such as proline content, membrane stability index (MSI), malondealdehyde (MDA) content, chlorophyll content, and antioxidant enzyme

activity Catalase (CAT) and Peroxidase (POD). The effect of salt stress reduced plant height, fresh and dry weight, LWC, radical and plumule length **(Lekshmi and Jayadev 2017).**

Physiological characteristic of salt tolerance in some plants (*Macrotyloma uniflorum, Vigna unguiculata*and *Vigna radiata).* Physiological traits such as germination percentage, percent mortality, fresh and dry weights, water status and mineral contents (Na, K and Ca) were measured. The growth experimental soil were salinized with NaCl at the rate of 50mM, 100mM and 150mM. Salinity caused a reduction in growth parameters such as relative growth rate (RGR), fresh and dry weights of leaves and stems. Whereas, roots growth was not affected by salinity. These changes were associated with a decrease in water content, K^+ and Ca^{2+} concentrations and a highly increased in Na+ contents in all organs. Thus, the K+/Na+ and Ca^{2+}/Na^+ ratios decreased with the applied of NaCl 150mM. Relationship between leaf water content and its Na^+ content, suggest that fenugreek developed a sodium inclusion mechanism to maintain its growth and consequently its survival under high salinity conditions (150mM NaCl) **(Hashini *et al*., 2009).**

NaCl induces the stress that results in plant reduced growth due to changes in internal mechanism of the plant.The effect of different salinity levels (50,100,150) on plant height, weight,number of leaves and viability of plants. Microscopic studies for root and stem anatomical attributes showed that salinity plays an important role in growth inhibition of plants. Xylem and phloem areas of root and stem were observed in decreasing trend under salt stress condition and the cortex also. While minimum cortex and epidermis area was observed in plants treated with 00 mgl^{-1} **(Younis *et al.,* 2014).**

To determine the effect of some plants growth regulators on stem anatomy of green gram seedlings grown saline (NaCl) conditions. Salt stress decreased the stem diameter, epidermis cell size, cortex zone thickness, vascular bundle width, cambium thickness, xylem width, trachea diameter and phloem width in the seedlings non-pretreated with the growth regulators, in comparison with the control seedlings grown in distilled water medium. In addition, it slightly increased the cuticle thickness. On the other hand, many of the growth regulator pretreatments more or less stimulated the stem diameter, epidermis cell width, cortex zone thickness, vascular bundle width, xylem width, trachea diameter and phloem width in comparison with the control seedlings grown on saline medium. Moreover, they generally reduced the cuticle thickness, epidermis cell length and cambium thickness **(Cavusoglu *et al*., 2008).**

Salt tolerance mechanism of *Macrotyloma uniflorum, Vigna unguiculata* and *Vigna radiata* were assessed by analyzing growth, nutrient uptake, anatomical modifications and alterations in levels of some organic metabolites in seedlings imposed to various levels of salinity (0, 50, 100, and 150mM NaCl) condition. After 15 days of salt treatment, plant height, leaf area, and shoot biomass decreased with increase in salinity whereas the leaf succulence increased significantly with increasing salinity in *Macrotyloma uniflorum*. There was significant alterations in leaf, stem, and root anatomy by salinity. The thickness of epidermis and spongy parenchyma of leaf increased in salt treated seedlings as compared to control, whereas palisade parenchyma decreased dramatically in extreme salinity (150 mM NaCl). There was a significant reduction in stomatal density and stomatal pore area of leaf with increasing salinity. Anatomical observations of stem showed that the epidermal cells diameter and thickness of cortex decreased by salinity whereas thickness of hypodermal layer, diameter of hypodermal cell, pith area and pith cell diameter increased by high

salinity. The root anatomy showed an increase in epidermal thickness by salinity whereas diameters of epidermal cells and xylem vessels decreased. There was no significant changes in polyphenols level of leaf at all levels of salinity **(Parida *et al*., 2016).**

Materials and Methods

MATERIAL AND METHODS

MATERIAL:

Three seeds of the family Fabaceae were selected for the present study. They were *Macrotyloma uniflorum* (Lam.) Verdc, *Vigna radiata* (L) Wilczek, and *Vigna unguiculata* (L) Walp**.** The seeds were shown in Plate 1

Botanical Name : *Macrotyloma uniflorum* (Lam.) Verdc

Common Name : Horse gram

Description:

Macrotyloma uniflorum (Lam.)Verdc. is a perennial climbing plant with a rhizome, growing to a height of about 60 cm (24 inch). The stem sprouts from the rhizome each year. It is clad in varying amounts of whitish hairs and bears alternate, trifoliate leaves with petioles up to 7 cm (2.8 inch) long. The leaflets are elliptical, and up to 7 cm (2.8 inch) long. The flowers are borne in twos or threes in the leaf axils, and are typical of the bean family with banner, wings and keel. They are cream, yellowish or green, often with a purple blotch inside. These are linear-oblong, upcurving pods up to 8 cm (3.1 inch) long, containing up to ten reddish-brown, speckled or black seeds.

Botanical Name :*Vigna radiata*(L.) R. Wilczek.

Common Name : Mung beans

Description:

The mung bean plant is an annual, erect or semi-erect, reaching a height of 0.15-1.25 m. It is slightly hairy with a well-developed root system. Wild types tend to

Plate 1

LEGUME SEEDS SELECTED FOR THE STUDY

Macrotyloma uniflorum

Vigna radiata

Vigna unguiculata

be prostrate while cultivated types are more erect. The stems are many-branched, sometimes twining at the tips. The leaves are alternate, trifoliolate with elliptical to ovate leaflets, 5-18 cm long x 3-15 cm broad. The flowers (4-30) are papillonaceous, pale yellow or greenish in colour. The pods are long, cylindrical, hairy and pending. They contain 7 to 20 small, ellipsoid or cube-shaped seeds. The seeds are variable in colour. They are usually green, but can also be yellow, olive, brown, purplish brown or black, mottled and ridged.

Botanical Name :*Vigna unguiculata* (L) Walp.

Common name : Cowpea

Description:

The cowpea is an annual herbaceous legume cultivated for its edible seeds or for fodder. It may be climbing and erect, as well as prostrate and creeping depending on the cultivar. Prostrate varieties grow to about 80 cm and climbing cultivars up to 2 m. It has a well developed root system. The leaves are trifoliate with oval leaflets, 6- 15 cm long and 4-11 cm broad. The papillonaceous flowers can be white, yellowish, pale blue or violet and are distributed along axillary clusters. Pods occur in pairs forming a V, mostly pendulous but they can be erect. They are cylindrical, 6 to 20 cm long and 3-12 mm broad, and contain 8 to 20 seeds. Seeds can be white, pink, brown or black.

METHODS:

The seeds of *Macrotyloma uniflorum* (Lam.) Verdc, *Vigna radiata* (L) Wilczek and *Vigna unguiculata* (L) Walp**.** were collected from the local shop in Thoothukudi market. The experiment was conducted in the Green House garden in

St. Mary's College (Autonomous), Thoothukudi. Twelve Pots were selected which is divided into three series of four pots each.

SALT CONCENTRATION:

The seeds of uniform size were surface sterilized with 0.1% Mercuric chloride for two minutes and rinsed thoroughly with distilled water. Various concentrations of Sodium chloride solutions (50mM, 100mM, 150mM) were prepared.

The selected pots were filled with soil and organic manures. About 25 seeds were sown in each pot. The pots were watered with 100ml of normal water in alternated days. Salt treatment was started from the 15^{th} day after sowing. The plants were treated with NaCl salt using the following concentrations 50mM, 100mM, 150mMonce in three days for 35 days. The control pot was watered with normal water.

PHYSICAL PARAMETERS:

The effects of salinity were studied using *Macrotyloma uniflorum* (Lam.) Verdc, *Vigna radiata* (L) Wilczek, and *Vigna unguiculata* (L) Walp. The plants were collected at the stages of 15^{th}, 25^{th}, 35^{th} days for growth measurements such as shoot length, leaf area, number of leaves, dry weight and fresh weight.

GROWTH DETERMINATION:

Germination Percentage:

From all the treatments, the number of seeds germinated were counted and recorded. Germination percentage was calculated by the following formula.

$$\text{Germination percentage} = \frac{\text{Number of seeds germinated}}{\text{Total number of seeds sown}} \times 100$$

Number of Leaves:

Five plants were collected and the number of leaves in each plant was calculated and the average was taken.

Leaf Area:

Leaves from each plant were collected and their outline was drawn in graph sheets to calculate the leaf area and expressed in cm^2.

Leaf Length:

The leaf length was measured in five seedlings of each treatment by measuring the length of the leaf from the tip of the petiole to the leaf tip and the average was taken.

Petiole Length:

The petiole length was measured in five seedlings of each treatment by measuring the length of the petiole from the node to the tip of petiole and the average was taken.

Root Length:

The root length was measured in five seedlings of each treatment by measuring the length of the root from the ground level to the tip of the root and the average was taken.

Shoot Length:

The shoot length was measured in five seedlings of each treatment by measuring the length of the shoot above the ground level to the tip of the shoot andthe average was taken.

Fresh Weight of Shoot:

The plants were collected from each pots and their fresh weight were measured in grams using chemical balance.

Dry Weight of Shoot:

Freshly weighed plants were kept in an oven at regulated temperature (65^{o}C) for three days. The plants were taken after proper drying and weighed.

MICROSCOPIC STUDIES

Plant material was cleaned with tap water, cutted into suitable parts, and fixed in FAA solution [formaldehyde + acetic acid + ethyl alcohol 50% (in ratios 5:5:90)] for suitable time. Free-hand sections were prepared with a razor blade, then sections dehydrated in different concentrations of ethyl alcohol and the sections were stained with safranine. After that sections were mounted in a slide using glycerine and were photographed using Trinocular microscope LV50U.

Results and Discussion

RESULT AND DISCUSSION

Some environmental stresses adversely affect plant growth, development and finally the crop yield. Salinity, drought, oxidative stress and nutrient imbalances are the major environmental stresses. Salinity tress remains one of the world's oldest and the most serious problem which substantially hampers crop productivity. Salinity has been described as a critical environmental problem especially when it adversely affects the plant growth. Salinity has been observed to be capable of restricting the growth of plants in large areas of the earth than other inhibitory substances encountered by plants in natural environment.

The growth performance of NaCl treated plants of *Macrotyloma uniflorum* (Lam.) Verdc, *Vigna radiata* (L) Wilczek, and *Vigna unguiculata* (L) Walp**.** on 20th , 25th and 30th day after treatment were shown in **Plate 2-4.**

Germination:

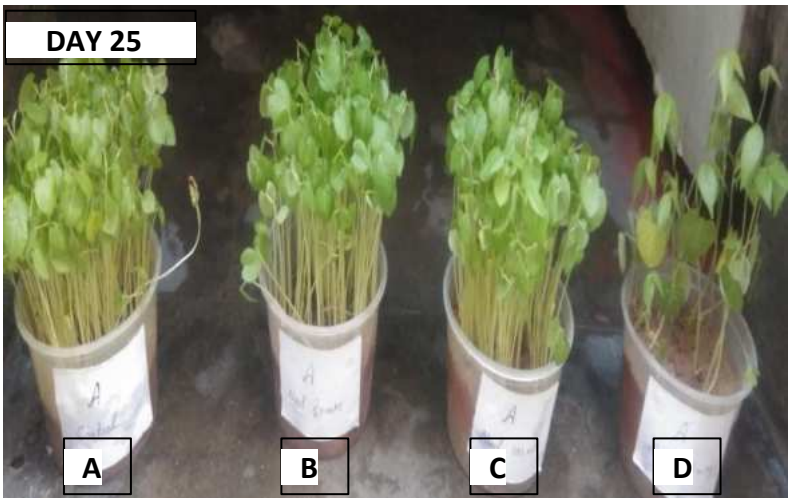
The germination percentage of the seeds *Macrotyloma uniflorum* (Lam.) Verdc, *Vigna radiata* (L) Wilczek, and *Vigna unguiculata* (L) Walp showed great variation and it is shown in **Table 1 and Figure1 - 2**. The seeds of *Vigna radiate* showed good germination rate with limited days.

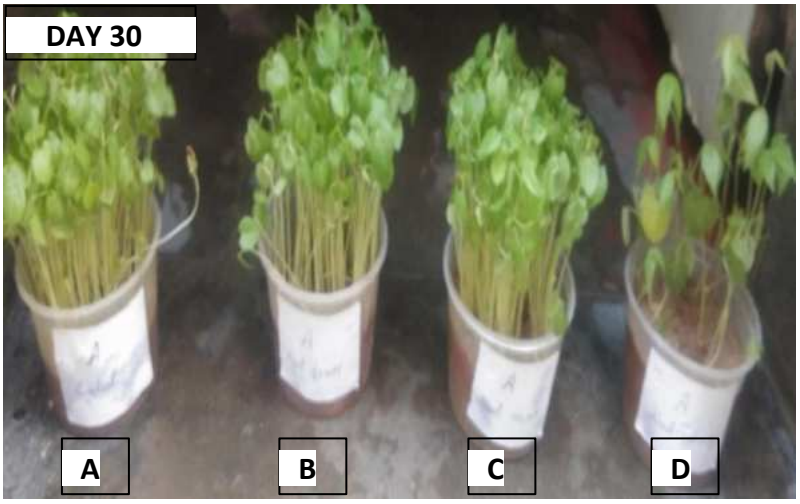
Growth Parameters:

Shoot length:

Plate 2

EFFECT OF DIFFERENT CONCENTRATION OF NACL ON THEGROWTH OF MACROTYLOMA UNIFLORUM

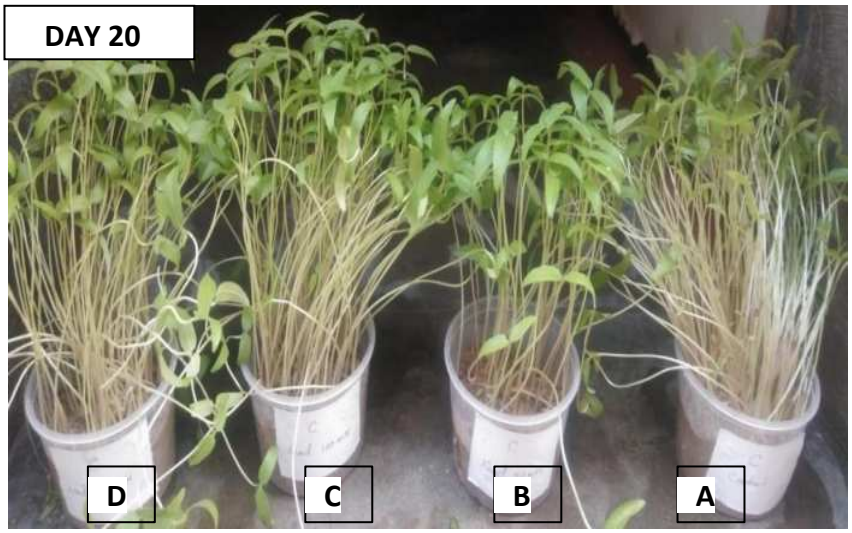


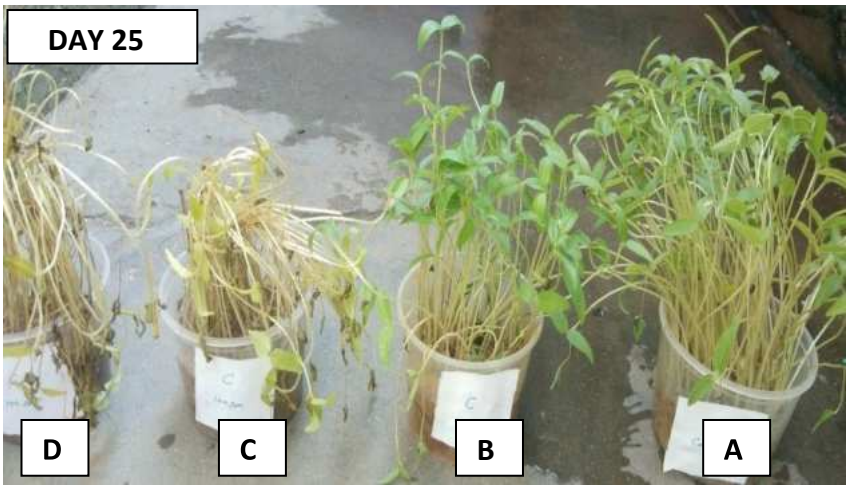


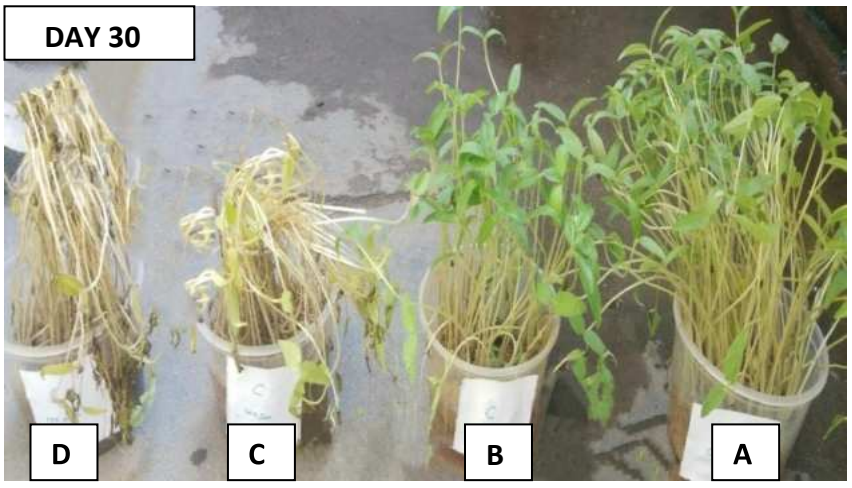
A – Control B – 50 mM C – 100 mM D – 150mM

Plate 3

EFFECT OF DIFFERENT CONCENTRATION OF NACL ON THEGROWTH OF VIGNA RADIATA



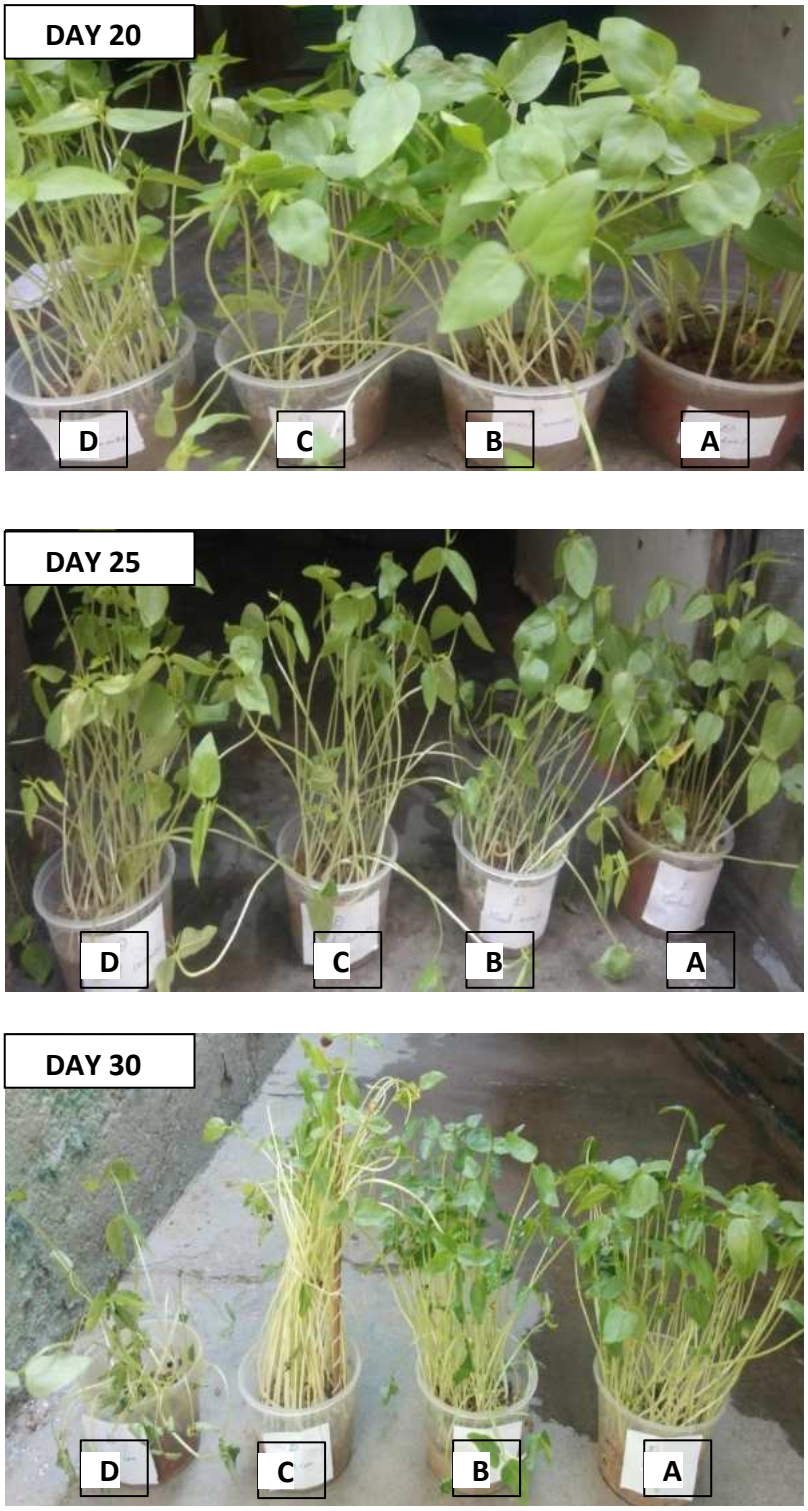




A – Control B – 50 mM C – 100 mM D – 150 m

Plate 4

EFFECT OF DIFFERENT CONCENTRATION OF NACL ON THEGROWTH OF VIGNA UNGUICULATA



A – Control B – 50 mM C – 100 mM D – 150 mM

TABLE 1: GERMINATION POTENTIAL OF SELECTED LEGUME PLANTS

S. No.	Seeds	Germination Percentage	Days Required for the Germination
1.	*Macrotyloma uniflorum*	83.13 ± 1.62	4 ± 0.51
2.	*Vigna radiata*	92.5 ± 2.2	1 ± 0.9
3.	*Vigna unguiculata*	85.63 ± 0.82	3 ± 0.6

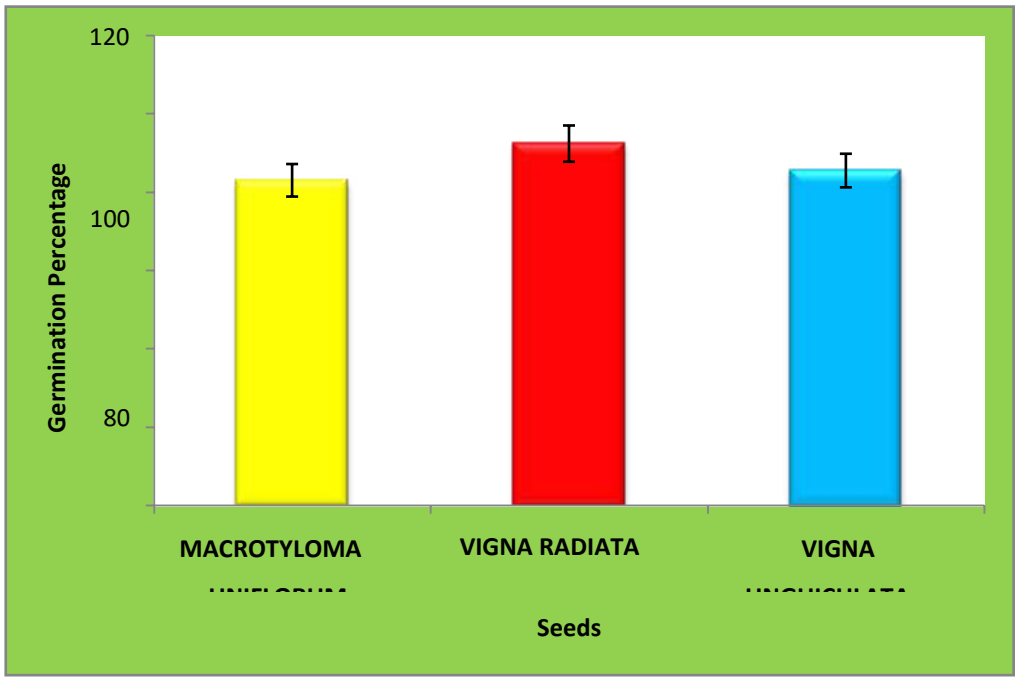


FIGURE 1 GERMINATION CAPACITY OF SELECTED LEGUME SEEDS

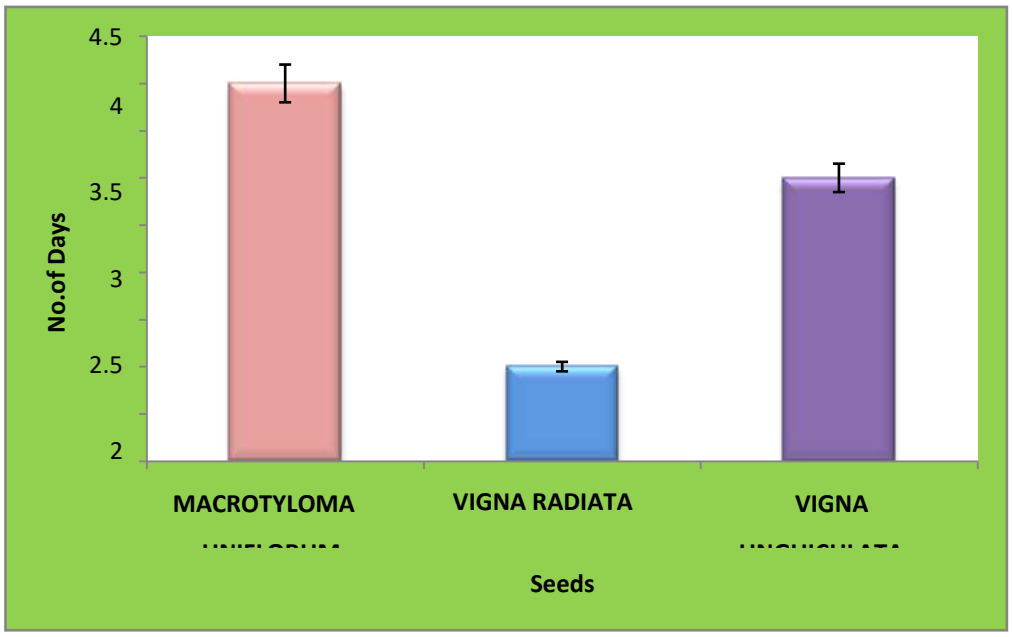


FIGURE 2: DAYS REQUIRED FOR THE GERMINATION OF SELECTED LEGUME SEEDS

Shoot length of *Macrotyloma uniflorum* (Lam.) Verdc, *Vignaradiata* (L) Wilczek, and *Vignaunguiculata* (L) Walp was severely affected with increasing concentration of NaCl. The higher shoot length was observed in the control series. Itgradually decreases with increasing concentration. The minimum shoot length was observed in 150mM concentration. Among the three seeds, the growth of *Macrotyloma uniflorum*is greatly affected by the increasing concentration of NaCl. This has been shown in **Table 2-10** and in **Figure 3**. Similar results were observed in Pea plants by **Ljubomira *et al*., 2017.**

Root length

Root length of *Macrotyloma uniflorum* (Lam.) Verdc, *Vigna radiata* (L) Wilczek, and *Vigna unguiculata* (L) Walp was severely affected with increasing concentration of NaCl. The higher shoot length was observed in the control series. It gradually decreases with increasing concentration. The minimum root length was observed in 150mM concentration. Among the three seeds, the growth of *Vigna radiata* is greatly affected by the increasing concentration of NaCl. This has been shown in **Table 2-10** and in **Figure 3.**

Leaf length

Leaf length of *Macrotyloma uniflorum* (Lam.) Verdc, *Vigna radiata* (L) Wilczek, and *Vigna unguiculata* (L) Walp was severely affected with increasing concentration of NaCl. The higher leaf length was observed in the control series. It gradually decreases with increasing concentration. The minimum leaf length was observed in 150mM concentration. Among the three seeds, the growth of *Vigna unguiculata*is greatly affected by the increasing concentration of NaCl. This has been shown in **Table 2-10** and in **Figure 4.**

TABLE 2: EFFECT OF DIFFERENT CONCENTRATION NACL ON THE GROWTH PERFORMANCE ON 20 DAY OLD SEEDLING OF MACROTYLOMA UNIFLORUM

Treatment	**Seedling Weight (g)**		**No. of Leaves**	**Leaf Area (cm^2)**	**Length (cm)**			
	Fresh	**Dry**			**Leaf**	**Petiole**	**Stem**	**Root**
Control	0.3 ± 0.023	0.15 ± 0.006	6 ± 1.1	1.25 ± 0.12	1.5 ± 0.11	1.1 ± 0.2	15 ± 1.91	2.6 ± 0.21
50 mM	0.38 ± 0.011	0.19 ± 0.013	6 ± 0.9	1.25 ± 0.09	1.9 ± 0.12	1.5 ± 0.31	11.5 ± 2.01	2.3 ± 0.16
100 mM	0.24 ± 0.018	0.12 ± 0.016	5 ± 0.8	1.10 ± 0.11	1.4 ± 0.04	1.3 ± 0.26	11.9 ± 1.44	1.9 ± 0.13
150 mM	0.29 ± 0.016	0.15 ± 0.02	4 ± 0.6	1.20 ± 0.09	1.5 ± 0.04	1.4 ± 0.12	12 ± 0.51	1.9 ± 0.17

TABLE 3: EFFECT OF DIFFERENT CONCENTRATION NACL ON THE GROWTH PERFORMANCE ON 20 DAY OLD SEEDLING OF MACROTYLOMA UNIFLORUM

Treatment	**Seedling Weight (g)**		**No. of Leaves**	**Leaf Area (cm^2)**	**Length (cm)**			
	Fresh	**Dry**			**Leaf**	**Petiole**	**Stem**	**Root**
Control	0.31 ± 0.02	0.15 ± 0.023	6 ± 1.1	1.25 ± 0.32	1.5 ± 0.22	1.1 ± 0.31	15.3 ± 2.01	2.8 ± 0.16
50 mM	0.33 ± 0.06	0.17 ± 0.016	6 ± 0.6	1.25 ± 0.15	1.9 ± 0.06	1.5 ± 0.24	11.5 ± 1.01	2.3 ± 0.09
100 mM	0.23 ± 0.023	0.11± 0.014	6 ± 0.4	1.25 ± 0.18	1.4 ± 0.08	1.3 ± 0.19	11.5 ± 0.79	1.7 ± 0.11
150 mM	0.23 ± 0.03	0.13 ± 0.021	5 ± 0.8	1.05 ± 0.19	1.5 ± 0.03	1.4 ± 0.16	11.7 ± 0.11	1.6 ± 0.06

TABLE 4: EFFECT OF DIFFERENT CONCENTRATION NACL ON THE GROWTH PERFORMANCE ON 20 DAY OLD SEEDLING OF MACROTYLOMA UNIFLORUM

Treatment	**Seedling Weight (g)**		**No. of Leaves**	**Leaf Area (cm^2)**	**Length (cm)**			
	Fresh	**Dry**			**Leaf**	**Petiole**	**Stem**	**Root**
Control	0.31 ± 0.033	0.15 ± 0.026	6 ± 1.4	1.25 ± 0.41	1.7 ± 0.26	1.2 ± 0.36	15.3 ± 2.25	2.8 ± 0.19
50 mM	0.34 ± 0.013	0.17 ± 0.023	6 ± 0.7	1.20 ± 0.21	1.5 ± 0.13	1.5 ± 0.21	11.5 ± 0.93	2.0 ± 0.12
100 mM	0.22 ± 0.019	0.11 ± 0.016	4 ± 0.4	1.26 ± 0.19	1.5 ± 0.05	1.3 ± 0.17	11.2 ± 0.67	2.1 ± 0.11
150 mM	0.21 ± 0.016	0.10 ± 0.012	4 ± 0.5	1.10 ± 0.2	1.5 ± 0.02	1.3 ± 0.11	11.0 ± 0.13	1.8 ± 0.08

TABLE 5: EFFECT OF DIFFERENT CONCENTRATION NACL ON THE GROWTH PERFORMANCE ON 20 DAY OLD SEEDLING OF VIGNA UNGUICULATA

Treatment	**Seedling Weight (g)**		**No. of Leaves**	**Leaf Area (cm^2)**	**Length (cm)**			
	Fresh	**Dry**			**Leaf**	**Petiole**	**Stem**	**Root**
Control	0.77 ± 0.26	0.39 ± 0.09	5 ± 1.01	9.5 ± 0.85	4.3 ± 0.82	0.5 ± 0.29	18 ± 2.41	2.7 ± 0.91
50 mM	0.87 ± 0.19	0.46 ± 0.05	5 ± 0.8	9.7 ± 0.31	4.4 ± 0.31	0.5 ± 0.24	18 ± 1.81	2.5 ± 0.82
100 mM	0.94 ± 0.11	0.47 ± 0.03	5 ± 0.6	9.3 ± 0.22	4.3 ± 0.27	0.4 ± 0. 24	17.7 ± 1.01	2.3 ± 0.62
150 mM	0.69 ± 0.15	0.34 ± 0.06	5 ± 0.42	7.3 ± 0.82	4.2 ± 0.16	0.4 ± 0.18	16.5 ± 0.81	1.9 ± 0.32

TABLE 6: EFFECT OF DIFFERENT CONCENTRATION NACL ON THE GROWTH PERFORMANCE ON 20 DAY OLD SEEDLING OF VIGNA UNGUICULATA

Treatment	**Seedling Weight (g)**		**No. of Leaves**	**Leaf Area (cm^2)**	**Length (cm)**			
	Fresh	**Dry**			**Leaf**	**Petiole**	**Stem**	**Root**
Control	0.77 ± 0.35	0.38 ± 0.09	5 ± 1.23	9.5 ± 0.93	4.3 ± 0.91	0.5 ± 0.36	18 ± 2.81	2.7 ± 0.93
50 mM	0.76 ± 0.16	0.38 ± 0.06	5 ± 0.84	8.9 ± 0.25	4.2 ± 0.27	0.5 ± 0.21	18 ± 1.98	2.5 ± 0.5
100 mM	0.83 ± 0.11	0.41 ± 0.09	5 ± 0.61	8.15 ± 0.15	4.2 ± 0.3	0.4 ± 0.26	17.5 ± 0.93	2.3 ± 0.43
150 mM	0.68 ± 0.09	0.34 ± 0.05	4 ± 0.4	7.23 ± 0.43	4.0 ± 0.13	0.4 ± 0.14	17.5 ± 0.14	2.3 ± 0.11

TABLE 7: EFFECT OF DIFFERENT CONCENTRATION NACL ON THE GROWTH PERFORMANCE ON 20 DAY OLD SEEDLING OF VIGNA UNGUICULATA

Treatment	**Seedling Weight (g)**		**No. of Leaves**	**Leaf Area (cm^2)**	**Length (cm)**			
	Fresh	**Dry**			**Leaf**	**Petiole**	**Stem**	**Root**
Control	0.78 ± 0.43	0.37 ± 0.06	5 ± 1.2	9.6 ± 0.12	4.5 ± 0.11	0.5 ± 0.39	18.2 ± 3.13	2.7 ± 1.16
50 mM	0.76 ± 0.09	0.35 ± 0.03	5 ± 0.8	8.9 ± 0.11	4.3 ± 0.14	0.5 ± 0.2	18 ± 1.13	2.5 ± 04
100 mM	0.73 ± 0.02	0.32 ± 0.01	5 ± 0.6	7.5 ± 0.16	4.2 ± 0.2	0.4 ± 0.3	17.5 ± 0.74	2.5 ± 0.2
150 mM	0.65 ± 0.012	0.33 ± 0.02	4 ± 0.4	7.4 ± 0.06	4.2 ± 0.07	0.4 ± 0.11	17.1 ± 0.16	2.1 ± 0. 17

TABLE 8: EFFECT OF DIFFERENT CONCENTRATION NACL ON THE GROWTH PERFORMANCE ON 30 DAY OLD SEEDLING OF VIGNA RADIATA

Treatment	**Seedling Weight (g)**		**No. of Leaves**	**Leaf Area (cm^2)**	**Length (cm)**			
	Fresh	**Dry**			**Leaf**	**Petiole**	**Stem**	**Root**
Control	0.39 ± 0.08	0.19 ± 0.02	4 ± 0.7	9.5 ± 0.94	4.5 ± 0.34	0.7 ± 0.08	17.2 ± 2.06	2 ± 0.36
50 mM	0.40 ± 0.06	0.20 ± 0.04	4 ± 0.62	7.2 ± 0.71	3.7 ± 0.29	0.7 ± 0.06	17.2 ± 1.91	2 ± 0.21
100 mM	0.43 ± 0.04	0.22 ± 0.03	4 ± 0.43	7.2 ± 0.63	3.5 ± 0.23	0.5 ± 0.07	17 ± 1.2	2 ± 0.14
150 mM	0.42 ± 0.03	0.21 ± 0.02	2 ± 0.5	6.8 ± 0.36	3.1 ± 0.19	0.4 ± 0.08	16 ± 0.92	1.9 ± 0.09

TABLE 9: EFFECT OF DIFFERENT CONCENTRATION NACL ON THE GROWTH PERFORMANCE ON 30 DAY OLD SEEDLING OF VIGNA RADIATA

Treatment	**Seedling Weight (g)**		**No. of Leaves**	**Leaf Area (cm^2)**	**Length (cm)**			
	Fresh	**Dry**			**Leaf**	**Petiole**	**Stem**	**Root**
Control	0.39 ± 0.16	0.19 ± 0.08	4 ± 0.9	1.7 ± 0.4	3.7 ± 0.34	0.7 ± 0.17	17.2 ± 2.16	2 ± 0.36
50 mM	0.40 ± 0.04	0.20 ± 0.03	4 ± 0.7	1.7 ± 0.14	3.7 ± 0.16	0.7 ± 0.04	17.2 ± 1.02	3 ± 0.16
100 mM	0.4 ± 0.03	0.20 ± 0.02	3 ± 0.4	1.7 ± 0.18	3.4 ± 0.17	0.5 ± 0.06	16.5 ± 0.96	2 ± 0.05
150 mM	0.42 ± 0.02	0.21 ± 0.02	3 ± 0.2	1.5 ± 0. 21	3.4 ± 0.09	0.4 ± 0.03	16.8 ± 0.81	1.9 ± 0.03

TABLE 10: EFFECT OF DIFFERENT CONCENTRATION NACL ON THE GROWTH PERFORMANCE ON 30 DAY OLD SEEDLING OF VIGNA RADIATA

Treatment	**Seedling Weight (g)**		**No. of Leaves**	**Leaf Area (cm^2)**	**Length (cm)**			
	Fresh	**Dry**			**Leaf**	**Petiole**	**Stem**	**Root**
Control	0.39 ± 0.19	0.13 ± 0.16	4 ± 0.5	1.7 ± 0.22	3.9 ± 0.41	0.7 ± 0.23	17.2 ± 2.3	2 ± 0.42
50 mM	0.39 ± 0.06	0.17 ± 0.02	4 ± 0.4	1.7 ± 0.13	3.9 ± 0.1	0.7 ± 0.06	17.2 ± 1.16	2 ± 0.26
100 mM	0.35 ± 0.16	0.18 ± 0.06	3 ± 0.4	1.5 ± 0.06	3.5 ± 0.12	0.5 ± 0.08	16.5 ± 0.56	2 ± 0.03
150 mM	0.3 ± 0.19	0.13 ± 0.04	3 ± 0.2	1.5 ± 0.02	3.5 ± 0.41	0.3 ± 0.09	16.8 ± 0.46	1.8 ± 0.14

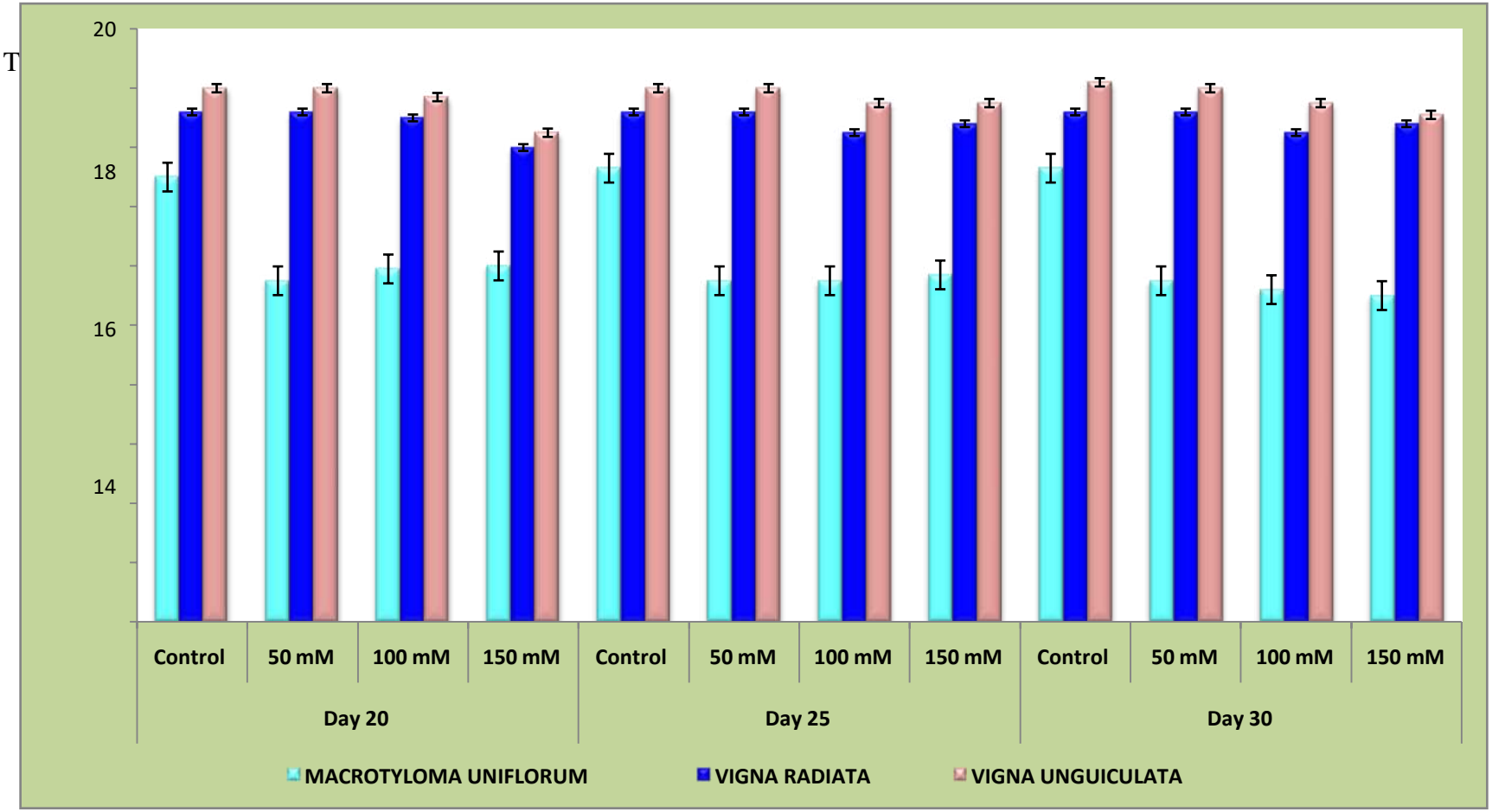


FIGURE 3: EFFECT OF DIFFERENT CONCENTRATION OF NACL ON STEM LENGTH OF SELECTED LEGUME PLANTS AT DIFFERENT TIME INTERVALS

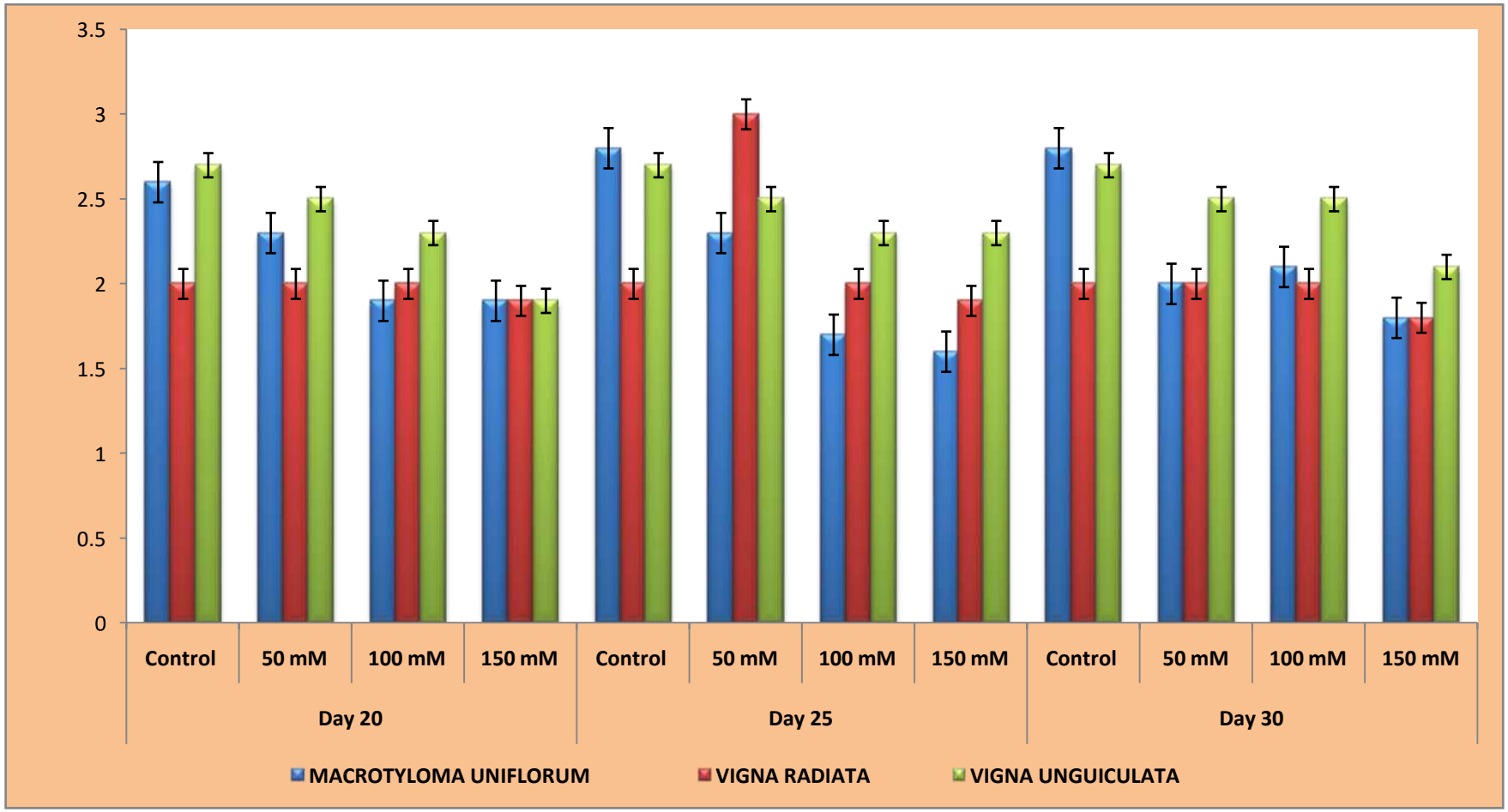


FIGURE 4: EFFECT OF DIFFERENT CONCENTRATION OF NACL ON ROOT LENGTH OF SELECTED LEGUME PLANTS AT DIFFERENT TIME INTERVALS

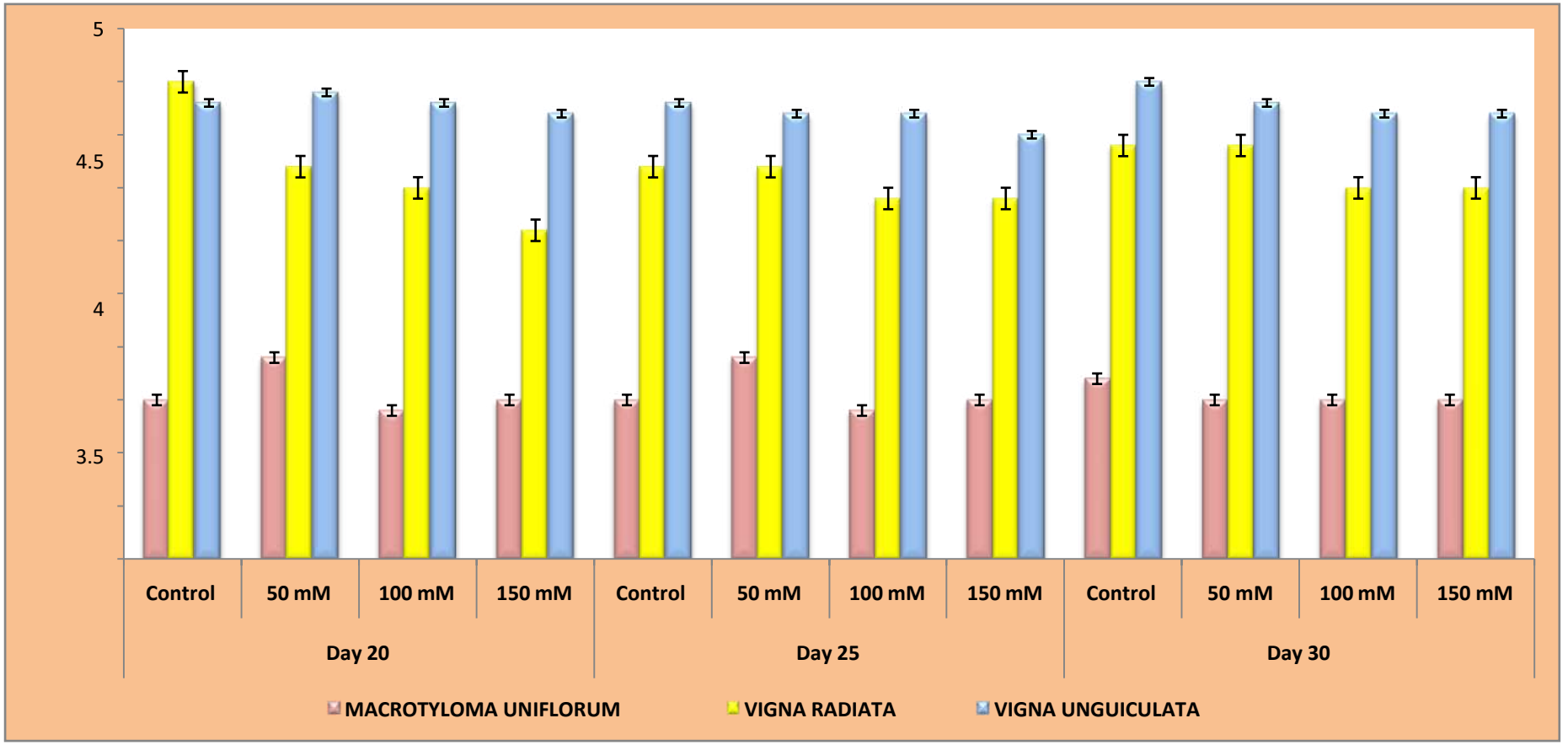


FIGURE 5: EFFECT OF DIFFERENT CONCENTRATION OF NACL ON LEAF LENGTH OF SELECTED LEGUME PLANTS AT DIFFERENT TIME INTERVALS

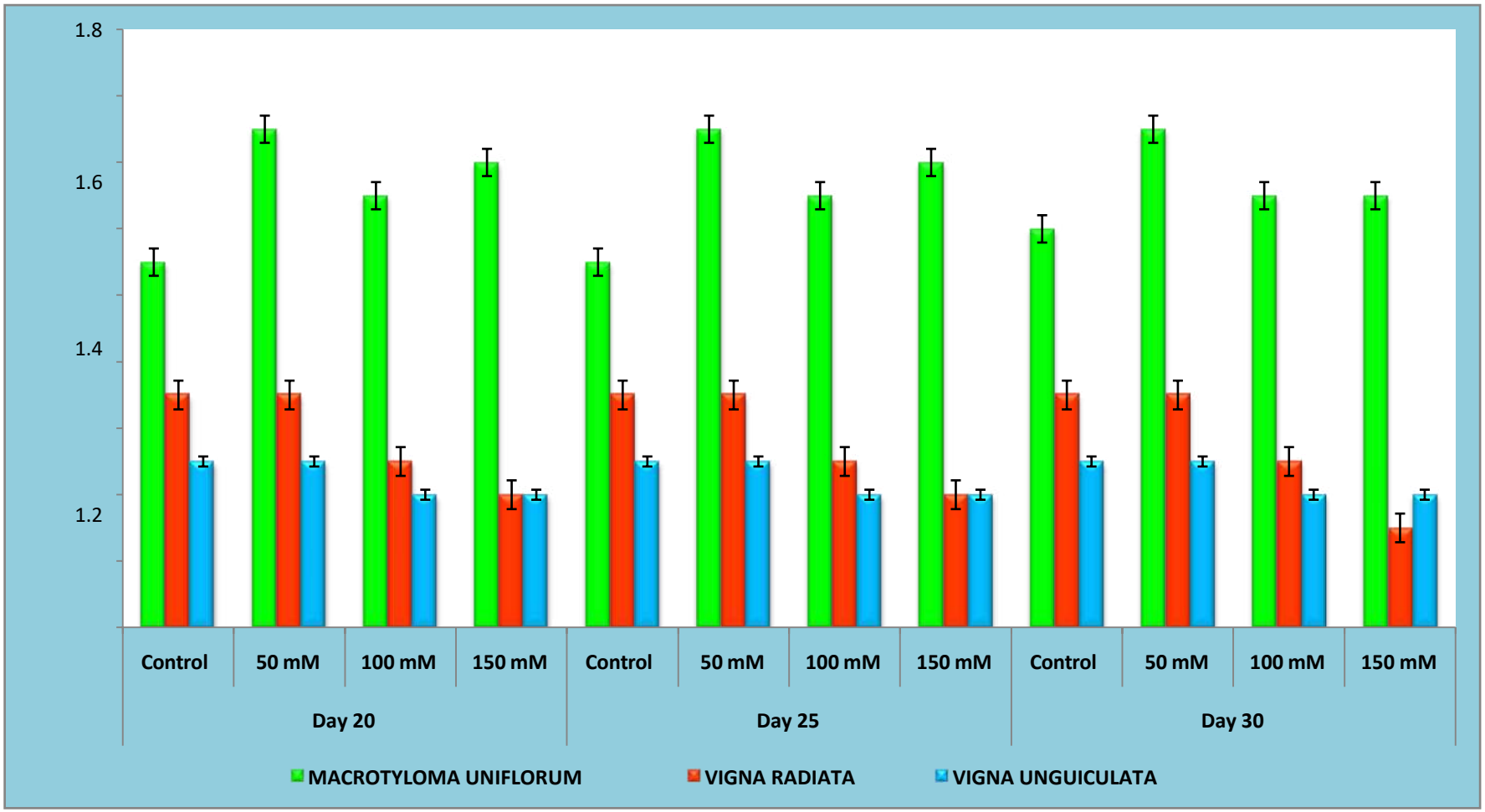


FIGURE 6: EFFECT OF DIFFERENT CONCENTRATION OF NACL ON PETIOLE LENGTH OF SELECTED LEGUME PLANTS AT DIFFERENT TIME INTERVALS

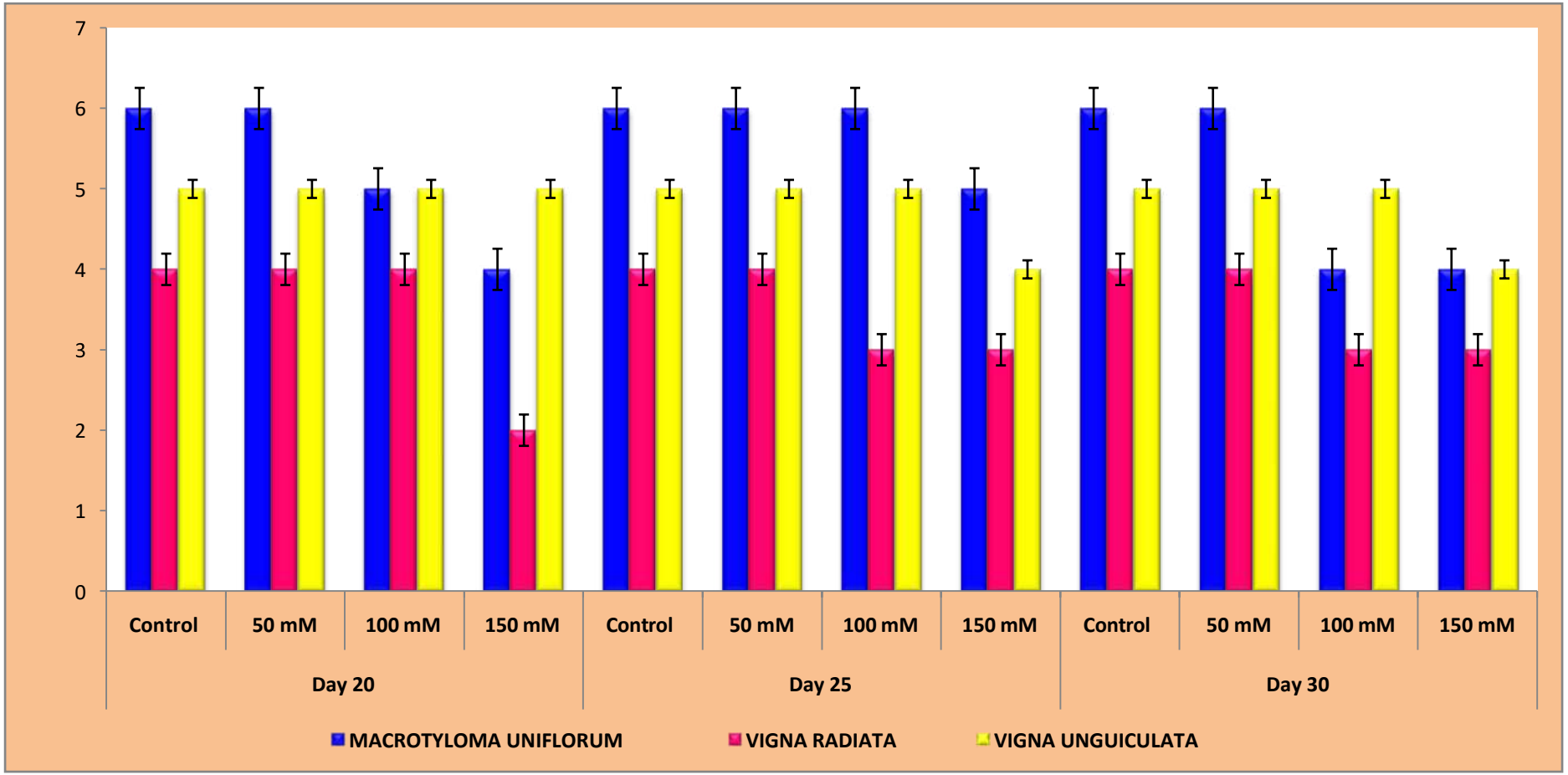


FIGURE 7: EFFECT OF DIFFERENT CONCENTRATION OF NACL ON NUMBERS OF LEAVES OF SELECTED LEGUME PLANTS AT DIFFERENT TIME INTERVALS

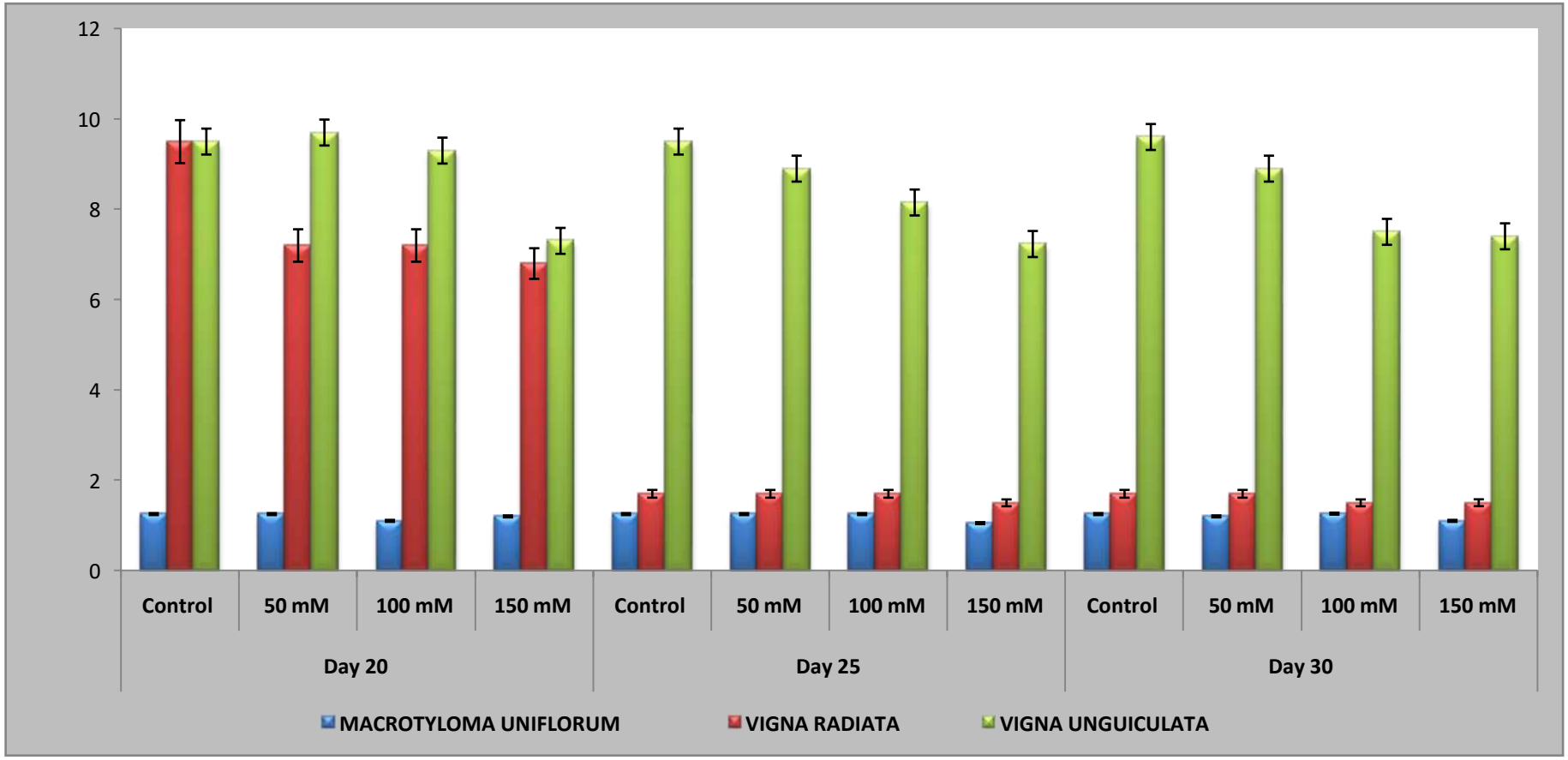


FIGURE 8: EFFECT OF DIFFERENT CONCENTRATION OF NACL ON LEAF AREA OF SELECTED LEGUME PLANTS AT DIFFERENT TIME INTERVALS

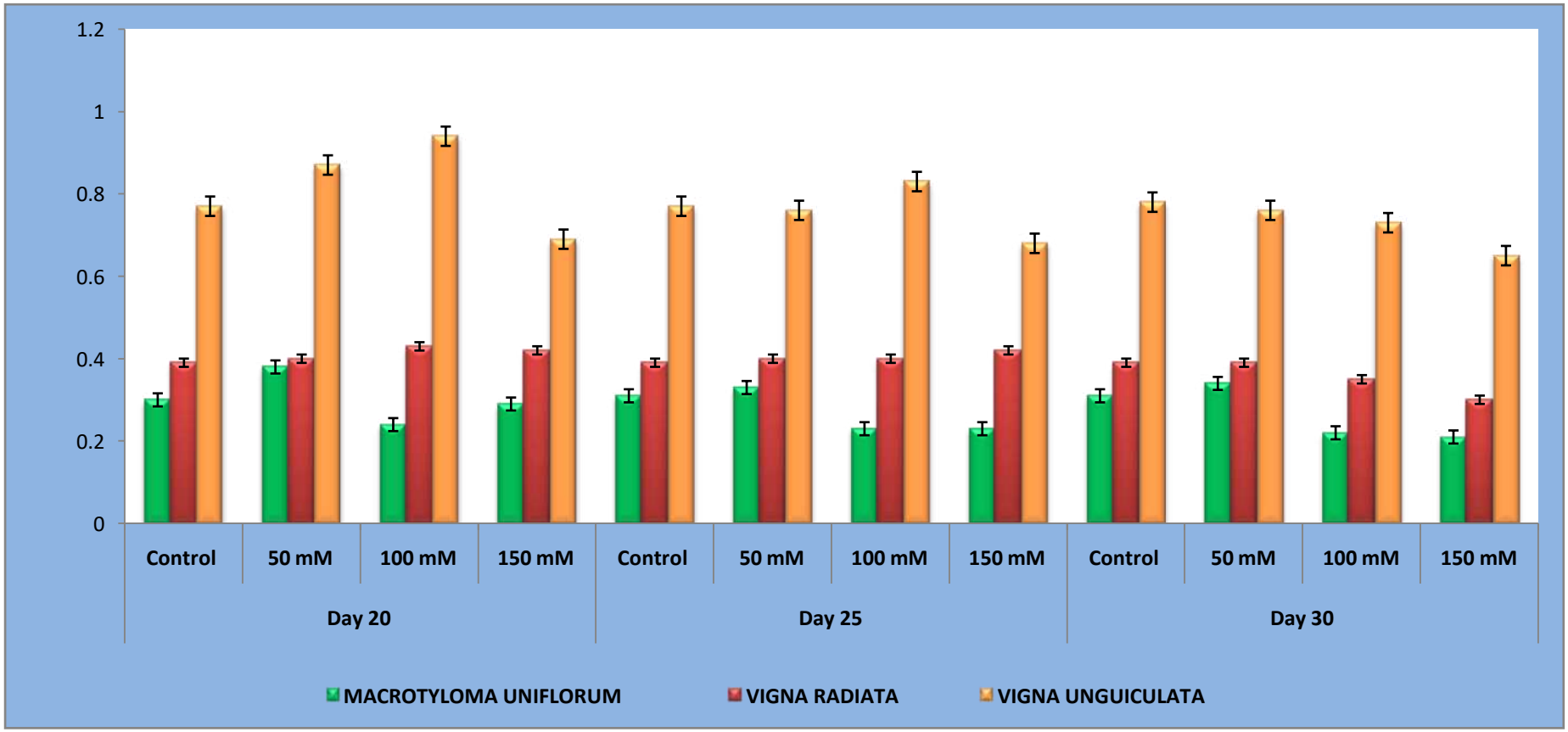


FIGURE 9: EFFECT OF DIFFERENT CONCENTRATION OF NACL ON THE FRESH WEIGHT OF SELECTED LEGUME PLANTS AT DIFFERENT TIME INTERVALS

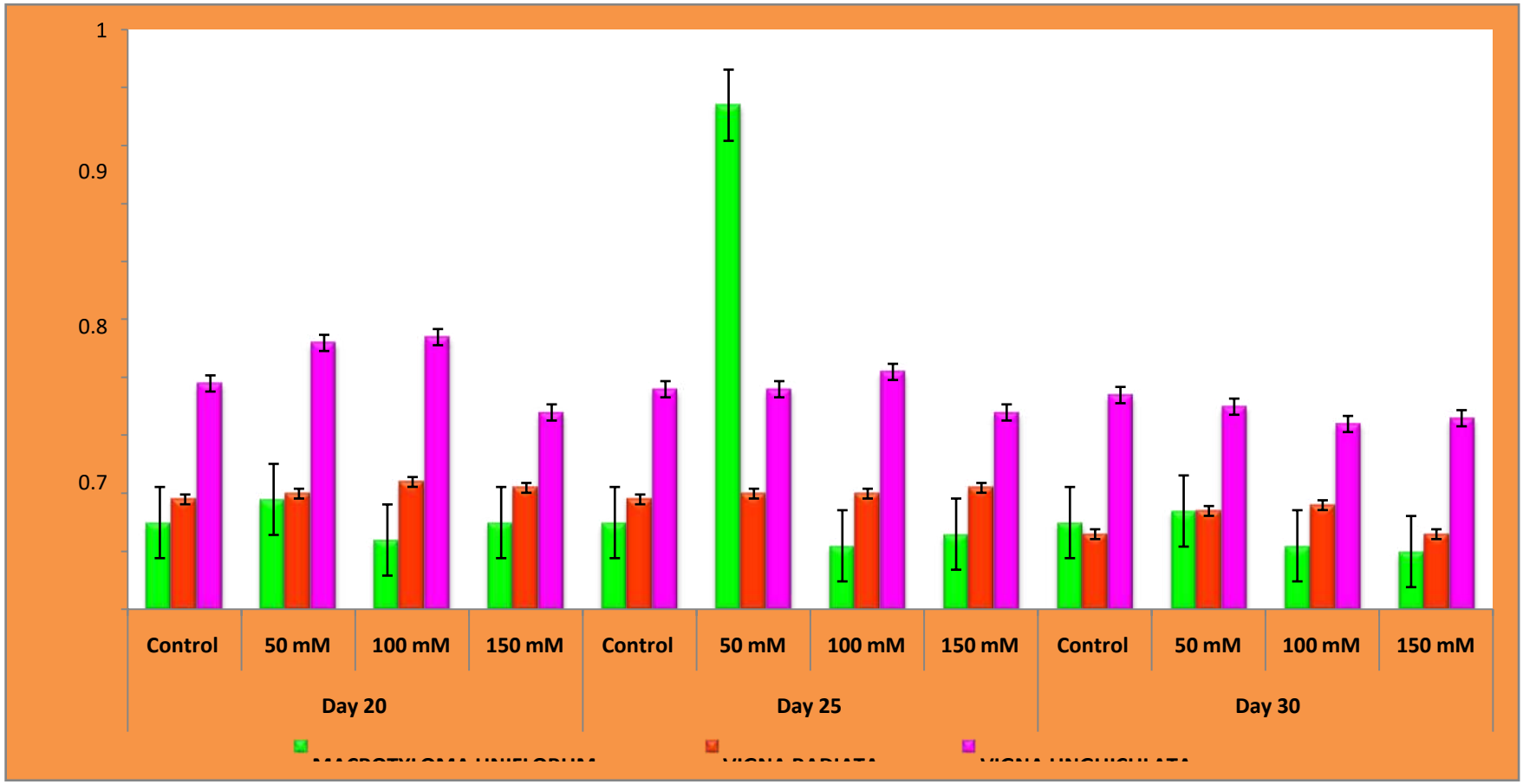


FIGURE 10: EFFECT OF DIFFERENT CONCENTRATION OF NACL ON THE DRY WEIGHT OF SELECTED LEGUME PLANTS AT DIFFERENT TIME INTERVALS

Petiole length

TABLE 19: EFFECT OF DIFFERENT CONCENTRATION NACL ON THE GROWTH PERFORMA

Petiole length of *Macrotyloma uniflorum* (Lam.) Verdc, *Vigna radiata* (L) Wilczek, and *Vigna unguiculata* (L) Walp seedlings affected with increasing concentration of NaCl. The higher petiole length was observed in the control series. It gradually decreases with increasing concentration. The minimum petiole length was observed in 150mM concentration. Among the three seeds, the growth of *Vigna unguiculata* is greatly affected by the increasing concentration of NaCl. This has been shown in **Table 2-10** and in **Figure 5.**

SEEDLINGS OF VIGNA RADIATA

Number of leaves:

The number of leaves in *Macrotyloma uniflorum* (Lam.) Verdc, *Vigna radiata* (L) Wilczek, and *Vigna unguiculata* (L) Walp were also greatly influenced by increasing concentration of NaCl. The leaf number was maximum in control series and minimum at 150mM. The result was shown in **Table 2-10** and in **Figure 6.** Among the three seeds, the number of leaves are very less in *Vigna radiate* as the concentration of NaCl increases with days. The reduction in number of leaves may be as a result of accumulation of ions especially sodium and chloride which may have been toxic to the leaves leading to the loss of leaves **(Abdul Qados, 2011)**. In *Helianthus annuus*, the increasing salt concentration reduced the leaf number **(Ahmad and Jhon, 2005; Manivannan *et al*., 2007).**

Leaf area:

Leaf area of NaCl treated *Macrotyloma uniflorum* (Lam.) Verdc, *Vigna radiata* (L) Wilczek, and *Vigna unguiculata* (L) Walp plants showed reduction with increasing concentration. This can be seen in **Table 2-10** and **Figure 7.** This

Plate 5

EFFECT OF NACL ON THE LEAF ANATOMY OF

might be because of the accumulation of sodium and chloride. In *Glycine max* and *Talinium triangularae*, the leaf area decreases with increasing salt concentration as reported by **(Ahmad and Jhon, 2005).**

Fresh and Dry weight:

Fresh and dry weight of *Macrotyloma uniflorum* (Lam.) Verdc, *Vigna radiata* (L) Wilczek, and *Vigna unguiculata* (L) Walp were decreased with increasing salt concentration as shown in **Table 2-10** and in **Figures 8 - 9.** Reduction in dry weights depended relatively on the decrease in the length of shoot and root **(Arif *et al.*, 2020)**.

Microscopic characters:

Leaf Anatomy:

The leaf anatomy of NaCL treated *Macrotyloma uniflorum* (Lam.) Verdc, *Vigna radiata* (L) Wilczek, and *Vigna unguiculata* (L) Walp was shown in **Plate 5 – 7**. The treated plants showed great variation in the epidermal cells, thickness of palisade parenchyma and in the number of xylem cells. The maximum destruction was observed in the leaves of *Vigna unguiculata* which is been treated with 150mM concentration of NaCl. reduction of leaf thickness might be attributed to that salinity reduces the ability of plants to take up water and this quickly causes reduction in the growth rate, if excessive amounts of salt enter the plant they will eventually rise to toxic levels in the older transpiring leaves and reduce the photosynthetic capacity of the plant **(Barclay, 2015)**.

Plate 6

EFFECT OF NACL ON THE LEAF ANATOMY OF MACROTYLOMA UNIFLORUM

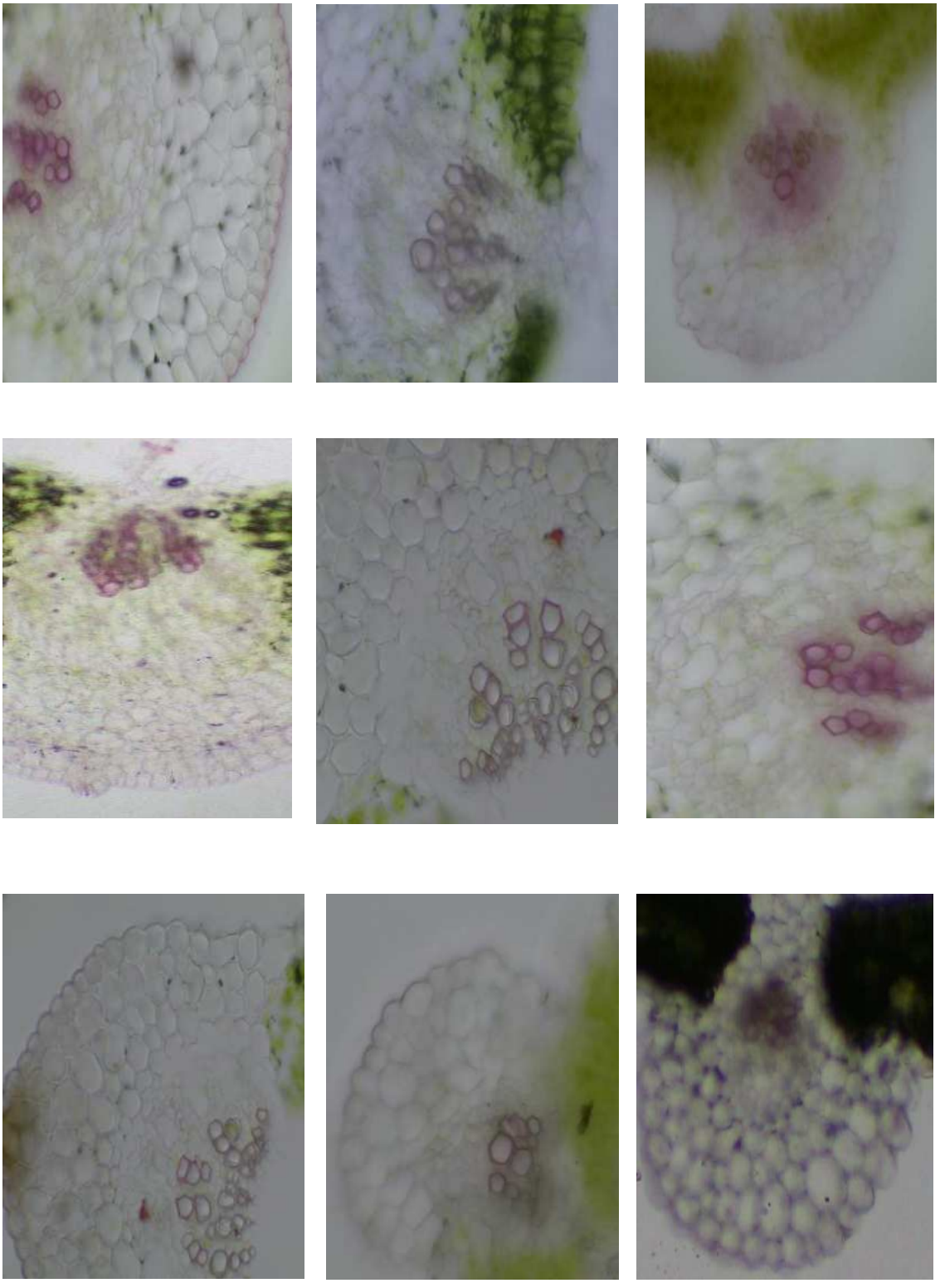


Plate 7

EFFECT OF NACL ON THE LEAF ANATOMY OF
VIGNA RADIATA

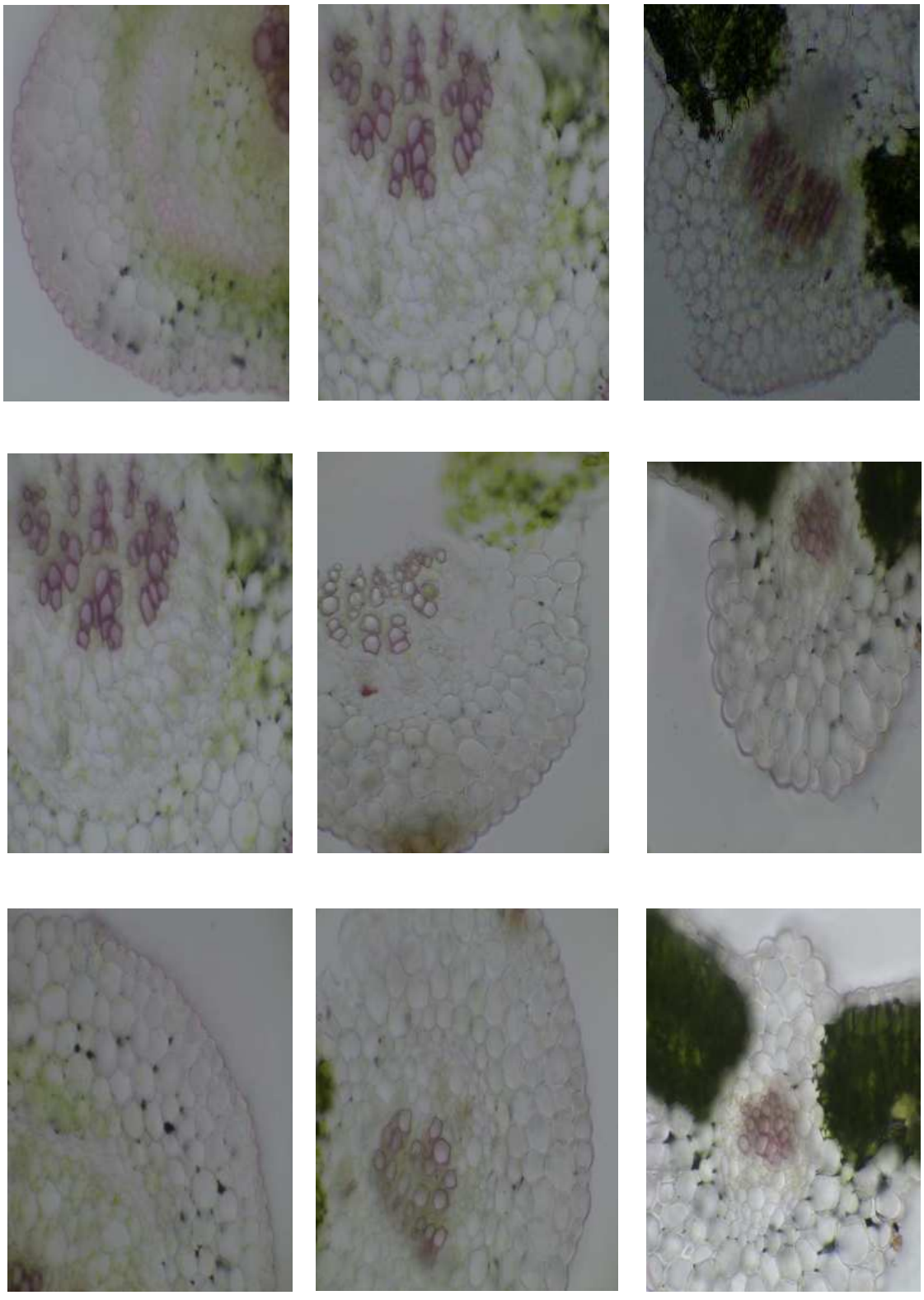


Plate 8

EFFECT OF NACL ON THE STEM ANATOMY OF
VIGNA UNGUICULATA

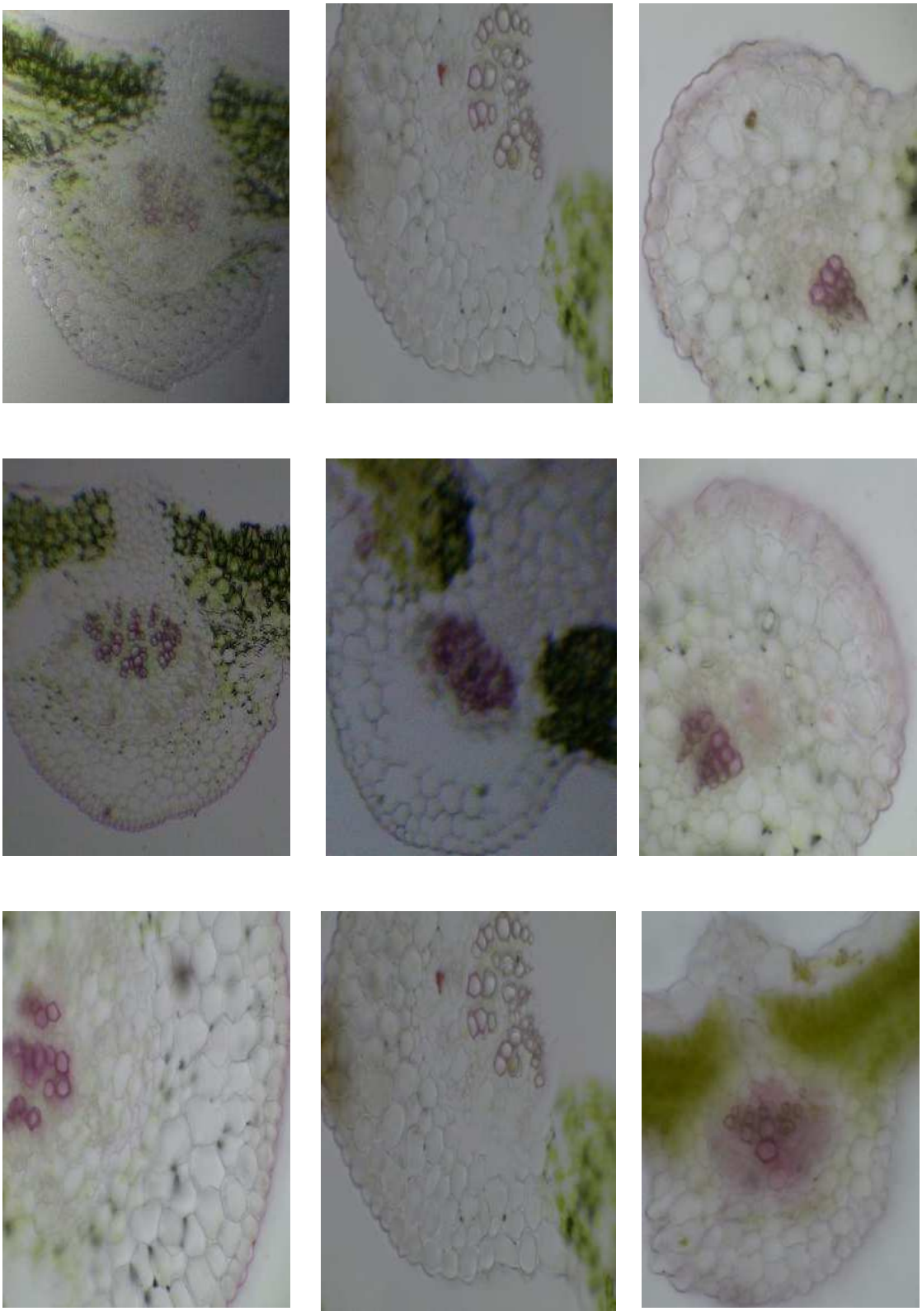


Plate 9

EFFECT OF NACL ON THE STEM ANATOMY OF

Stem Anatomy:

he stem anatomy of NaCl treated *Macrotyloma uniflorum* (Lam.) Verdc,

Plate 8 – 10. The treated plants showed great variation in the epidermal cells, thickness of palisade parenchyma and in the number of xylem cells. The maximum destruction was observed in the stem of *Vigna unguiculata* which is been treated with 150mM concentration of NaCl. **Yang and Guo (2018)** stated that the width of vascular bundles and diameters of rice stems decreased in NaCl medium.

Root Anatomy:

The root anatomy of NaCl treated *Macrotyloma uniflorum* (Lam.) Verdc, *Vigna radiata* (L) Wilczek, and *Vigna unguiculata* (L) Walp was shown in **Plate 10 – 13.** The treated plants showed great variation in the epidermal cells, thickness of palisade parenchyma and in the number of xylem cells. The maximum destruction was observed in the leaves of *Vigna unguiculata* which is been treated with 150mM concentration of NaCl. Salt stress increased the flowing resistance of water from roots to leaves, reduced vascular tissue transportation efficiency, and restricted the transportation of water due to dissolved salt ions absorbed by the roots (**Narsing Rao *et al.*, 2019).**

Plate 10

EFFECT OF NACL ON THE STEM ANATOMY OF
MACROTYLOMA UNIFLORUM

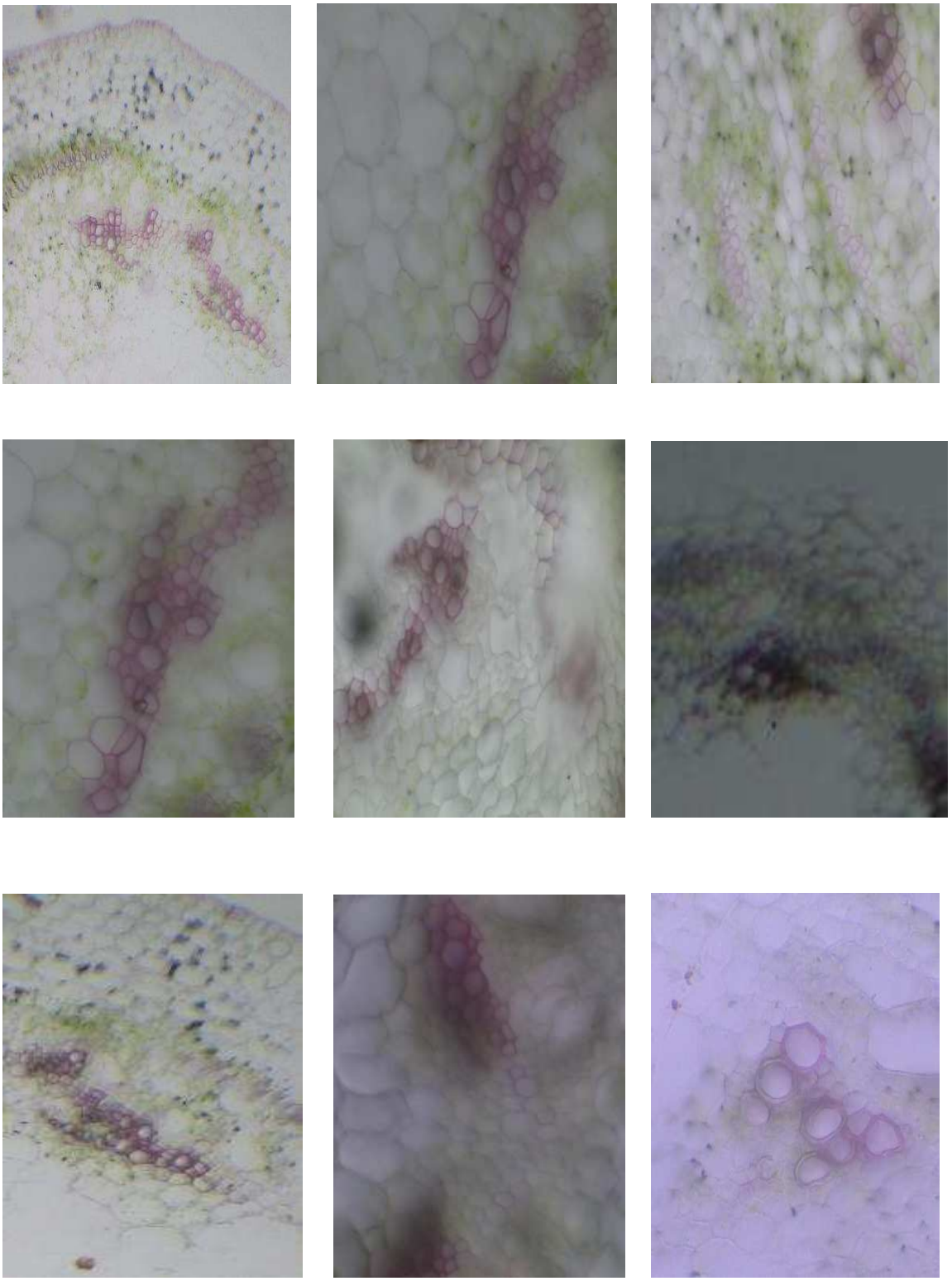


Plate 11

EFFECT OF NACL ON THE STEM ANATOMY OF

VIGNA RADIATA

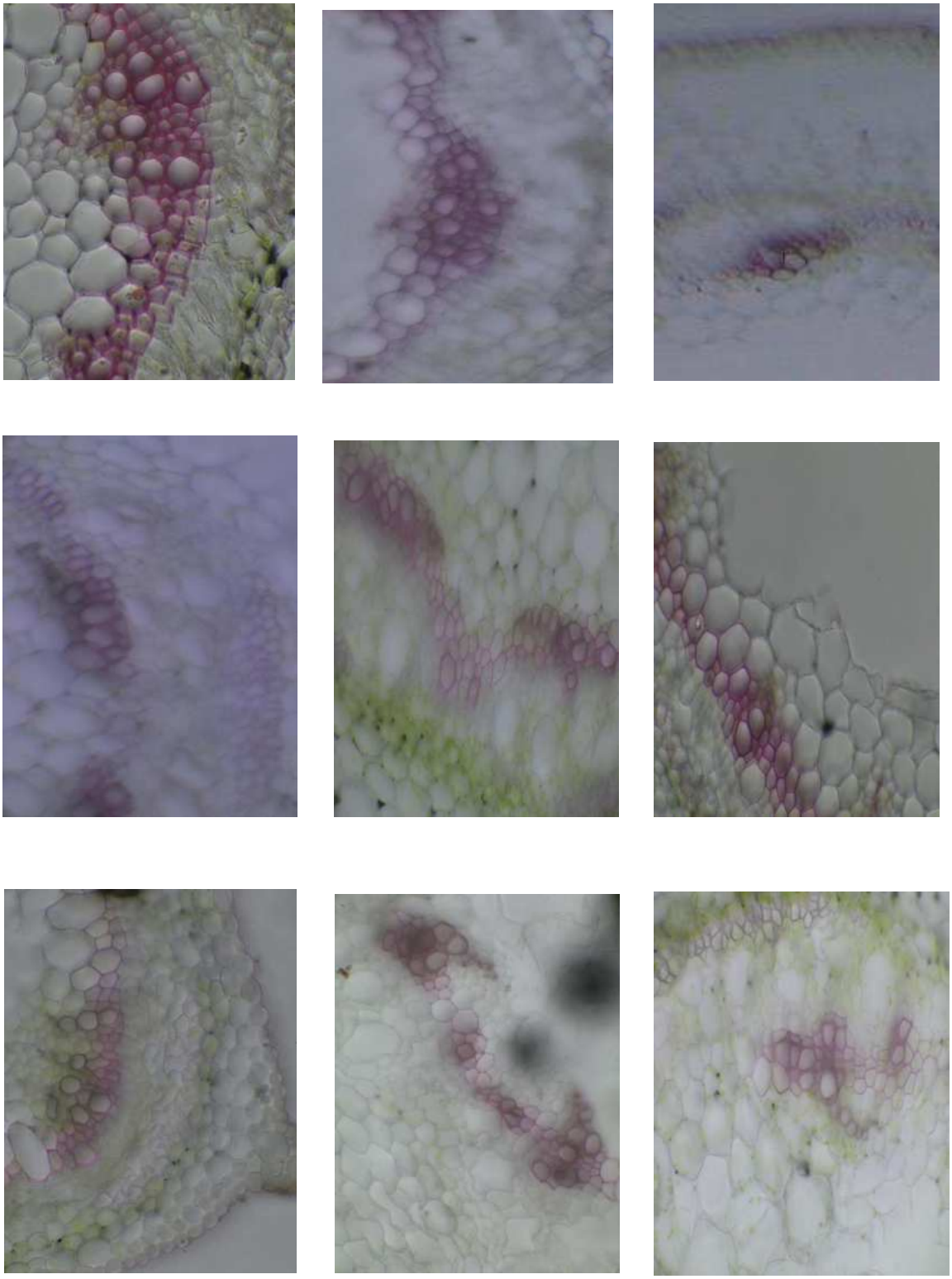


Plate 11

EFFECT OF NACL ON THE ROOT ANATOMY OF

VIGNA UNGUICULATA

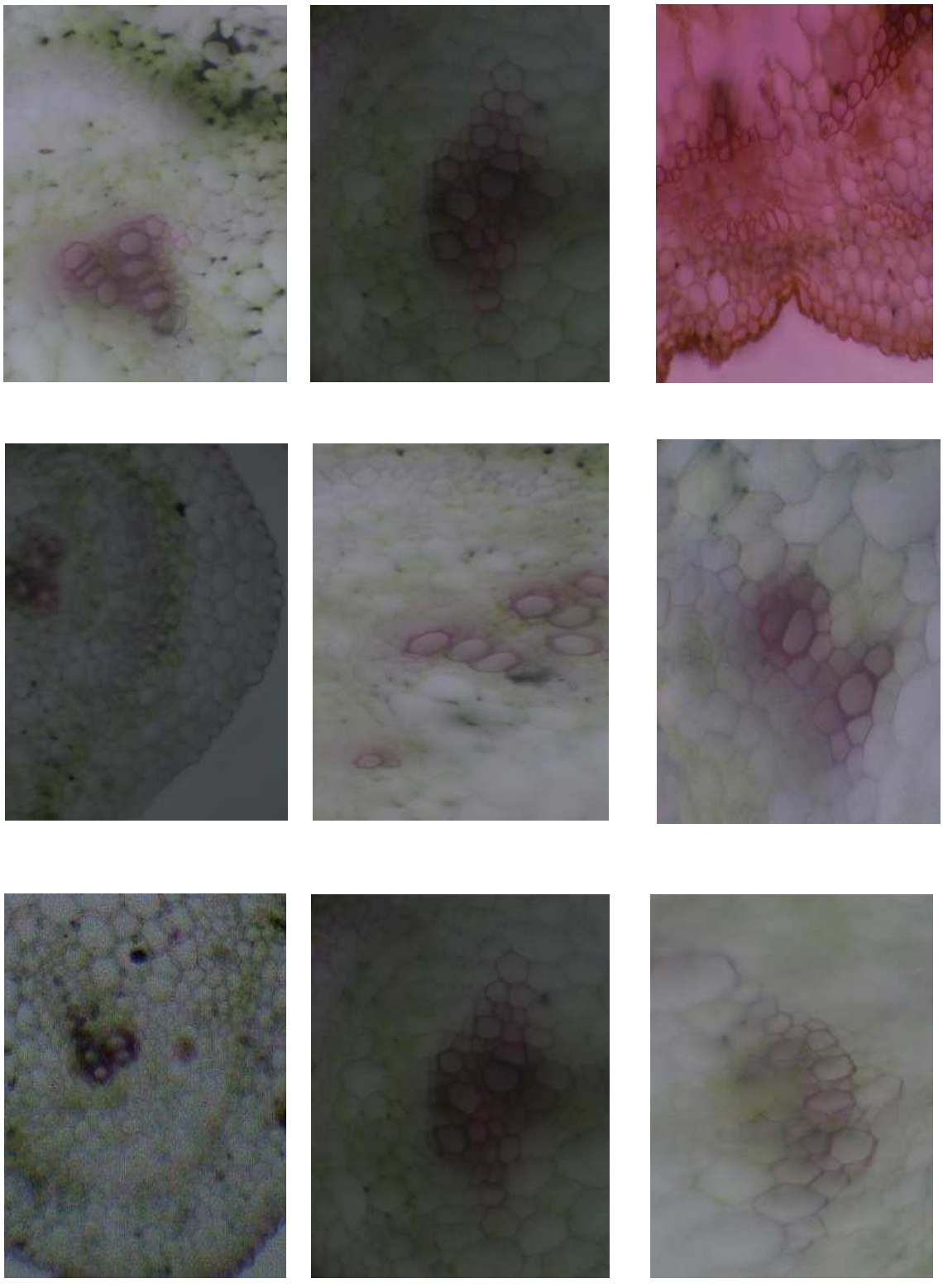


Plate 12

EFFECT OF NACL ON THE ROOT ANATOMY OF
MACROTYLOMA UNIFLORUM

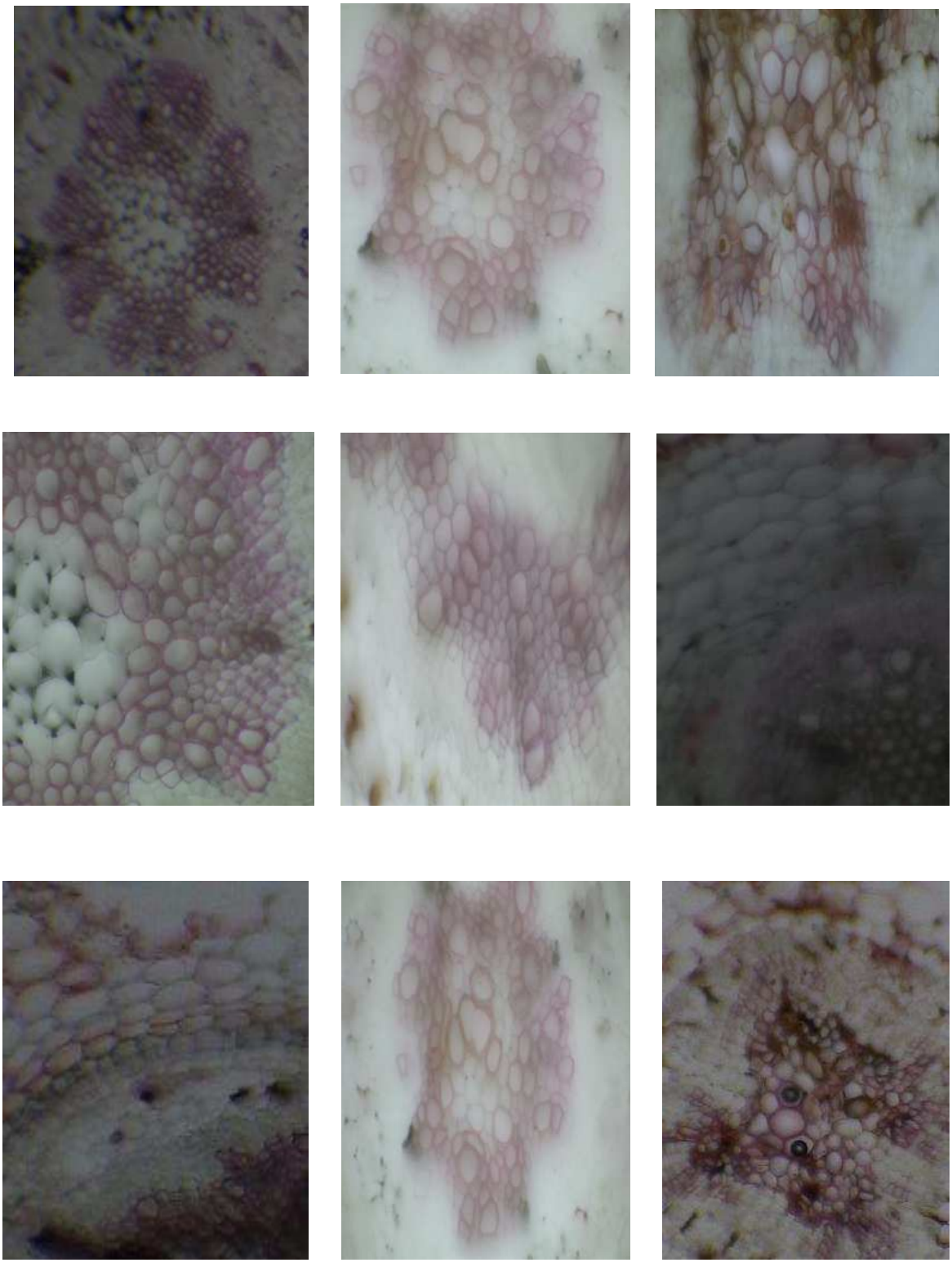


Plate 13

EFFECT OF NACL ON THE ROOT ANATOMY OF

VIGNA RADIATA

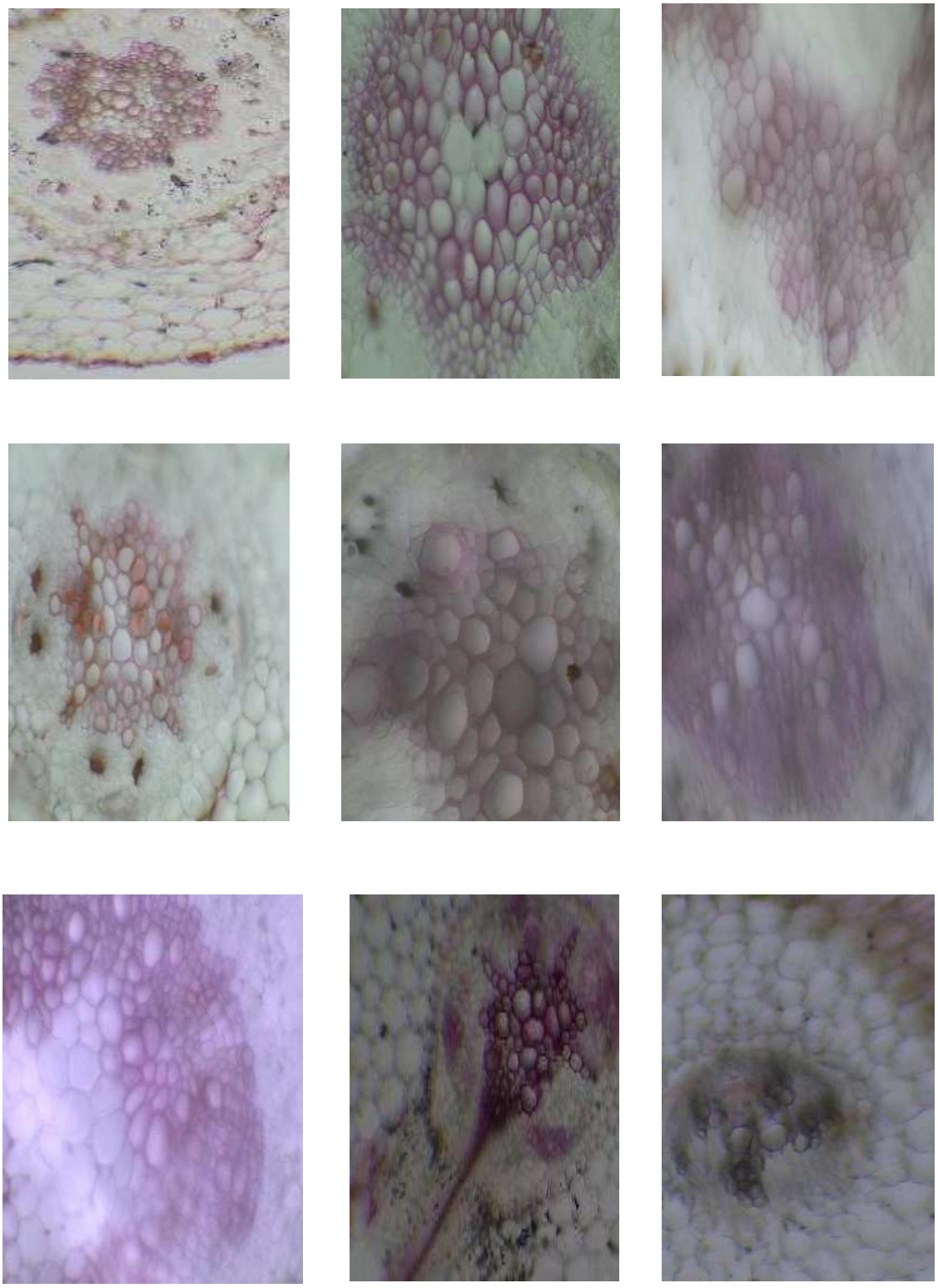
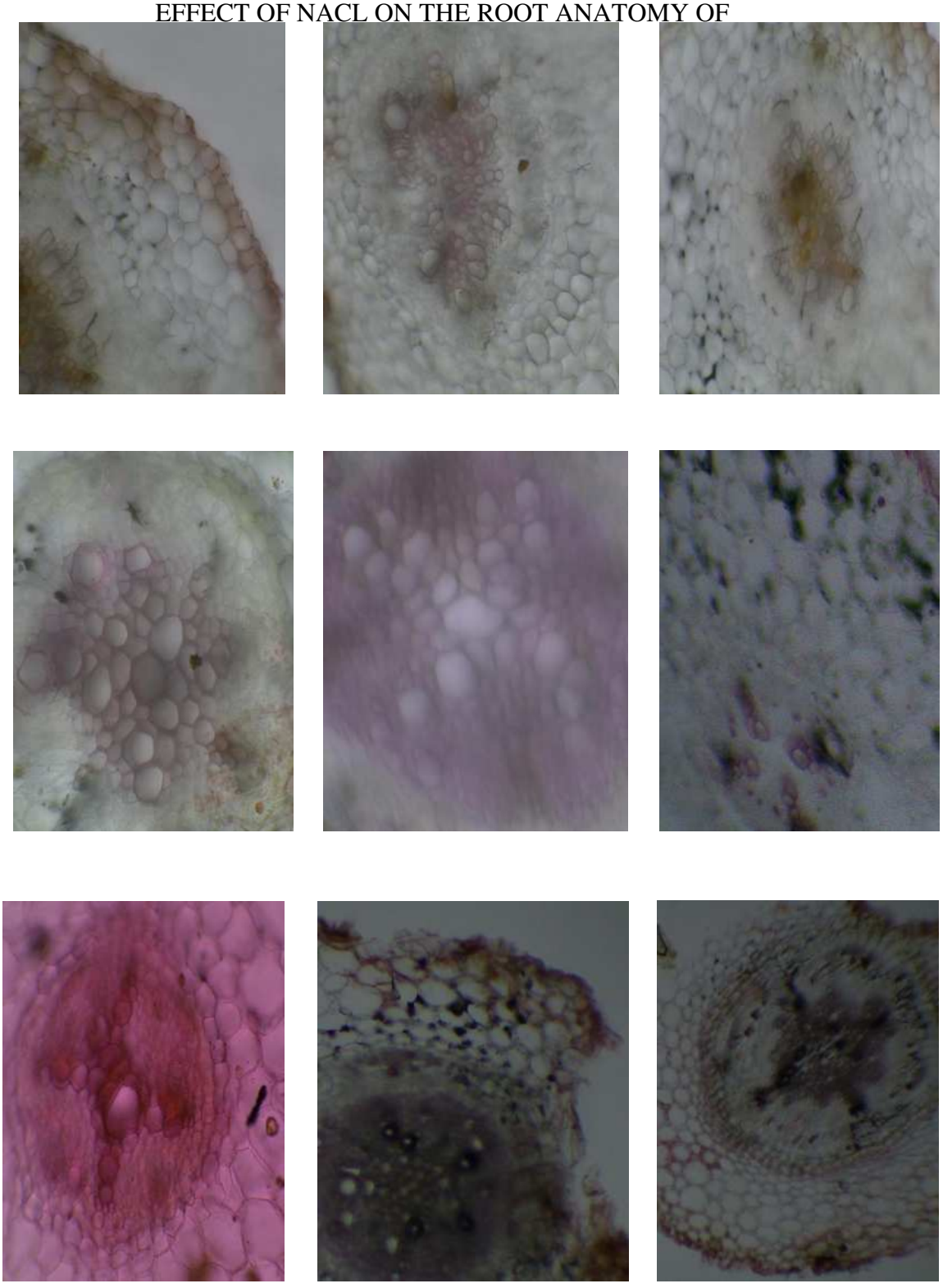


Plate 14

VIGNA UNGUICULATA

EFFECT OF NACL ON THE ROOT ANATOMY OF



SUMMARY AND CONCLUSION

Plate 15

EFFECT OF NACL ON THE ROOT ANATOMY OF

Salt stress is a condition where excessive soil solution causes inhibition of plant growth and leading to plant death. Soil salinization is one of the major factors of soil degradation. It has reached 19.5 % of the irrigated land and 21 % of the dryland agriculture. Salinity also affects the germination of seeds by creating an external osmotic potential that prevents water uptake due to the toxic effects of Na^+ and Cl^- ions on the germinating seeds.

The effect of NaCl salinity on morphological and anatomical parameters of *Macrotyloma uniflorum* (Lam.) Verdc, *Vigna radiata* (L) Wilczek, and *Vigna unguiculata* (L) Walp were studied in this project work. Different concentration of sodium chloride was prepared and applied to the plants grown in the pots.

The morphological characters such as root length, shoot length, leaf length, petiole length, leaf number, fresh weight, dry weight and leaf area decreased with increasing salt concentrations. The characters were studied after 20^{th}, 25^{th} and 30^{th} day of treatment with NaCl.

The morphological and anatomical parameters of *Macrotyloma uniflorum* (Lam.) Verdc, *Vigna radiata* (L) Wilczek, and *Vigna unguiculata* (L) Walp showed many variations when they are subjected to salt stress. The plants can tolerate low concentration of NaCl such as 50mMand 100mM as the concentration increases it shows adverse effects.

As the concentration of the salt increases the anatomy of the selected plants also showed great variation the epidermal thickness the number of palisade parenchyma cells and others xylem cells a varied in all the parts of the selected plants

as the concentration increases they show adverse effects in the air conduction tissues also

Plate 1

EFFECT OF NACL ON THE ROOT ANATOMY OF

As the scarcity of the fresh water gets increasing then becomes in the work done here may not provide sufficient information to raise a model crop for Salt resistance since the parameter analysed here assured and negative impact on the growth parameters of the plant, a new saline resistant variety may be a best attempt in the future.

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A COMPARATIVE STUDY OF PHYTOCHEMICAL ATTRIBUTES ANTIBACTERIAL, ANTHELMINTIC AND ANTICANCER ACTIVITIES OF SELECTED ACANTHACEAE MEMBERS

A dissertation submitted to

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MASTER OF SCIENCE IN BOTANY

By

L.KISHANIYA

Reg.No.19APBO08

DEPARTMENT OF BOTANY

ST. MARY'S COLLEGE (Autonomous)

THOOTHUKUDI-628001

APRIL - 2021

CERTIFICATE

This is to certify that this dissertation entitled, **A COMPARATIVE STUDY OF PHYTOCHEMICAL ATTRIBUTES ANTIBACTERIAL, ANTHELMINTIC AND ANTICANCER ACTIVITIES OF SELECTED ACANTHACEAE MEMBERS** submitted by **L.KISHANIYA, Reg.No. 19APBO08** to ST. MARY'S COLLEGE (Autonomous), THOOTHUKUDI in partial fulfillment for the award of the degree of **"Master of Science in Botany"** is done by her under my supervision. It is further certified that this dissertation or any part of this has not been submitted elsewhere for any other degree.

GUIDE

HOD M. Glory
HOD of Botany
St. Mary's College (Autonomous)
Thoothukudi - 628 001.

EXAMINER

Lucia Rose
PRINCIPAL
Mary's College (Autonomous)
Thoothukudi - 628 001.

DECLARATION

I do here by declare that this dissertation entitled **A COMPARATIVE STUDY OF PHYTOCHEMICAL ATTRIBUTES ANTIBACTERIAL, ANTHELMINTIC AND ANTICANCER ACTIVITIES OF SELECTED ACANTHACEAE MEMBERS** submitted by me in partial fulfilment for the award of the degree of **'Master of Science in Botany'**, in the result of my original and independent work carried out under the guidance of **Dr. Mrs. F. Dayana Lobo, M.Sc., M.Phil., Ph.D.,** Assistant Professor, Department of Botany, St. Mary's College (Autonomous). Thoothukudi and it has not been submitted elsewhere for the award of any other degree.

L. Bli

Station : Thoothukudi **(L. KISHANIYA)**

Date: 15.04.21

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CONTENT

Indian has an ancient heritage of traditional medicine. Materia Medica of India provides lots of information on the folklore practices and traditional aspects of therapeutically important natural products (Umesh et al., 2010). Out of 17.000 species of higher plants reported to occur within India, 7500 are known to have medicinal use. The various indigenous systems such as siddha, ayurveda, unani and allelopathy use several plant species to treat different ailments (Perumal,1997).

Medicinal plants possess therapeutic properties or exert beneficial pharmacological effects on the human and animal body (Ghani, 2003). Plants are the source of about 25% of prescribed drugs in the world (Rate *et al.,* 2001). In developing countries about 80% people rely on traditional plant based medicines for their primary health care needs. There is abundant number of medicinal plants and only small amounts of them were investigated for its biological and pharmacological activities. The wide range of medicinal plant parts like flowers, leaves, barks, stems, fruits, roots extracts are used as powerful raw drug, possessing a variety of pharmacological activities. Discovery of new pharmaceutical agents from

medicinal plants can combat the drastic increase in infectious diseases in many countries especially in rural areas and it has been used as an economic reason as well.

Nowadays, there is widespread interest of drugs derived from plants which reflect its recognition of the validity of many traditional claims regarding the value of natural products in health care (Nair *et al.*, 2005). Thus, in order to determine the potential use of medicinal plants, it is essential to intensify the study of medicinal plants that finds place in folklore. The application of herbs and medicinal plants in traditional medicine to diagnose, prevent or treat diseases dates back to many centuries among rural communities throughout the world (Conco *et al.*, 1999). The active Phytochemical produced by plants include, alkaloids, glycocide, betecyanin, anthraquinonen, flavonoids, phenols, saponins, steroid, tannins, terpenes, quinines, protein, carbohydrates (Cahular, 2010). In recent years, advances have been made in the development of antimicrobial compounds in an effort to check the harmful effects of microorganisms (Rao, 1995).

Bacterial disease results when the harmful bacteria enter the organism then multiply and invade the body's defence mechanism. These pathogenic bacteria enter the body through inhalation, ingestion or damaged skin tissue.

The inability of the immune system to stop the bacteria from reproducing and spreading consequently results in the symptoms of bacterial disease (Namukobea *et al.*, 2011). The antimicrobial resistance is the foremost problem all over the world with present antibiotic therapy in treating infectious diseases (Manikandan *et al.*, 2011). The development of drug resistance by microorganisms reduces the effectiveness of modern drugs (WHO, 2000). Thus, resistance to antibacterial agents poses threat in many areas of the world especially in the developing countries (Shears, 2000). The integration of traditional and modern medicine is gaining increase recognition globally (Abebe, 1996, WHO, 2000).

Adathoda vasica belongs to the family Acanthaceae. It comprises almost 250 genera with 2500 species, Its species are widespread in tropical regions of the world and are poorly represented in temperate regions (Geone *et al.*, 2011). *Adathoda vasica* has mucolytic, expectorant and bronchodilator action, so it is greatly used in respiratory troubles, it relieves a cough, fights off respiratory infections and helps in the management of asthma. (Amandeep kaur *et al.*, 2011)

Andrographis paniculata called periyanagai belongs to the family Acanthaceae. *Andrographis paniculata* used in ancient oriental and

ayurvedic medicine. A paniculata, commonly known as king of Bitters or Kalmegh, is an annual, branched, erect handsome herb running half to one meter in height. (Vincent Imieje; et al 2014). So it is essential to work on medicinal plants (A. vasica and A. paniculata) to bring pharmaceutical values.

Plants derived natural products such alkaloids, flavonoids, terpenoids have received considerable attention in recent years due to their diverse pharmacological properties including antioxidant and anticancer activities. According to World Health Organisation (WHO), medicinal plants would be greatest source to obtain an array of drugs. Thus, such plants should be investigated to better understanding for their properties, safety practices in addition to usefulness. Selected plants such as *Adathoda vasica* and *Andrographis panniculata* are rich in medicinal properties and many people are not aware of the therapeutic activities of such medicinal plants. Hence the present investigation was undertaken with the following

Objectives:

1. To qualitatively screen the presence of different phytochemicals of ethanol, petroleum ether, chloroform and acetone extracts of leaves (*Adathoda vasica* and *Andrographis paniculata*).
2. To elucidate the effectiveness of medicinal plants in controlling human pathogenic bacteria such as (*Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus* and *Vibrio chlorae*).

3. To evaluate anthelmintic activity in leaf of *Adhatoda vasica* and *Andrographis paniculata* against *Eisenia fetida*.

4. To evaluate the anticancer potential of the *Andrographis paniculata* recent research has thrown light on the cultivation of this plants on a large scale because of its high therapeutic values.

Phytochemical Activity:

Medicinal plants are consumed as food by many Indian tribes in addition to being used as medicines to protect their health. Plants' medicinal properties are due to phytochemicals. Several pharmaceutical industries have sprung up as a result of this basic knowledge. Plant crude extracts/drugs can be used to identify phytochemical constituents that play a significant role in medicine (Savithramma *et al.*, 2011). There are non-nutritive chemicals that protect people from a variety of ailments. Phytochemicals are categorised into two categories: primary and secondary metabolites, which are graded according to their function in the body.

Poonam kulyal *et al.* (2010) have done the phytochemical investigation of the aerial parts of *Andrographis paniculata*, gives diterpenic constituents andrographolide, 14-deoxy-11,12-didehydroandrographolide, 14-deoxyandrographolide, 3,14-dideoxyandrographolide, 14-deoxy-11-oxoandrographolide, 14-deoxy-12-hydroxy andrographolide, neoandrographolide, andrographiside and 14-deoxyandrographiside. The

structures of these compounds have been established on the basis of spectral data analysis.

Muntha K. Reddy *et al.* (2003) have done phytochemical investigation of the roots and aerial parts of *Andrographis paniculata* Nees yielded a new flavone, 5-hydroxy 7, 2', 6'-trimethoxyflavone and an unusual 23-carbon terpenoid, 14-deoxy-15-isopropylidene-11, 12-didehydroandrographolide together with five known flavonoids and four known diterpenoids. The structures of these compounds were determined on the basis of spectral and chemical studies.

Antimicrobial Activity:

Karthikeyan *et al* (2009) studied the effect of ethanol, petroleum ether and water extracts were tested on *Staphylococcus aureus, Staphylococcus epidermidis, Bacillus subtilis, Enterococcus faecalis, Escherichia coli, Pseudomonas aeroginosa, Proteus vulgaris, Klesiella pneumoniae* and *Candida albicans* . The minimum inhibitory concentration of the crude extracts was determined for various organisms and found effective.

Kamlesh *et al*(2011) Antimicrobial screening of hot aqueous, methanolic and chloroform extracts at 125, 250 and 500 mg/ml concentrations by disc diffusion assay method (25 µL/disc) against selected Gram positive (*Staphylococcus aureus* -MTCC 7405, *Bacillus sp.* MTCC 4666) and Gram negative (*E. coli* – MTCC 1680, *Klebsiella sp.*- MTCC 4032) bacteria revealed that methanolic extract was moderately effective against *Staphylococcus aureus* and the zones of inhibition at 250 and 500 mg/ml concentrations were found to be 12.33+0.88 and 14.00+0.57 mm, respectively compared to the zone of inhibition of 19.33+0.57 mm of 0.02µg levofloxacin against *Staphylococcus aureus*. But hot methanolic extract almost lacked any such activity against rest of the three microbes. Hot chloroform and hot aqueous extracts were also found to be almost devoid of any antibacterial activity against these microbes.

Sarker *et al* (2011) reported anti microbial activity of the oil against *Bacillus subtilis, Salmonella typhimurium, Staphylococcus aureus,* and *E.coli.* It was found that all mentioned microorganisms were more or less sensitive to this essential oil.

Josephin Sheeba *et al.,*(2012) assesed the antimicrobial activity (MIC) of *Adhatoda vasica* against clinical pathogen solvents like methanol,

ethanol, acetone, chloroform, diethyl ether and water were used for the preparation of plant extracts in various concentrations by disc diffusion method the antimicrobial activity (MIC) was measured. From this, solvents showed higher activity in the order of diethyl ether > methanol > ethanol > acetone > Chloroform> water. The plant extract of *Adhatoda vasica* showed higher activity for different clinical pathogens in the order of *Klebsiella pneumoniae>Staphylococcus aureus > Proteus vulgaris >Pseudomonas aeruginosa > Streptococcus Pyogens.* ,

Rashmi *et al* (2012) investigated the antimicrobial activity of methanolic leaf extracts of *Justicia adhatoda* and vasicine against *Staphylococcus aureus, Streptococcus pyogenes, Serratia marcescens, Klebsiella pneumoniae, Escherichia coli, Pseudomonas aeruginosa, Candida albicans, Cryptococcus neoformans* and *Aspergillus flavus*. Studies on the minimum inhibitory concentration of the extracts on the test organisms showed that the lowest minimum inhibitory concentration and minimum microbicidal concentrations were demonstrated against *Serratia marcescens, Escherichia coli and Pseudomonas aeruginosa* and the highest minimum inhibitory concentration was exhibited against *Staphylococcus aureus, Streptococcus pyogenes, Klebsiella pnuemoniae.*

Sermakani *et al.* (2011) have designed with the objective to examine the petroleum ether, acetone, chloroform and methanol extracts of *Andrographis paniculata* leaves and stems, in order to evaluate the chemical composition, investigate it's *in vitro* antimicrobial potential against strains of *Enterococcus faecalis, Streptococcus pyogenes, Klebsiella pneumonia, Proteus vulgaris, Candida albicans and Aspergillus flavus.* These results may justify the popular use of this species as it has antimicrobial activity. However, in order to evaluate possible clinical application in therapy of infectious diseases, further clinical trials are required.

Singha *et al.* (2003) have evaluated the antimicrobial activity of aqueous extract, andrographolides and arabinogalactan proteins from *Andrographis paniculata.* The aqueous extract showed significant antimicrobial activity.

Ignacimuthu *et al. (2010),* evaluated Antimycobacterial activity of two natural alkaloids, vasicine acetate and 2-acetyl benzylamine, isolated from Indian shrub *Adhatoda vasica* Ness. leaves In folk medicine, *Adhatoda vasica* Ness. (Acanthaceae) is used to treat asthma and cough. The leaves of *A.vasica* were powdered and extracted with hexane, ethyl acetate and methanol. The hexane extract showed 97% reduction in colony-forming

units (CFU) at 100 µg/ml. The hexane extract was subjected to column chromatography. Two natural compounds, vasicine acetate and 2-acetyl benzylamine, were isolated from it.

Abubacker and Vasantha Ratha 2011 studied the antibacterial effect of ethanolic leaf extract of *A. paniculata* against *Escherichia coli; Klebsiella pneumonia, Proteus vulgaris* and *Streptococcus pneumonia* by disc diffusion method were identified. The results revealed that the ethanolic leaf extract and andrographolide compound isolated from the leaves are potent in inhibiting these bacteria and the work highlights that the inhibitory effect is on par with standard antibiotics.

Bobbarala *et al.*, 2009 reported the antibacterial activity of the Hexane, Chloroform and Methanolic extracts of *Andrographis paniculata* against tested organisms. The growths of all bacterial pathogens were highly inhibited by methanolic extracts of *Andrographis paniculata* than chloroform and hexane extracts respectively. The methanolic extracts inhibited the growth of 95% organisms tested, followed by chloroform extracts inhibited 80%. Hexane extracts inhibited 65% growth of the tested organisms. In vitro screening of the aqueous extract of *A. paniculata* posses'

potential antibacterial activity towards both gram-positive and gram-negative microorganisms.

The antimicrobial activity of aqueous extract of *Andrographis paniculata* andrographolides and arabinogalactan proteins from *A. paniculata* when evaluated, showed significant antimicrobial activity, which may be due to the combined effect of the isolated arabinogalactan proteins and andrographolides . Similar work conducted by Chakraborty *et al.* on the aqueous extract of *Andrographis paniculata* is more effective against *Staphylococcus aureus* than *Escherichia coli.* Minimum inhibitory concentration (MIC) value of *Andrographis paniculata* is 0.0009 mg/ml against *Staphylococcus aureus* and 0.001mg/ml against *Escherichia coli* (Zaidan *et al.*,2005).

Youhong *et al.*, 2009 investigated the extract of *A. paniculata* against nine human bacterial pathogens. They concluded that the observed antimicrobial activity was due to other active principles present in the extracts that were used in the investigation.

Antioxidant Activity :

Verma and Vinayak compared the antioxidant effects of the aqueous extract on liver defense systems in lymphoma bearing mice. The aqueous extract significantly increased the activities of catalase, superoxide dismutase and glutathione-S-transferase enzymes and reduced lactate dehydrogenase activity. Extracts prepared from cultivated *A. paniculata* and their active constituent andrographolide were evaluated for antioxidant, antioedema and analgesic activities. The results showed that the aqueous *A.paniculata* extract *(A. paniculata* water) exhibited a greater antioxidant activity than the ethanol *A. paniculata* extract (*A. paniculata*-Ethanol) in all model systems tested. At a concentration of 50 µg/ml, the free radical scavenging xanthine oxidase inhibition and antilipid peroxidation activities for *A. paniculata*-water were 66.8%, 57.3% and 65.3%, respectively, and for *A.paniculata*-Ethanol were 57.8%, 52.6% and 34.2% respectively. It has been reported that *A. paniculata*-water was more potent than *A.paniculata* - Ethanol in antioxidant activities Verma *et al.*, 2008).

A rapid method based on HPTLC and RP-HPLC with UV detection for quantitative determination of two major bioactive compounds in *A. paniculata,* andrographolide and 14-deoxy-11, 12-didehydroandrographolide is described. The recoveries of the two

compounds were between 96.8–99.0% by HPTLC method and 98.1–99.2% by HPLC assay. The relative standard deviations of the two compounds ranged between 0.89–0.99 (intra-day) and 0.86–0.98 (inter-day) for the HPTLC method and 0.86–1.02 (intra-day) and0.87–1.12 (inter-day) for HPLC method. The methods were used for routine analyses and to obtain relative amount of the two compounds in the leaves of the plant cultivated in different locations of Malaysia. The extracts and isolated compounds exhibited lipid peroxidation inhibition and free radical activities (Akowuah et al., 2006).

Sharma *et al.*, 2011 focused on the anti-oxidant potency of aqueous, methanol and ethanol extracts of *Andrographis paniculata*. The methanolic extracts of leaves of *Andrographis paniculata* showed promising anti-oxidant activity. Results suggest that the active antioxidant compounds are better extracted in methanol for *Andrographis paniculata*. Results also suggest that there is a direct co-relation between the total polyphenols extracted and anti-oxidant activity. Free radical scavenging potential of various extracts (methanol, ethanol and aqueous) of *A. paniculata*. In this method, ascorbic acid was used as a standard of determining reducing power. The methanol extract of the leaves of *A. paniculata* exhibited

appreciable activity as compared to the aqueous and ethanol extracts, indicating that *A. paniculata* has promising free radical scavenging activity Anuratha *et al.*, (2010).

Anticancer Activity :

Chun and his co-workers (2010) reported that elevated interleukin-6 (IL-6), a major mediator of the inflammatory response, has been implicated in androgen receptor (AR) activation, cellular growth and differentiation, plays important roles in the development and progression of prostate cancer, and is a potential target in cancer therapy. Through drug screening using human prostate cancer cells expressing IL-6 autocrine loop, they found that andrographolide, a diterpenoid lactone isolated from a traditional Chinese and Indian medicinal plant Andrographis paniculata, could inhibit IL-6 expression and suppress IL-6–mediated signals. Andrographolide inhibits IL-6 expression at both mRNA and protein levels in a dose-dependent manner. Andrographolide suppresses both IL-6 autocrine loop– and paracrine loop–induced cell signaling including Stat 3 and Erk phosphorylation. Furthermore, andrographolide inhibits cell viability and induces apoptotic cell death in both androgen-stimulated and castration-

resistant human prostate cancer cells without causing significant toxicity to normal immortalized prostate epithelial cells. Moreover, treatment of andrographolide to mice bearing castration-resistant DU145 human prostate tumors that express constitutive IL-6 autocrine loop significantly suppresses tumor growth. These results demonstrate that andrographolide could be developed as a therapeutic agent to treat both androgen-stimulated and castration-resistant prostate cancer possibly by suppressing IL-6 expression and IL-6 induced signaling.

Rajeshkumar *et al* ., 2015 studied the *Invitro* anticancer properties of *Andrographis* paniculata leaves against neuroblastima (IMR-32) and human colon (HT-29) cancer cell line. The leaves were shade dried and extracted with water, ethanol and acetone solvents. Anticancer property of *A. paniculata* leaf extract was analyzed by Spectrophotometric MTT assay method. The results were found that ethanol extract showed nearly 50% i.e. inhibition concentration (IC50) for IMR-32and HT-29cell lines at 200 µg/ml, where other extracts display 50% inhibition at 250 µg/ml concentration for HT-29cell lines. Anticancer activity of water, ethanol and acetone extracts of A. paniculata leaves against HT-29 cancer cell lines shows 50% inhibition at 200 µg/ml concentration. The significant

difference is statistically analyzed as $p<0.01$ for ethanol extract and acetone extracts. From the analysis we found that extracts of A. paniculata shows excellent anticancer activities against different cancer cell lines, it is alternatives medicines for cancer would replace side effect causing chemotherapeutic agent.

Daryush Talei *et al* ., 2013 to investigate the potential of salt stress to enhance the accumulation of the anticancer phytochemicals in *Andrographis paniculata* accessions. For this purpose, 70-day-old plants were grown in different salinity levels (0.18, 4, 8, 12, and 16 dSm^{-1}) on sand medium. After inducing a period of 30-day salinity stress and before flowering, all plants were harvested and the data on morphological traits, proline content and the three anticancer phytochemicals, including andrographolide (AG), neoandrographolide (NAG), and 14-deoxy-11,12-didehydroandrographolide (DDAG), were measured. The results indicated that salinity had a significant effect on the aforementioned three anticancer phytochemicals. In addition, the salt tolerance index (STI) was significantly decreased, while, except for DDAG, the content of proline, the AG, and NAG was significantly increased. Furthermore, it was revealed that significant differences among accessions could happen based on the total dry weight, STI, AG, and NAG.

Finally, we noticed that the salinity at 12 dSm^{-1} led to the maximum increase in the quantities of AG, NAG, and DDAG. In other words, under salinity stress, the tolerant accessions were capable of accumulating the higher amounts of proline, AG, and NAG than the sensitive accessions.

Vidhaya Menon and Sujata bhat, 2010 tested andrographolide **1**, a diterpene lactone of *Andrographis paniculata*, displays *in vitro* and *in vivo* antitumor activity against breast cancer models and mouse myeloid leukemia (M1) cells. In the present study, we report the semi-synthesis of andrographolide derivatives and there *in vitro* activity against A549 (ATCC) (NSCL cancer) cell line. Amongst the derivatives tested, compounds **3-5** displayed maximum activity, with IC_{50} values of 22-31 μg/mL.

Riffat Battol *et al.*, 2017 studied the ethanol extract of *Foeniculum vulgare* exhibited significant inhibition of cancer cells proliferation. Methanol extract of *Justicia adhatoda* also showed considerable inhibition of cancer cells. Future studies must converge on detailed investigation of modes of action of extracts of tested plants.

Adhatoda vasica is one such plant which is used widely for a variety of purposes which forms part of many other traditional herbal medicines. Biologically active compounds present in the plants are responsible for the such curative activity. These compounds could be identified by dissolving them in appropriate solvents. In the present study water, methanol and ethanol were used as solvents. Most of the secondary compounds were found in all the solvents but methanol gave positive result more than the other. Quantification of important solvents showed that high tannin content 65.61 µg/ml followed by saponins 19.09 µg/ml and alkaloids 12.87 µg/ml with methanol crude extract of *A. vasica*. GCMS analysis of methanol crude extract of A. vasica showed 21 compounds of alcohols, steroids, ester, etc. mostly having antimicrobial and antioxidant property. Antimicrobial property with well diffusion method showed effective antimicrobial activity for *B. subtilis* 12.17mm followed by *V. cholera* 11.83mm and K. pneumonia 11.50mm. Antioxidant activity was performed with DPPH assay and ABTS assay with DPPH assay 81.81% antioxidant activity was recorded at 160µg/ml and 94.84% antioxidant activity with 160µg/ml using methanol crude extract of *A. vasica*. Anticancer property was estimated through cytotoxicity study with cell lines showed least IC50 with HeLa 88.24 µg/ml

followed by MCF 92.80 µg/ml HepG2 111.08 µg/ml cell lines using methanol crude extract of A. vasica (Gopinath *et al* ., 2018)

Anti-helminthic activity:

The ethyl acetate, methanol and aqueous extracts from the whole plant of *Andrographis echioides* were investigated for their anthelmintic activity against *Pheretima posthuma*. The plant parts were used for cuts, scorpion sting, snake bite and stomach ache. Various concentrations (10, 25 and 50 mg/ml) of each extract were tested in the bioassay, which involved the determination of time of paralysis and death of the earth worms. Distilled water and Albendazole were used as control and standard respectively. The results revealed that the test extracts of *Andrographis echioides* exhibited significant anthelmintic activity at concentration of 50 mg/ml. The use of *A. echioides* as an anthelmintic has been confirmed and further studies are suggested to isolate the active principles responsible for the activity. (Padma *et al.*,2012).

Al-Shaibani *et al.*, 2011 studied the ovicidal and larvicidal properties of AV extracts against gastrointestinal nematodes of sheep *in vitro*. The aqueous and ethanolic extracts of the plant at 25-50 mg/ml concentration

were studied and shown to be ovicidal and larvicidal. The effect was dose dependent and ethanolic extract was more effective. The highest ED50 values of AV extracts were recorded against the eggs of *Chaberita ovina* (18.2 mg/ml for both the extracts). The lowest values were recorded against the eggs of *O. circumcinta* as 12.59 and 11.48 mg/ml for ethanolic and aqueous extracts, respectively. Similarly, the ED50 values of AV extracts against larvae, the highest ED50 values for *O. Columbianum* was 19.5 and 18.62 mg/ml and lowest against the *H. contotus* larvae : 15,14 and 12.88 mg/ml for aqueous and ethanolic extracts respectively.

Muraliu *et al.,* 2014 studied the antihelminthic activity of the aqueous extract of *Andrographis paniculata* Nees. leaves against the adult Indian earthworms *Pheretima posthuma* due to its anatomical and physiological resemblance with the intestinal roundworm parasites of human beings. Several plant species are administered orally to control the various diseases in our country. Some of these plants have been pharmacologically provided to be of some value and maybe a popular remedy for the treatment of various ailments. The growth of knowledge to cure disease continues at an accelerating pace and number of new plant-derived drugs increase likewise. Herbal medicine is currently experiencing a revival in Western society along

with other complementary therapies such as traditional Chinese Medicines, Osteopathy and Homeopathy. In this context, the present study is the first milestone with particular emphasis on the application of *Andrographis paniculata* Nees. (Kalmegh) the medicinal plant, their antihelminthic effect, and phytochemical screening, for their better formulation and controlling the various diseases in future. The aqueous extract was found to be more potent, and activities are compared with the drug piperazine citrate as a reference drug.

Padma *et al.*, (2012) investigated the ethyl acetate, methanol and aqueous extracts from the whole plant of *Andrographis echioides* were investigated for their anthelmintic activity against *Pheretima posthuma*. The plant parts were used for cuts, scorpion sting, snake bite and stomach ache. Various concentrations (10, 25 and 50 mg/ml) of each extract were tested in the bioassay, which involved the determination of time of paralysis and death of the earth worms. Distilled water and Albendazole were used as control and standard respectively. The results revealed that the test extracts of *Andrographis echioides* exhibited significant anthelmintic activity at concentration of 50 mg/ml. The use of *A. echioides* as an anthelmintic has

been confirmed and further studies are suggested to isolate the active principles responsible for the activity.

Somnath *et al.*, (2015) studied that the ethanolic, chloroform, acetone, and aqueous extracts of the leaves of *Adhatoda vasica Nees* produced anthelmintic activity against african earthworm *Eudrilus eugeniae.* Various concentrations (50 mg/ml) of aqueous and ethanolic extracts were evaluated in the bioassay involving determination of time of paralysis (P) and time of death (D) of the worms. Albendazole was used as standard anthelmintic drug and distilled water was used as negative control. The results of the present study indicated that the ethanolic and aqueous extracts significantly exhibited paralysis of worms in lower doses (10, 25 and 50 mg/ml) and also caused death of worms at higher concentration of 50 mg/ml, as compared to standard drug. Further studies are in process to isolate the active principle responsible for the activity.

Collection of plants:

Fresh leaves of *Adhathoda vasica* and *Andrographis paniculata Nees* were collected from St. Mary's College (Autonomous) Thoothukudi, Tamilnadu, India. Fresh leaves of *Adhathoda vasica* and *Andrographis paniculata* were washed thoroughly to remove the dust adhered than shade dried. The dried sample was ground to a fine powder. The powdered sample was bagged in a polythene bag and placed in an airtight container until extraction.

Preparation of plants Extraction:

The 10g of powered sample (*Adhathoda vasica* and *Andrographis paniculata*) were successively extracted with the help of polar and non polar solvent like 200ml of ethanol, acetone, chloroform and petroleum ether by Soxhlet apparatus. The filtrate was evaporated to dryness at reduced pressure in vaccum evaporator

Qualitative Phytochemical Activity:

Phytochemical analysis was carried out for all extracts using standar methods

(Horbone1984, Kokate *et al.*, 1995, Harborne, 1998).

Test for Alkaloid (Wagner's test):

About 1 ml of extract was taken and few drops of Wagner's reagent was added and the formation of a reddish brown precipitate indicate the presence of alkaloids.

Test for Flavonoid (Shinoda Test):

About 1 ml of extract was added to pinch of magnesium turnings and 1-2 drops of concentrated hydrochloric acid was added. Formation of pink color indicated the presence of Flavonoids.

Test for Phenol (Lead acetate test):

About 1 ml of extract was taken and 0.5 ml of 1% lead acetate solution was added and the formation of precipitate indicated the presence of tannins and phenolic compounds.

Test for Quinines:

A small amount of extract was treated with concentrated HCl and observed for the formation of yellow colour precipitate.

Test for Tannin (Ferric chloride test):

About 1 ml of extract was taken and 0.5 ml of 5% ferric chloride was added. The development of dark bluish black color indicated the presence of tannins.

Test for Steroid:

About 1 ml of extract was dissolved in 2 ml of chloroform and equal volume of concentrated sulphuric acid was added along the sides of the test tube. Appearance of brown ring indicated the presence of steroids.

Test for Carbohydrate (Benedict's test):

About 5 ml of Benedict's solution was added to 1 ml of extract and boiled in water bath. The appearance of red or yellow or green precipitate indicated the presence of reducing sugars.

Test for Saponin (Foam test):

About 0.5 mg of extract was diluted with 20 ml distilled water and shaken well in a graduated cylinder for 15 min. The formation of foam to a length of 1cm indicated the presence of saponin.

Test for Glycoside:

About 0.5 ml of extract was dissolved in 1 ml of water and then aqueous NaOH solution was added. Formation of yellow color indicated the presence of glycosides.

carbonate solution was added, and mixed. The control contained all the reagents except the sample. After 30 min of incubation at 37°C, absorbance was measured at 750 nm (UV-visible spectrophotometer – Model expressed No: UV 2371). Total phenolics were expressed as gallic acid equivalent (GAE) per gram dry weight

Total Flavonoid (Zhinshen *et al.*, 1999)

100 mg of plant sample was homogenized with 10 ml of distilled water and filtered through muslin cloth. 250 µl aliquot of the extract was mixed with 1.25 ml of distilled water and 75 µl of 5% $NaNO_2$ solution. After 6 minutes, 150 µl of 10% $AICI_3.H_2O$ solution was added. After 5 minutes, 0.5 ml of IM NaOH solution was added and then the total volume was made up to 2.5 ml with distilled H_2O Following thorough mixing of the solution, absorbance against blank was determined at 510 nm. Quercetin was used as standard and the results were expressed as mg quercetin equivalents (QEVg dry weight).

Antibacterial activity- Disc diffusion Assay :

Antibacterial activity of each plant extract was analysed using human pathogens, Gram positive bacteria, *B.subtilis* and *S.aureus* and Gram negative bacteria *E.coli* and *V.chlorae* obtained from the Department of

Microbiology St. Mary's College (Autonomous), Thoothukudi. Each bacterial pathogen was subcultured in agar medium and maintained. What man No:1 sterile filter paper discs (5mm) were impregnated with 1 mg/ mlconcentration and dried aseptically at room temperature. The spread plates were prepared by proper concentration of inocula. Each sample loaded disc was placed in the seeded agar plate. After 24-48 hrs of ± 37° C incubation, the diameter of the inhibition zone was measured, for positive control, streptomycin disc (100µg/ml) was used; where as for negative control, respective solvents were loaded on sterile discs.

Anthelmintic Activity (Kumar *et al., 2010*):

Preparation of extract:

Dried plant powder (*Adhathoda vasica* and *Andrographis paniculata*) (10 gm) was extracted with 200 ml of petroleum ether, acetone, ethanol and chloroform for 24 hrs. The extracts were filtered through filter paper (What man No.1). The filtrate was collected and concentrated till a syrupy mass was obtained and dried at room temperature. The dried extracts were dissolved in normal saline and used for anthelmintic activity.

...experimental animal:

... its anatomical and physiological similarity to human intestinal ... anthelmintic operation was conducted on adult earthworms *Eisenia fetida*. The *Eisenia fetida* were collected and identified from the department of Agriculture, St. Mother Theresa Engineering college, Thoothukudi.

Experimental design :

In the present investigation the earthworms were divided into the following 6 groups. Each group consists of 3 earthworms.

Group I : Earthworms were placed in normal saline and served as control

Group II : Earthworms were placed in acetone extract of plants (*Adhathoda vasica, Andrographis paniculata) at* the dose of 50mg/ml.

Group III : Earthworms were placed in chloroform extract of (*Adhathoda vasica, Andrographis paniculata)*at the dose of 50 mg/ml.

Group IV: Earthworms were placed in ethanol extract of (*Adhathoda vasica, Andrographis paniculata)at* the dose of 50 mg/mL.

Group V: Earthworms were placed in petroleum ether extract of (*Adhathoda vasica, Andrographis paniculata)* at the dose of 50 mg/ml.

Group VI: Earthworms were placed in standard drug albendazole at the dose of 50 mg/ml served as standard.

Anticancer activity:

IN VITRO STUDIES

CYTOTOXICITY ACTIVITY

MTT ASSAY

Principle

This Colorimetric assay is based on the capacity of Mitochondria succinate dehydrogenase enzymes in living cells to reduce the yellow water soluble substrate 3- (4, 5-dimethyl thiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) into an insoluble, coloured formazan product which is measured spectrophotometrically. Since reduction of MTT can only occur in metabolically active cells, the level of activity is a measure of the cells (Wilson, 1983 & Masters, 2000).

MTT-Assay-Chemicals

3-(4,5–dimethyl thiazol–2–yl)–5–diphenyl tetrazolium bromide (MTT), Fetal Bovine serum (FBS), Phosphate Buffered Saline (PBS), Dulbecco's Modified Eagle's Medium (DMEM) and Trypsin were obtained from Sigma Aldrich Co, St Louis, USA. EDTA, Glucose and antibiotics from Hi-Media Laboratories Ltd., Mumbai. Dimethyl Sulfoxide (DMSO) and Propanol from E.Merck Ltd., Mumbai, India.

Cell Lines and Culture Medium

MCF-7 (Human, Breast cancer), cell cultures were procured from National Centre for Cell Sciences (NCCS), Pune, India. Stock cells were cultured in Dulbecco's modified Eagle's medium (DMEM). Medium was supplemented with 10% inactivated Fetal Bovine Serum (FBS), penicillin (100 IU/ml), streptomycin (100 ⌈g/ml) and amphotericin B (5 ⌈g/ml) in an humidified atmosphere of 5% CO_2 at 37°C until confluent. The cells were dissociated with TPVG solution (0.2% trypsin, 0.02% EDTA, 0.05% glucose in PBS). The stock cultures were grown in 25 cm^2 culture flasks and all experiments were carried out in 96 microtitre plates (Tarsons India Pvt. Ltd., Kolkata, India).

Preparation of Test Solutions

For cytotoxicity studies, each weighed test drugs were separately dissolved in distilled DMSO and volume was made up with DMEM supplemented with 2% inactivated FBS to obtain a stock solution of 1 mg/ml concentration and sterilized by filtration. Serially two fold dilutions were prepared from this for carrying out cytotoxic studies.

Determination of Cell Viability by MTT Assays

The monolayer cell culture was trypsinized and the cell count was adjusted to 1.0×10^5 cells/ml using medium containing 10% FBS and were used for the determination of cell viability by MTT assays as described by Francis and Rita (1986) respectively. The absorbance was measured using a microplate reader at a wavelength of 540 nm. The percentage growth inhibition was calculated using the following formula and concentration of test drug needed to inhibit cell growth by 50% (CTC_{50}) values is generated from the dose-response curves for each cell line.

$$\% \text{ Growth inhibition} = 100 - \frac{\textit{Mean OD of individual test group}}{\textit{Mean OD of control group}} \times 100$$

Phytochemical Analysis:

Phytochemicals are chemical compounds that are naturally found in plants. They are responsible for the colour and organoleptic properties of the plant. It is also referred to as those chemicals that may have biological significance but are not established as an cuentials nutrient in plant. Phytochemicals could be available as dietary supplements, but the parential health benefits of phytochemicals are derived from consumption of the whole plant. Several phytochemicals have a wide range of activities, which helps to give immunity against long term disease.

The phytochemicals like alkaloids, flavonoids, tannins, saponins, carbohydrates, glycosides, phytosterols, phenols, protein and amino acid, diterpens etc. are known to show medicinal activity as well as exhibit physiological activity. These chemicals are produced by plants, particularly the secondary metabolites which are synthesized as a measure for self-defense against insects, pests, pathogens, herbivores, UV exposure and environmental hazards. Phytochemistry takes into account the structural compositions of these metabolites, the biosynthetic pathways, functions, mechanisms of actions in the living systems and it's medicinal, industrial,

and commercial applications. The proper understanding of phytochemical is essential for drug discovery and for the development of novel therapeutic agents against major diseases.

The results of the phytochemical analysis of the leaf extracts in various solvents has shown a remarkable variation in the presence the above studied phytochemical compounds in the studied taxa. The detailed investigations phytochemicals in various solvents are shown in (Table 1 to 2). The study revealed that the leaf extracts of *Adhathoda vasica* was showing maximum presence of alkaloids, proteins, glycosides, phenols and steroids in all solvents. Steroids in modern clinical studies have supported their role as anti-inflammatory and analgesic agents (Perumal, 2012). Tannins reduce the risk of coronary heart diseases (Ghani *et al.*, 2003). Tannin was highly present in acetone and ethanol extracts of *A. vasica*. Quinines were completely absent in all extracts. Flavonoids, proteins and glycosides were found in all extracts of *A.paniculata* Flavonoids are useful in reduce body heat and remarkable activities in cancer prevention and anticancer activities (Veerachari, 2011). Phenols were absent in all extracts of *A. paniculata*. Tannin was adequately present in acetone, aqueous and ethanolic extracts but completely absent in chloroform and petroleum ether.

Table 1 : Preliminary phytochemical screening of different extracts of *Adathoda vasica* (leaves)

S.No	Compounds	Acetone	Chloroform	Ethanol	Petroleum Ether	Aqueous
1.	Tannins	+	-	+	-	+
2.	Saponins	+	+	+	-	+
3.	Flavonoids	+	-	+	+	+
4.	Glycosides	+	+	+	+	+
5.	Phenols	+	+	+	+	+
6.	Terpenoides	+	-	+	-	-
7.	Coumarins	-	+	-	-	-
8.	Quinines	-	-	-	-	-
9.	Proteins	-	+	-	-	-
10.	Alkaloids	+	+	+	+	+
11.	Carbohydrates	+	-	+	-	-
12.	Steroids	-	+	+	+	+

'-' Absence, '+' Presence

Table 2 : **Preliminary phytochemical screening of different extracts of *Andrographis paniculate* (leaves).**

S.No	Compounds	Acetone	Chloroform	Ethanol	Petroleum Ether	Aqueous
1.	Tannins	+	-	+	-	+
2.	Saponins	+	-	+	-	+
3.	Flavonoids	+	+	+	+	+
4.	Glycocides	+	+	+	+	+
5.	Phenols	-	-	-	-	-
6.	Terpenoides	+	-	+	-	-
7.	Coumarins	+	-	-	+	-
8.	Quinines	-	-	-	-	-
9.	Proteins	+	+	+	+	+
10.	Alkaloids	+	+	-	-	+
11.	Carbohydrates	-	-	-	-	-
12.	Steroides	-	-	+	-	-

- Absence , + Presence

Our results show that saponin was present in higher amounts in ethanol, acetone, chloroform extracts and absent in petroleum ether extract of plants. Terpenoids were present in acetone and ethanol extracts of both plants. Terpenoids are known to possess a wide range of biological activities including antimicrobial, antifungal, antiparasitic, antiviral, antimalarial, antispasmodic, antihyperglycemic, antiinflammartory, immunmodulatroy properties.(Wagner and Elmadfa, 2003).

The Medicinal plants are rich in secondary metabolites which include alkaloids, flavonoids, saponins and related active metabolites which are of great medicinal value and have been active metabolites which are of great medicinal value and have been extensively used in the drug and pharmaceutical industry. These secondary metabolites are reported to have many biological and therapeutic properties. Recently number of studies had been reported on the phytochemistry of medicinal plants, particularly on the vegetative parts like leaves (Kaur *et al.*, 2015).

Antioxidant Activity :

Phenolic compound are effective hydrogen doners, making them good antioxidant .(Amic et al.,2003). Plant derived polyphenolic flavonoid reduce free radical by quenching upregulating, or protecting antioxidant defences and chelating radical intermediate compounds. (Naresh kumar et al., 2019).

Total phenol :

Total phenol was estimated by folin-ciocalteu method and was expressed as mg.gallic acid equivalent per gram. The total phenolic content of studied plants ranged from 0.56 to 1.54 mg GAE/g. *Andrographis paniclata* contained comparatively higher amount of total phenol whereas *Adhathoda vasica* had very low quantity. Both the plants were found to have significantly higher concentration of total phenolics. Phenolic compounds in plants have been reported to have a wide range of biological activities including antioxidant properties. (Komal mahindrakar *et al.*, 2020)

Total Flavonoid :

The total flavonoid content was determined as mg. QE/g extract after comparison with a quercetin standard graph. Total flavonoid level was ranged between 0.13 to 0.33mg QE/GW. The study revealed that *Adhathoda*

Table 3 : Antioxitant chemicals presented in the leaf of *Andrographis paniculata* and *Adhathoda vasica*

S.No	Plants	Phenol (mgGAE/gDW)	Flavonoid (mgGAE/Gdw)
1.	*Adhathoda vasica*	0.56±0.79	0.33±0.029
2.	*Andrographis paniculata*	1.54±0.81	0.13±0.017

Values are the mean of 3 replicate ± SD

mg GAEs/gDW = milligram, gallic acid equivalents per gram dry weight

mg QEs /g DW = milligram quercetin equivalent per gram dry weight

vasica leaf extract possessed high quality of flavonoid than *Andrographis paniculata*. Flavonoids are useful in reduce body heat and remarkable activity in cancer prevention and anticancer activity.(Veerachari 2011).

In Antibacterial Activity :

The antibacterial activity of two medicinal plant leaf extracts (*Adhathoda vasica and Andrographis paniculata*) in four different solvents (ethanol, acetone, chloroform, and Petroleum ether) was measured against four human pathogenic bacteria (*E. coli, B. subtilis, S. aureus, and V. chlorea*) and the results were presented in (Table: 4 and 5).The inhibition zones against these species ranged in size from 3 to 16mm in diameter. All of the extracts examined prevented the growth of all of the pathogens in the sample. Ethanol extract of *Adhathoda vasica* had the highest activity against *E. coli* (8mm) and *V. chlorea* (7mm). Petroleum ether and chloroform extract of *Adhathoda vasica* had lower inhibitory activity against *V.chlorea, B.sustilis* and *S.aureus*. The plant leaf extracts of *A.vasica* was found to have vasicine acetate showed significant antimycobacterial activity (Ignacimuthu and Shanmugam, 2010). In our present study ethanol leaf extract of *Andrographis paniculata* was found to be active against *S.aureus*. Mishra et al., 2013 reported that 75% of methanol extracts of *A.paniculata* leaves with

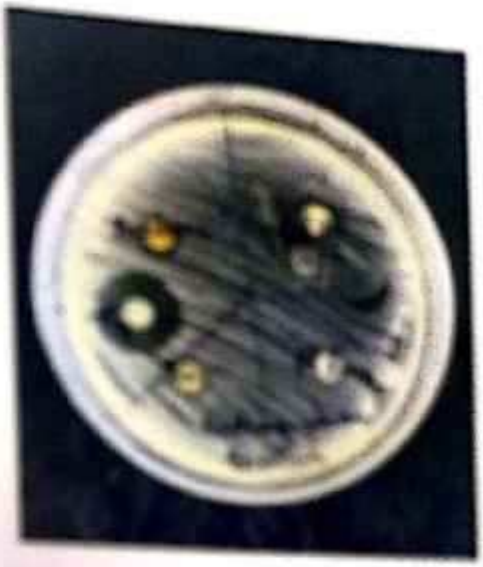
Table 4 : **Antibacterial Activity of Adhathoda vasica extracted with different solvent against human pathogen**

S.No	Organism	Zone of inhibition (mm)				
		Acetone	Ethanol	Chloroform	Petroleum Ether	Amoxicillin
1.	*E.Coli*	7	8	6	5	16
2.	*B. subtilis*	5	6	5	3	10
3.	*S.aureus*	4	4	3	6	14
4.	*V.chlorea*	4	7	6	3	12

Table 5 : **Antibacterial Activity of *Andrographis paniculata* extracted with different solvent against human pathogen**

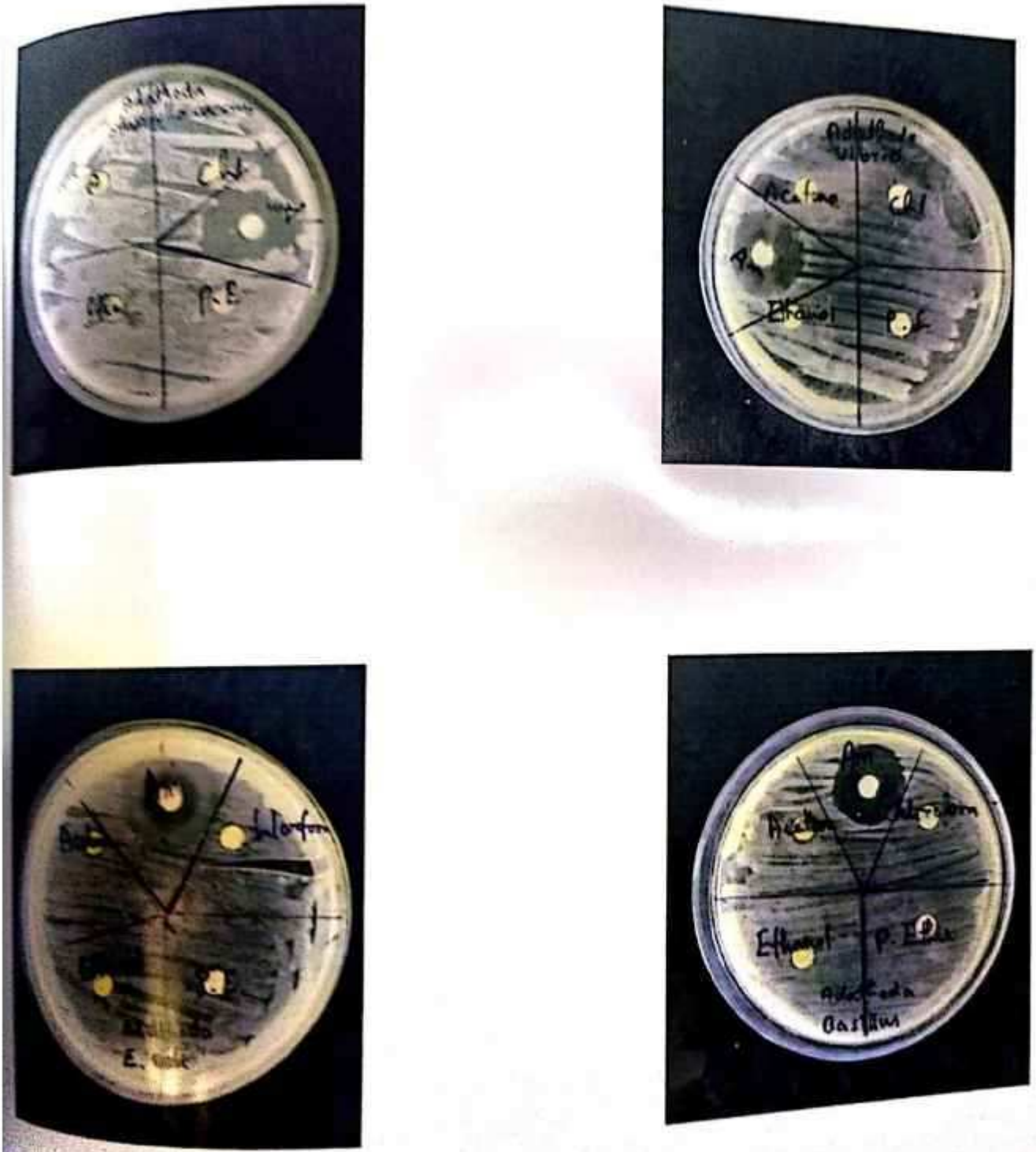
S.No	Organism	Zone of inhibition (mm)				
		Acetone	Ethanol	Chloroform	Petroleum Ether	Amoxicillin
1.	*E.Coli*	4	5	6	4	15
2.	*B.subtilis*	5	4	4	3	10
3.	*S.aureus*	6	7	5	3	10
4.	*V.chlorea*	3	4	5	3	16

Figure 1



Andrographis paniculata

Figure 2



Adhathoda vasica

higher inhibitory activity against *S.aureus*. The chloroform and acetone extracts of *A.paniculata* showed maximum antibacterial activity against *E.coli* and *S.aureus*.

Hosamani *et al*., 2011 have reported the acetone and alcohol extracts of *A.paniculata* with higher inhibitory against *B.subtilis* and *S.aureus*. The antibacterial activity of *A.paniculata* may due to the presences of active principle called andrographoloid. The petroleum ether showed less inhibitory activity against *B.subtilis, S.aureus* and *V.chlorea*. In this study all the *A.vasica* and *A.paniculata* extracts exhibited varying degree of inhibitory activity against the growth of all microorganisms. So this results supports both plants as antimicrobial potential.

Anthelmintic Activity :

The word "helminth" comes from the greekword, 'helminths,' which means 'worm'. Helminth is a general word that refers to a variety of parasitic worm that live with the body (Patel *et al*., 2010). According to the World Health Organization (WHO), over two billion people are infected with parasitic worms. (Mulla *et al*., 2010). By 2025, it is projected that 57 % of the population in developing countries will be affected(M anoj Salhan *et al*., 2011) Anthelmintics are drugs that work locally to remove worms

from the GIT or systemically to eliminate adult helminths or development forms that infect organs and tissue.(Mohamad aaer *et al.*,1999) Abdominal pain, loss of appetite, nausea, vomiting, headache, and diarrhoea are commen side effects of current anthelmintics.(Devi *et al.*, 2009) Nature anthelmintics can play an important role in the treatment of these parasite infection.(Aswar *et al.*, 2008)

The present study deals with the anthelmintic property of *Andrographis paniculata* and *Adhathoda vasica* different leaf extracts against Eisenia fetida with 50 mg/ml concentration were presented in table 7. Observations were made for the time taken to paralyze and death of individual worms. Paralysis was said to occur when worms do not move even in normal saline. Death was concluded when the worms lost their motility followed with fading away of their body color.(Arnold and Harry 1968).

As shown in table(7) the chloroform extract of *Andrographis paniculata* was more potent than ethanol, acetone and petroleum ether extracts. The chloroform extract at 50 mg/ml concentration shows paralysis at 11.63±0.23 minutes and death at 15.92±0.32 minutes. The acetone extract of *A. paniculata* at 50mg/ml concentration was taken 20.36±1.31 minutes for paralysis and death at 22.58±0.64 minutes. The chloroform leaf extracts

Table 6 : **Anthelmintic Activity of the leaves extracts of *Adhathoda vasica***

Treatment	group	Concentration mg/ml	Time taken for paralysis (min.)	Time taken for death (min.)
Control (Saline)	I	50	---	---
Acetone	II	50	18	20
Ethanol	III	50	15	18
Chloroform	IV	50	12	17
Petroleum Ether	V	50	28	32
Albendazole	VI	50	13	18

Values are the mean of 3 replicants ± SD

Control = Albendazole (50mg/ml)

Leaf extract = 50mg/ml

Table 7 : Anthelmintic Activity of the leaves extracts of *Andrographis paniculata*

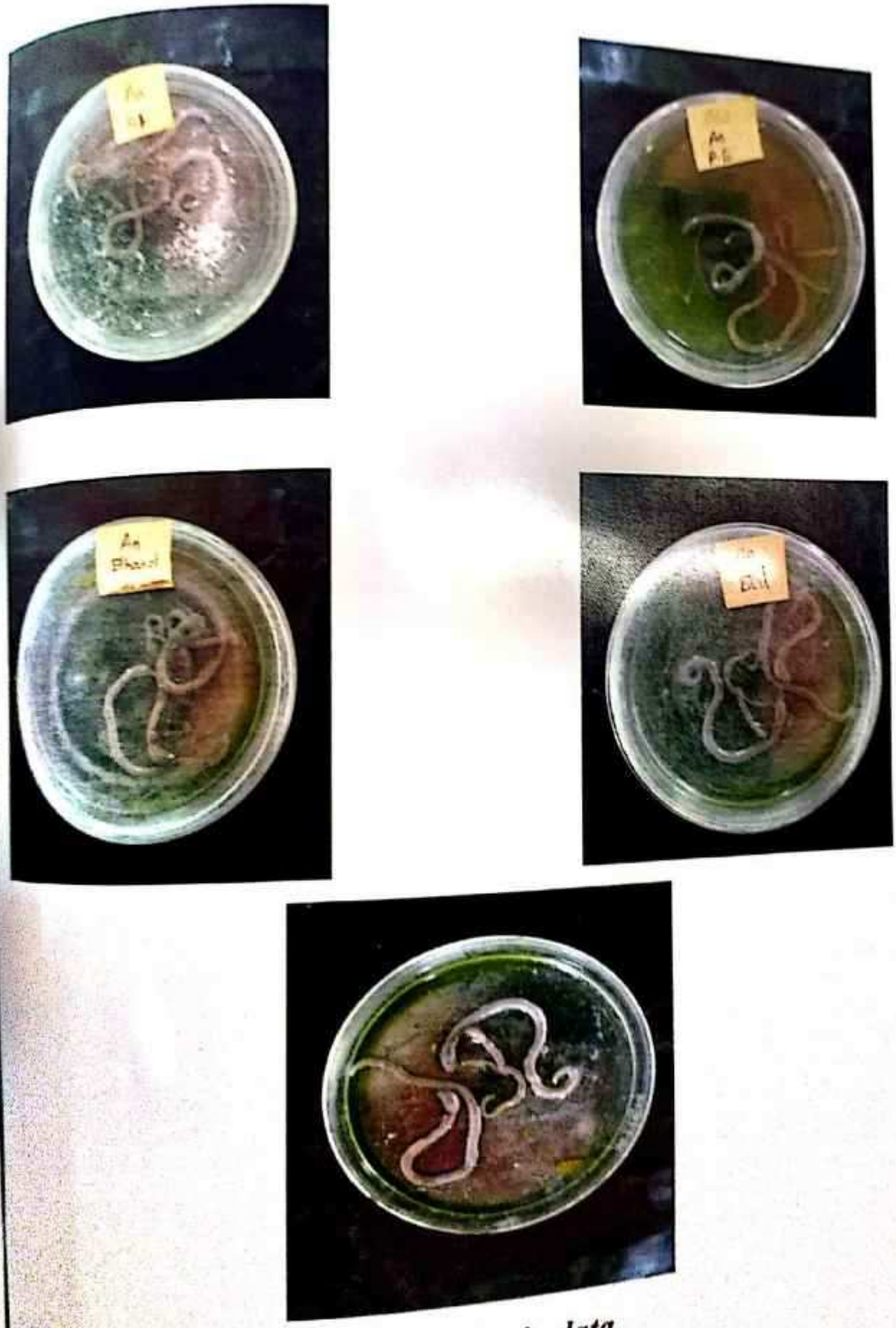
Treatment	group	Concentration mg/ml	Time taken for paralysis (min.)	Time taken for death (min.)
Control (Saline)	I	50	---	---
Acetone	II	50	20	22
Ethanol	III	50	19	23
Chloroform	IV	50	11	15
Petroleum Ether	V	50	17	21
Albendazole	VI	50	13	18

Values are the mean of 3 replicants ± SD

Control = Albendazole (50mg/ml)

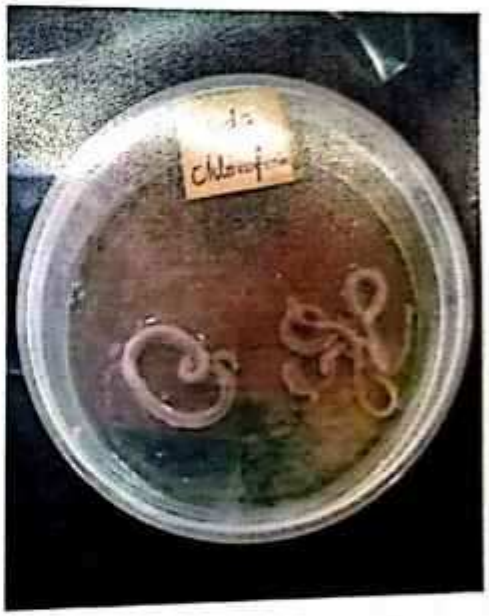
Leaf extract = 50mg/ml

Figure 3



Andrographis paniculata

Figure 4



Adhathoda vasica

of *Adhathoda vasica* caused paralysis at 12.27±0.43 minutes and time of death at 17.42±0.58 minutes whereas ethanol extract of *Adhathoda vasica* showed paralysis at 15.82±0.38 minutes and death at 18.68±0.34 minutes. Petroleum ether extracts of *A.vasica* at 50mg/ml concentration showed paralysis at 28.23±0.72 minutes and death at 32.24±2.43 minutes. The control (saline solution treated) earthworms were observed for 24hrs in which no paralysis and death was noticed.

Phytochemical analysis of the leaf extracts of *A.vasica* and *A.paniculata* revealed the presence of tannins as one of the predominant phytoconstituents. Tannins, the polyphenolic compounds, that can bind to free proteins in the gastrointestinal tract of host animal(Bate smith, 1962; Anthnasiaddou *et al.,* 2001) or glycoprotein on the cuticle of the parasite and cause death (Thompson and Geary,1995). Some synthetic phenolic anthelmintics, e.g. niclosamide, oxyclozanide, bithionol, nitroxynil, etc, are shown to interfere with energy generation in helminth parasites by uncoupling oxidative phosphorylation (Martin, 1997).

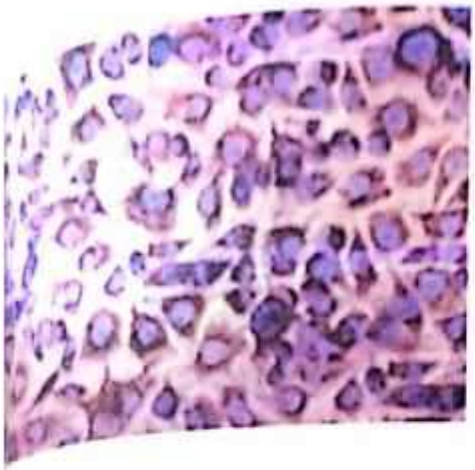
system in India for multiple clinical applications. In our present work, ethanolic leaf extract of *A. paniculata* were tested against MCF-7 human breast adenocarcinoma cell line with different concentrations (50 µg/ml, 250 µg/ml, 500 µg/ml, 750 µg/ml and 1000µg/ml). MTT assay was performed which is the technique utilized for survival determination measurements and the concentration required for a 50% inhibition of viability (IC_{50}) was determined graphically. The effect of the samples on MCF-7 proliferation was measured as a percentage of cell viability. Table 8 contains the results. With increasing concentrations of test compounds, the percentage of growth inhibition of *A. paniculata* was found to increase. The CTC50 values observed in the leaf extract of *A. paniculata* was 13.99, 20.57, 41.97, 57.81 and 59.67 µg/ml against MCF-7. The American National Cancer Institute assigns a significant cytotoxic effect of promising anticancer product for future bio-guided studies if it exerts an IC50 value ≥30 µg/ml (Suffness and Pezzuto, 1990). Our qualitative phytochemical analysis revealed the presence of terpenoids, flavonoids, phenols, glycosides, tannins and alkaloids in the ethanolic extracts of *A. paniculata*, which could be responsible for this activity. The phytochemical constituents such as flavonoids and terpenoids are the main components that are responsible for the potential cytotoxic activity (Rathinam Prema *et al.*, 2012). The

Table 8 : Anticancer Activity of *Andrographis paniculata*

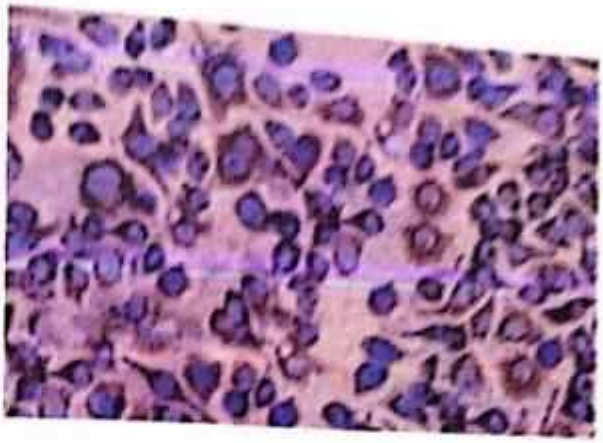
MCF-7 cell line studies;

S.No	Concentration	CTC_{50}	% CTC_{50}
1	50	13.99	718.54
2	250	20.57	
3	500	41.97	
4	750	57.81	
5	1000	59.67	

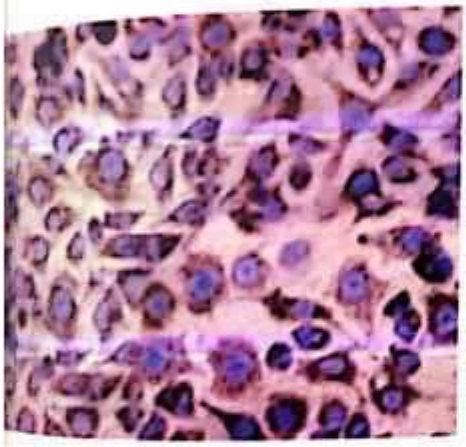
Figure: MCF- 7 cell line studies



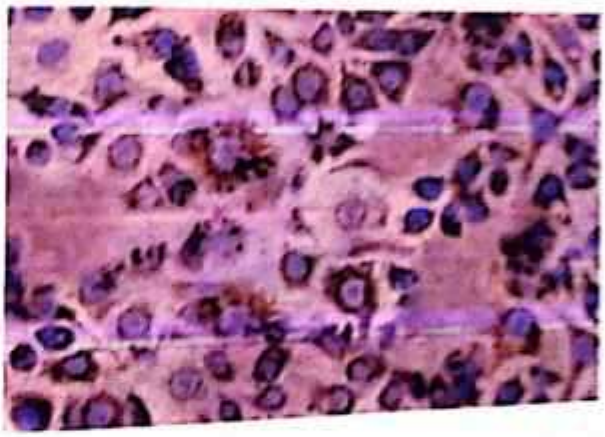
50 µg/ml



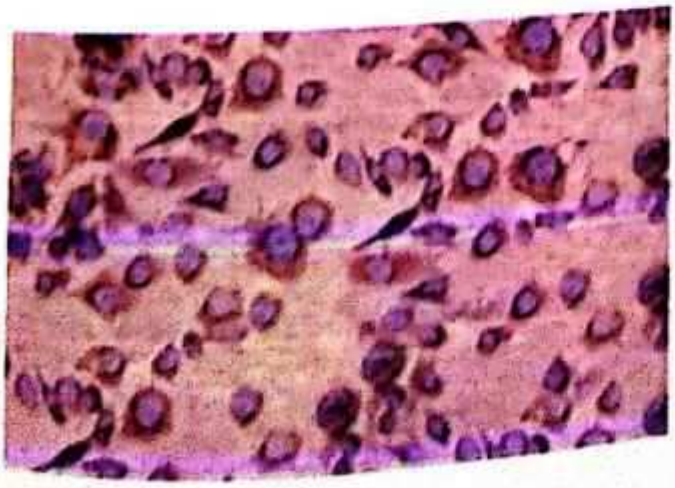
250 µg/ml



500 µg/ml



750 µg/ml



1000 µg/ml

flavonoids have reported for their cytotoxic activity due to presence of phenolic groups (Matsuo *et al.*, 2005) The plant contains some diterpenoids. However the major bitter constituent is andrographolide, which is diterpene lactone. Diterpenoids and flavonoids are the main chemical constituents of *Andrographis paniculata* which are believed to be responsible for the most biological activities of plant (Beena *et al.*,2011). Two flavonoids, identified as 5,7,2',3' –tetramethoxyflavone, as well as several other flavonoids were obtained from the whole plant. The bitter principle andrographolide was isolated in pure form by Goiter. The presence of flavonoids in *A. paniculata* extracts reduces the risk of cancer (Ferguson *et al.*, 2004). Terpenes have been shown to prevent the growth of cancerous cells, reduce tumor size, lower cholesterol levels, and lower the concentration of microorganisms (Neerja Gupta *et al.*, 2011). Similarly, the findings of this study agree with Park *et al* (2008) and Reed and Pellecchial (2005), who believed that flavonoids cause apoptosis through DNA fragmentation, nuclear condensation, and cell shrinkage. In this sense, the plant is capable of inhibiting cell viability. This research provides a crucial framework for future research into the isolation, characterization, and mechanism of cytotoxic compounds extracted from plant extract.

phytochemical constituents in plant samples are biologically active compounds of antioxidant, antimicrobial, antifungal, hypoglycaemic, anti-diabetic, anti-inflammatory, anticarcinogenic, antimalarial, and anticholinergic properties, among others. alkaloids, glycosides, flavonoids, phenols, saponins, steroids, tannins, terpenoids, coumarins, quinines, and carbohydrates were present in *Adhathoda vasica* and *Andrographis paniculata* leaf extracts .

The study revealed that the leaf extracts of *Adhathoda vasica* was showing maximum presence of alkaloids, proteins, glycosides, phenols and steroids in all solvents. Quinines were completely absent in all extracts. Flavonoids, proteins and glycosides were found in all extracts of *A.paniculata.* Terpenoids were present in acetone and ethanol extracts of both plants.

Recently number of studies had been reported on the phytochemistry of medicinal plants, particularly on the vegetative parts like leaves. *Andrographis paniclata* contained comparatively higher amount of total phenol whereas *Adhathoda vasica* had high quantity. The study revealed that *Adhathoda vasica* leaf extract possessed high quality of flavonoid than *Andrographis paniculata.* Ethanol extract of *Adhathoda vasica* had the highest activity against *E. coli* (8mm) and *V. chlorea* (7mm). Petroleum

and chloroform extract of *Adhathoda vasica* had lower inhibitory against *V.chlorea*, *B.sustilis* and *S.aureus*. The plant leaf extracts of was found to have vasicine acetate showed significant bacterial activity (Ignacimuthu and Shanmugam, 2010).

The chloroform extract of *Andrographis paniculata* was more potent than ethanol, acetone and petroleum ether extracts. The chloroform extract at 50 mg/ml concentration shows paralysis at 11.63±0.23 minutes and death at 15.92±0.32 minutes. The chloroform leaf extracts of *Adhathoda vasica* caused paralysis at 12.27±0.43 minutes and time of death at 17.42±0.58 minutes. Tannins, the polyphenolic compounds, that can bind to free proteins in the gastrointestinal tract of host animal(Bate smith, 1962; Athanasiaddou *et al.*, 2001) or glycoprotein on the cuticle of the parasite and cause death (Thompson and Geary,1995). Table 8 contains the results. With increasing concentrations of test compounds, the percentage of growth inhibition of *A. paniculata* was found to increase. The CTC50 values observed in the leaf extract of *A. paniculata* was 13.99, 20.57, 41.97, 57.81 and 59.67 µg/ml against MCF-7. Our qualitative phytochemical analysis revealed the presence of terpenoids, flavonoids, phenols, glycosides, tannins and alkaloids in the ethanolic extracts of *A. paniculata*, which could be

responsible for this activity. The phytochemical constituents such as flavonoids and terpenoids are the main components that are responsible for the potential cytotoxic activity. This research provides a crucial framework for future research into the isolation, characterization, and mechanism of cytotoxic compounds extracted from plant extract.

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COMPARATIVE STUDY ON METABOLITES PROFILING AND BIOACTIVITY ANALYSIS OF MANGROVE SPECIES COLLECTED FROM THOOTHUKUDI AND PUNNAKAYAL

A dissertation submitted to

ST. MARY'S COLLEGE (AUTONOMOUS), THOOTHUKUDI

Affiliated to

MANONMANIAM SUNDARANAR UNIVERSITY, THIRUNELVELI

in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE IN BOTANY

By

LIBIGA P

Reg. No. 19APBO09

DEPARTMENT OF BOTANY

ST. MARY'S COLLEGE (AUTONOMOUS)

THOOTHUKUDI - 628001

2020 - 2021

CERTIFICATE

It is certified that this short term project work entitled **"Comparative Study on Metabolites Profiling and Bioactivity Analysis of Mangrove Species Collected from Thoothukudi and Punnakayal"** submitted to **St. Mary's College (Autonomous)** affiliated to **Manonmaniam Sundaranar University** in partial fulfillment of the requirements for the degree of **Master of Science in Botany** and is a record of work done in the Department of Botany, St. **Mary's College (Autonomous)**, Thoothukudi during the year 2020-2021 by **Libiga P (19APBO09).**

Arockia Jereis
GUIDE

M. Glory
HEAD OF THE DEPARTMENT

HOD of Botany
St. Mary's College (Autonomous)
Thoothukudi- 628 001.

M. Glory
EXAMINER

Lucia Rose.
PRINCIPAL
St. Mary's College (Autonomous)
Thoothukudi - 628 001.

DECLARATION

I do hereby declare that this dissertation entitled **"Comparative Study on Metabolites Profiling and Bioactivity Analysis of Mangrove Species Collected from Thoothukudi and Punnakayal"** submitted by me in partial fulfillment for the award of the degree of **Master of Science in Botany**, is the result of my original and independent work carried out under the guidance of **Dr. Sr. A. Arockia Jenecius Alphonse,** Assistant Professor of Botany, St. Mary's College (Autonomous), Thoothukudi and it has not been submitted elsewhere for the award of any other degree.

Station: Thoothukudi - 628001

Date: 15/4/21

P. Libiga

LIBIGA P

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CONTENT

INTRODUCTION

The mangroves are nature's prodigy, ecological miracle and visual treat. These plants grow and develop well in mud flats and shallow water coastal areas where the water is generally brackish. These areas are tough place for ordinary terrestrial plants to grow sustain however, this is not a big deal for the mangrove plants. Highly adoptable features in morphology, physiology and anatomy of mangroves help them to survive successfully in the highly complicated environment. Primary and secondary metabolic process of mangrove plants releases many major and minor bioactive compounds. Occurrence of these bioactive compounds which posses numerous pharmacological and ecological importance attracts the people and scientists towards them. Secondary metabolites produced from mangrove plants are chemically diverse compounds which could help the human being to overcome from life threatening diseases.

Mangroves are the plants that grow in the ecosystems situated between land and sea enclosing almost 18.1 million hectares of the Globe **(Sebastianes *et al.,* 2013).** Mangroves are halophytic plant species which can grow in saline coastal sediment habitats in the tropical and subtropical region. This salt tolerant forest ecosystem highly supports ecology, economy and ecosystem of the area **(Singh *et. al.,* 2012).** Nearly three quarters of tropical coastlines is reported to dominate by mangroves. These plants are considered one amongst the most prolific ecosystems on the earth to function as protectors of their juvenile stock and form the most prized biomass **(Singh *et. al.,* 2012).**

According to **Duke (1998),** mangrove vegetation comprises almost 70 species from 20 different angiosperm families**.** It consists of sixty eight woody halophytes

community which posses the capacity to withstand extreme transitional zone, which embodies divergent habitats between marine and terrestrial environments **(Badola and Hussain, 2003).** Mangroves adaptations are unpalatable in terms of adapting to regular fluctuations in tides, higher saline conditions and nil or near nil oxygenic conditions. Presence of specialized stilt roots makes mangroves to cope-up with tidal fluctuations. Evolving xeromorphic and halophytic conditions enables mangroves to adapt to higher saline conditions and existence of pneumatophores helps the plant to overcome nil oxygenic conditions. Mangrove accompanies elaborated spectrum of services like protection of coast, carbon sequestration, land accretion, water quality maintenance, pollution control, food, timbers and dyes etc. Mangrove coastal protection role is incredible in which mangrove plants protect the coastal regions from natural calamities which includes tsunamis, cyclones, floods and sea inundations **(Miththapala, 2008).**

India has a long coastline of approximately 7516.6 km which also comprises of the island territories. It has a fourth largest mangrove area in the world covering an area of about 6,749 km^2 **(Naskar and Mandal, 1999)**. These mangrove territories (69°E-89.5°E longitude and 7°N-23°N latitude) are made up of three distinct zones namely East coast habitats corresponding to a coast line of about 2700 km, in front of Bay of Bengal, West coast habitats corresponding to a coast line of about 3000 km, in front of Arabian sea and Island territories comprising of about 1816.6 km coastline **(Singh *et al.,* 2012).**

Indian mangrove consists of 125 species with 39 true mangroves and rest are associates **(Kathiresan, 2010).** Thirty-eight mangrove areas have been designated in India, sprawling the coastal states of Gujarat, Maharashtra, Goa, Karnataka, Kerala, Tamilnadu, Andra Pradesh, Odisha, West Bengal and Andaman and Nicobar Islands. Apart from these, mangroves are also present in Puducherry and Daman and Diu **(MOEF 2011-2012)**. Tamilnadu comprises both major and minor mangroves namely Mangroves of Pichavaram (1357 ha), Pudhupattinam (800 ha), Muthupet (12000 ha), Gulf of Mannar (148 ha) and Palk strait (700 ha) **(Selvam *et al.,* 2002).**

Nutrient content of the soil and their availability to the plant is one of the pillar factors influencing mangrove forest distribution, structure and productivity **(Reef *et al.,* 2010).** Soil of mangrove habitat has extremely low nutrient availability **(Lovelock *et al.,* 2005)** and also it varies greatly from location to location **(Feller *et al.,* 2003).** Mangrove soil is found nutrient limited, particularly in nitrogen and phosphorous **(Reich and Oleksyn, 2004; Lovelock *et al*., 2007).** Most previous investigations of nutrient limitations to mangrove have focused on macronutrients N and P, which most likely limiting structure and productivity of mangroves **(Krauss *et al.,* 2008).** Limitations to structure and productivity imposed by iron are also likely, but not yet to be assessed in the field **(Alongi, 2010)**. In mangrove soil, nitrogen was considered as the primary nutrient that affects species composition and structure of the mangrove forest. More recent analysis reported that nitrogen and phosphorous influences the structure and composition in approximately equal proportions **(Elser and Hamilton, 2007).** Under high salinity conditions in mangroves, potassium is also important for osmotic regulation **(Downton, 1982).** The availability of potassium in

mangrove soil is variable and there is some evidence for potassium limitation in some mangroves affecting forest structure and productivity **(Ukpong, 1997).**

Mangrove plants are well known to tolerate a number of biotic and abiotic stresses. Stress signals modulate the secondary metabolites in plant cells **(Akula and Ravishankar, 2011)** by changing the metabolic signalling and regulatory networks **(Krasensky and Jonak, 2012).** Nitric oxide (NO) modulation **(Zhang *et. al.,* 2012)** and reactive oxygen species homeostasis **(Miller *et. al.,* 2010)** play crucial role in various stresses which might contribute to the secondary metabolite composition in plants. Mangroves are continuously exposed to high salt, flood, high temperature and irradiance. They are always coping up with these abiotic stresses which might contribute to its diverse and novel secondary metabolite composition as well as increased bioactivity.

Mangrove forests not only play an essential role as the source of food for marine organisms but are also a good source of food for human consumption based on their nutrient potential. Several mangrove plants are used as medicinal plants in traditional medicine for many years. Research in the field of mangrove evidenced that these plants provide numerous contributions for human life. Studies on the bioactive compounds of mangrove plants lead to the discovery of new therapeutic agents. The mangrove plants are reported to have various biological activities such as antibacterial, antioxidant, anticancer, cytotoxic, antiproliferative, insecticidal, antimalarial, antifungal, antifeedant, antidiarrheal, central nervous system depressant, antimitotic, antileukemic and anti-plasmodial **(Patra and Thatoi. 2011).** The phytochemical constituents identified from

mangrove plants are known to exhibit medicinal properties. For example, alkaloids separated from mangroves are known to have biological activities like anti-inflammatory **(Augusto *et al.,* 2011),** antimalarial **(Dua *et al.,* 2013),** antimicrobial **(Benbott *et al.*, 2012)**, cytotoxicity, antispasmodic and pharmacological effects **(Ameyaw and Duker-Eshun, 2009; Thite *et al.,* 2013)**. Likewise, steroids obtained from mangrove plants are reported to have cardiotonic effect and also exhibited antibacterial and insecticidal properties **(Alexei *et al.,* 2009)**. They are very often used in medicines because of their well identified biological activities. Based on the results of experiments, tannins are known to have antibacterial **(Hisanori *et al.,* 2001),** antitumor and antiviral activities **(Kumari and Jain 2012).** Alkaloids show bioactivity against Gram-positive bacteria and cytotoxicity against leukaemia and HeLa cell lines **(Omar *et al.,* 1992).** Alkaloids, flavonoids and xanthones that are potent inhibitors of various oxidative processes in both *in vitro* and *in vivo* system.

The ever-rising threat of microbial resistance against many common antibiotics is the chief cause of global medical hazards. Currently, contagious pathogens are generally resistant to numerous antibiotics. This challenges the capacity of the antibiotics in arresting contaminations efficiently **(Sood and Gupta, 2012).** Pharmacological investigations have revealed antimicrobial, antioxidant, anticancer, antidiabetic, antiinflammatory activities and so on in *Avicennia* plants. This genus possesses some unique metabolites of varied chemical classes, which are responsible for their wide range of pharmacological activities. The presence of different bioactive compounds such as alkaloids, flavonoids, phenols, saponins, tannins, glycosides and terpenoids has been

detected. Hence, there is a great scope to discover new biological active phytochemicals from different mangrove species of the genus *Avicennia*.

Mangroves have shown potential and promising therapeutic applications to treat a variety of ailments as reported by many ethnomedical studies. Various parts of the plants such as roots, barks or stems and leaves have been used in folk medicines. They are mainly used medicinally to treat diabetes, hypertension and gastrointestinal disorders such as constipation, diarrhoea, dysentery, dyspepsia, haematuria and stomach pain. The plants are mostly used in Asian countries, namely India (45.8%), Bangladesh (5.1%), Malaysia (5.1%), China (5.1%), Indonesia (3.4%), Philippines (3.4%), and other countries with 16.9% **(Sadeer *et al.,* 2019).**

Mangrove plants have been used in medicine and recently extracts from mangrove and mangrove-dependent species have proved activity against human, animal and plant pathogens but only limited investigations have been carried out to identify the metabolites responsible for the therapeutic activities. Hence the present study was carried out

- To document the mangrove associates in polluted and unpolluted sites.
- To study the physio-chemical parameters of mangrove soil collected from polluted and unpolluted sites.
- To identify the phytochemicals, present in various solvents of stem and leaf extracts of *Avicennia marina and Rhizophora mucronata* collected from polluted and unpolluted sites.
- To quantify the selected primary and secondary metabolites of methanolic stem and leaf extracts of *Avicennia marina* and *Rhizophora mucronata* collected from polluted and unpolluted site.
- To evaluate the antioxidant activity of stem and leaf extracts of *Avicennia marina* and *Rhizophora mucronata* collected from polluted and unpolluted site.
- To analyse the functional groups of bioactive compounds, present in the leaf extracts of *Avicennia marina* and *Rhizophora mucronata* collected from polluted and unpolluted sites by FTIR spectroscopic analysis.

- To investigate the bioactive compounds, present in the leaf extracts of *Avicennia marina* and *Rhizophora mucronata* collected from polluted and unpolluted sites by GC-MS analysis.
- To explore the antibacterial activity of stem and leaf extracts of *Avicennia marina* and *Rhizophora mucronata* against human pathogenic bacteria.

REVIEW OF LITERATURE

Mangroves have long been big resource of interest for scientist for lead developments. Mangroves are biologically and chemically distinct, developing in a wide collection of new natural products. Natural products and flora in mangrove have been widely worn in folk remedy to extravagance various disorders. Though the chemical constituents of mangrove sows still have not been considered widely, explorations have led so remote to detection of numerous new composites with potential therapeutic significance for discovery of novel chemo-therapeutic representatives.

MANGROVES AND MANGROVE ASSOCIATES

Distribution of mangrove and mangrove associated plants depends upon various factors including the soil type, tidal moment, water temperature, pH, macro and micro nutrient content. Due to these reasons number of mangroves and mangrove associates varies location to location. In the Pichavaram mangroves there are 13 exclusive mangroves namely *Acanthus ilicifolius*, *Avicennia marina, Avicennia officinalis, Bruguiera cylindrica, Rhizophora apiculata* and others. Out of these, *Avicennia marina* dominates the mangroves amounting to 74% of the entire population**.** *Acanthus ilicifolius, Aegiceras corniculatum, Avicennia marina, Excoecaria agallocha, Rhizophora mucronata* and *Lumnitzera racemosa* are found to be present in the Muthupet mangroves. *Avicennia marina* overwhelmingly dominates the entire population amounting to 95% **(Selvam, 2002).** Bhitarkanika mangroves comprises of 64 plant species in which 28 are true mangroves and 4 are mangroves associates

(Badola and Hussain, 2003). Sunderbans mangroves are the World's largest surviving mangroves extends in both India and Bangladesh with majority of 62% located in Bangladesh. Total 24 mangrove species have been reported from sunderbans, out of which *Heritiera fomes* and *Excoecaria agallocha* are predominant mangrove species **(Kathiresan and Rajendran, 2005).**

SOIL ANALYSIS

Soil is the backbone for the plants to start their life. Physicochemical properties like pH, electrical conductivity and nutrient parameters such as N, P, K etc. are the major factors determining a plants growth. Mangrove forests cover about 160,000 km^2 all over the world. Countries like India, Brazil, Malaysia, Bangladesh, Nigeria, Venezuela and Senegal have the largest mangrove forests **(Giri and Muhlhausen, 2008).** From the findings of the previous studies conducted by several authors underscored that mangrove vegetation is influenced by critical ecological events like denitrification, nitrogen fixation **(Pelegrai *et al.,* 1997, Pelegrai *et al.,* 1998),** phosphorus sedimentary processes **(Chen and Twilley, 1999)** and mangrove water column nutrient exchange **(Childers *et al.,* 1999; Davis *et al.,* 2003).**

Studies conducted in different tropical mangrove forests reported that mangrove soils may be either acidic or alkaline. **Ferreira *et al.* (2010) and Moreno and Calderon (2011**) found that the soil pH ranging from 2.87-6.40. Some other researchers reported soil pH above 7.0 ranging from 7.4-8.22 **(Hossain *et al.,* 2012; Das *et al.,* 2012).** Several authors have studied the nutrient profile of the mangrove soil. **Rambok *et al.* (2010)** reported the nitrogen and phosphorous content of the forest Wildlife Sanctuary Sibuti

Mangrove Malaysia. They reported 0.22% of nitrogen and 25.27% of phosphorous in the soil. Nitrogen and phosphorous content of soil collected from Awat-Awat Lawas Mangrove Malaysia was 0.15 % and 12.32% respectively (**Rambok *et al.,* 2010**). **Hossain *et al.* (2012)** reported 0.09% of soil nitrogen in Sundarbans mangrove, Bangladesh.

Hassan and Razzaque (1981) found that the pH value of soil in Sundarbans is neutral to mildly alkaline under field conditions but in some localities the pH value of dried up sub soil samples drops to 6.5. **Mohamood and Saikat (1995)** reported the acidic pH values in the soil of Chakaria mangrove area and consequently, this area has a rich reserve of pyrite in its soil. **Muhibbullah *et al.* (2005)** reported the average pH values were found to be 6.3, 6.73, 7.13 and 6.8 in the Sharankhola, Chandpai, Nalianala and Burigoalini respectively for Sundarbans mangrove in Bangladesh.

PHYTOCHEMICAL ANALYSIS OF MANGROVE PLANTS

Plants are good source of biologically active compounds known as phytochemicals. The phytochemicals act as antioxidants by scavenging free radicals and many have therapeutic potential for free radical associated disorders **(Lee *et al.,* 2000).** Phytochemicals such as alkaloids, flavonoids, tannins, phenols, saponins and several other aromatic compounds in the plants serve a defence mechanism against prediction by many microorganisms, insects and other herbivores **(Shihabudeen *et al.,* 2010).** The phytochemicals are grouped into two main categories **(Krishnaiah *et al.,* 2009)** namely primary constituents which includes amino acids, common sugars, proteins and chlorophyll etc., and secondary constituents consisting of alkaloids, essential oils,

flavonoids, tannins, terpenoids, saponins and phenolic compounds etc. **(Krishnaiah *et al.,* 2007)**

Phytochemical screening is of paramount importance in identifying new source of therapeutically and industrially valuable compound having medicinal significance. The available literatures have reported that most of the *Avicennia* species have been traditionally used as a medicine for a wide array of diseases worldwide by the local communities inhabiting the mangrove forests. From many of the ethnomedicinal uses of the genus, the widest applications have been in the treatment of rheumatism, pregnancy, ulcer and smallpox (**Bandaranayake, 2002**). Different parts of the mangrove plants such as leaf, bark, stem, seeds, roots and fruits have been exploited over the years for the treatment of various diseases **(Shilpi *et al.*, 2012; Simlai and Roy 2013).** Plants like *Avicennia alba, A. marina, A. nitida* and *A. officinalis* are known for its potential activity against treatment of many diseases. A number of reports are available for ethnomedicinal uses of different species of *Avicennia* plants for the treatment of different diseases **(Thirunavukkarasu *et al.,* 2011).**

Phytochemical analysis of well-known mangrove plants namely *Avicennia marina* and *Rhizophora mucronata* have been studied by **Khattab *et al*. (2012)**. They reported that *Avicennia marina* leaves, seeds, flowers, stems and *Rhizophora mucronata* leaves are a source of steroids, tannins, glycosides, carbohydrates saponins, sterols, terpenoids and phenol. Many reports have documented that the genus *Avicennia* possesses some unique metabolites of varied chemical classes, which may be responsible for their wide range of pharmacological activities **(Ganesh and Jannet 2011;**

Shanmugapriya *et al.,* 2012; Poompozhil and Kumarasamy, 2014). Phytochemical screening of various solvent extracts from the genus of *Avicennia* such as methanol, ethanol, ethyl ether, acetone, hexane, chloroform, benzene, aqueous and ethyl acetate has confirmed the presence of diverse and novel phytochemicals like alkaloids, terpenoids, steroids, phenolics, saponins, flavonoids, tannins, steroid and glycosides.

Phytochemical studies on *Avicennia marina* leaf extract have revealed the presence of secondary metabolites such as alkaloids, flavonoids, triterpenoids and steroids, which could also be expected to be responsible for its bioactivity. Apart from economic uses, *Rhizophora stylosa* also possesses therapeutic properties. It contains 17 amino acids of which seven falls under the essential category. In traditional medicinal culture, *R. stylosa* is used for treating many diseases. Phytochemical investigations showed that the leaves of *Rhizophora stylosa* are rich in bioactive metabolites and are also used for treating diseases **(Abdel-Aziz *et al*., 2016; Aljaghthmi *et al*., 2018).**

Plants of this genus *Rhizophora* rich in phytochemicals and thus have high medicinal potential and are also used in traditional medicinal practice. The leaves, roots and bark of this tree are used for treating hemorrhages, angina and haematuria and even for inducing contractions in pregnant women. These medicinal properties are due to the rich store of phytochemicals **(Seepana *et al.,* 2016).** Phytochemical studies have revealed that most chemically investigated *Avicennia* species are rich in phytochemicals, namely terpenoids, glucosides and naphthalene derivatives **(Han *et al.,* 2007)**. These naturally occurring compounds are found to be concentrated in the plant's leaf, stem, bark and aerial roots.

Lima *et al.* (2010) studied the phytochemical constituents of ethanol extract of *Phoenix paludosa* leaves. Steroids, glycosides, tannins, flavonoids and gums were found to be present in the extract. **Shanmugapriya *et al.* (2012)** investigated the phytochemical characterization of leaves of *Avicennia marina* and *Avicennia officinalis*. Ethyl acetate, ethyl ether, ethanol and aqueous extracts of *Avicennia marimai* and *Rhizophora stylosa* leaves were evaluated for their qualitative phytochemical analysis. The qualitative phytochemical analysis revealed that extracts of mature leaves contained tannins, flavonoids, terpenoids, alkaloids, steroids, phenol and cardiac glycosides **(Mouafi *et al.,* 2014). Khushi *et al.* (2016)** reported the phytochemical constituents of ethanol extract of *Avicennia officinalis* leaf. Phytochemical screening confirmed the presence of carbohydrate, reducing sugars, glycosides, tannins, alkaloids, protein, terpenoid and flavonoid.

Gawali *et al.* (2017) studied the phytochemical constituents of ethanol extract of stem and fruit of *Derris trifoliata* and leaf, stem and fruit of *Sonnera alba.* **Thirunavukkarasu *et al.* (2018)** investigated qualitative and quantitative phytochemical profile of ethanol and n-butanol extracts of *Decandra* leaves. Proteins, coumarins, phenols, flavonoid, saponin, glycoside, alkaloid, terpenoid and tannin were present in the both the solvent extracts. **Syahidah and Subekti (2019)** reported total phenol, flavonoid and tannin content of *Rhizophora* leaves. Their experiment showed maximum number of secondary metabolites in acetone extract of *Rhizophora* leaves. Phenol, flavonoids and tannin content was 10.024 mg GAE/g, 2.641 mg QE/g and 6.703% respectively.

METABOLITIES AND ITS BIOLOGICAL ACTIVITIES OF MANGROVES

The development of pharmaceuticals begins with the identification of active principles, detailed biological assays and dosage formulations, followed by clinical studies to establish safety, efficacy and pharmacokinetic profile of the new drug. The same follows for mangrove plant therapeutic agents. The mangrove plants possess a number of biological activities such as antibacterial, antioxidant, anticancer, cytotoxic, antiproliferative, insecticidal, antimalarial, antifungal, antifeedant, antidiarrheal, central nervous system depressant, antimitotic, antileukemic and antiplasmodial activities.

Plants possess efficient antioxidant defence systems to scavenge the reactive oxygen species and protect the plants from destructive reactions. The mangrove plants and their associates possess a strong antioxidant activity as they grow in the environmental stress conditions. **Ramadan *et al.* (2009)** reported that methanolic extracts of the *Avicennia marina* exhibited strong antiradical potential against DPPH assay. Antioxidants from natural sources play a paramount role in helping endogenous antioxidants to neutralize oxidative stress. Antioxidants inhibit or prevent oxidation of substrates and evolve to protect cells against the damage effects of reactive oxygen species, such as singlet oxygen, superoxide, hydroxyl radical etc. **(Gulcin, 2010).**

Free radicals are electrically charged molecules that are produced as by-products of our own metabolism. They are continuously produced by our body's use of oxygen such as in respiration and some cell mediated immune functions. In normal metabolism, the levels of oxidants and antioxidants in humans are maintained in balance, for sustaining optimal physiological conditions **(Temple, 2000).**

In recent times, there is an increasing interest in the role of free radical mediated damage in the etiology of human diseases. Over production of free radicals in certain conditions can cause an imbalance, leading to oxidative damage to large biomolecules such as lipids, DNA and proteins **(Liu, 2002)** and thus leads to a range of chronic diseases, such as cardiovascular disease, neuronal disease, cataracts and several forms of cancer. It is established that the intake of antioxidant substances reinforces defences against free radicals. Therefore, it is urgent to find natural antioxidants. Plants are the basis of life on earth and are central to people's livelihoods **(Albert and Kuldip, 2006)**.

Ravikumar and Gananadesigan (2011) evaluated the antioxidant activity of *Lumnifera racemosa* leaf extract against DPPH, hydroxyl radical scavenging activity Sen ***et al*. (2013**) reported the antioxidant activity of methanol and ethyl acetate extract of *Exoecarthia agallocha* and *Avicennia* leaf using DPPH assay. Ethanol, methanol, chloroform, ethyl acetate and water extract of *Sonneratia alba* was analysed for their antioxidant capacity using DPPH radical scavenging assay **(Haq *et al.,* 2014)**. **Shanmugapriya *et.al.* (2012)** reported the methanol extracts of *Avicennia marina* and *Avicennia officinalis* to be rich in secondary metabolites and possess antioxidant and antibacterial activity. Acetone, methanol, ethyl acetate and ethanol extracts of *Avicennia marina* fruit and leaf were reported for their antioxidant potential, as these could scavenge the ABTS (2,2- azinobis (3-ethylbenzothiazoline-6-sulphonic acid)), CrO_5 (Chromium peroxide) and FRAP (Ferric reducing ability of plasma) molecules **(Sharief *et al*., 2014a, Sharief *et al.,* 2014b).** In another study, the ethyl acetate, ethanol, methanol extracts of pneumatophores along with aqueous and ethanolic bark extracts **(Packia Lincy *et al.,* 2013)** of *Avicennia* plant exhibited strong antioxidant activity by

scavenging DPPH (2,2- diphenyl-1-picrylhydrazyl), superoxide and ABTS radicals **(Selvasundhari *et al.,* 2014).** Similarly, the acetone, methanol and ethanol fruit extracts of *Avicennia* officinalis were reported for their capacity to scavenge ABTS, CrO_5, DPPH and FRAP **(Sharief *et al*. 2014b).**

The methanolic leaf extract of *Rhizophora* exhibited a strong anti-cholinesterase activity (AChE assay) with an IC_{50} value of 59.31 ± 0.35 µg/mL and potent antioxidant activity (DPPH) with an IC_{50} value of 47.39 ± 0.43µg/mL. These significant results could be attributed to the presence of a high number of flavonoids, particularly catechin **(Vadlapudi, 2009).** With regards to antidiabetic activity, *Rhizophora mucronata* is considered as an excellent natural antidiabetic agent due to the presence of phenolics, flavonoids, gallic acid, quercetin, and coumarin **(Sur *et al.,* 2016).**

GC-MS AND FTIR ANALYSIS

Gas Chromatography – Mass Spectrum (GC-MS) technique has been increasingly employed to analyse the presence of secondary metabolites in medicinal plants, as this technique has been proved to be a best valuable method for the analysis of essential oil, alcohols, acids, esters, alkaloids, steroids, amino groups, nitro compounds etc. **(Dineshkumar and Rajakumar, 2016).** Secondary metabolites from mangrove plants serves as a reservoir for the production of novel drug compounds. Most researchers are screening thousands of plants for the discovery of novel compounds, it is thus of high importance to scrutinize mangrove species with that very aim to isolate new phytochemicals which can be potential candidates for the development of pharmaceutical

drugs. Nearly about 200 bioactive metabolites have already been identified from mangroves **(Saranraj and Sujitha, 2015; Eldeen and Effendy, 2013)**

Revathi *et al* (2014) evaluated the phytochemical screening and GC-MS analysis of ethanol extract of *Bruguiera cylindria* leaf. Forty-eight phytocompounds were identified by GC-MS analysis. Ten bioactive photochemical compounds were identified namely 2- propenoic acid, 3-phenyl ester was found to be a major constituent with a peak area of 93.00 % and retention time 5.46, followed by Ethanone, 1-3-methoxyphenyl with a peak area of 80 % and retention time 4.43 and 4H- Pyran- 4- one, 2, 3- dihydro-3, 5-dihydroxy-6-methyl with a peak area of 78 % and 2.95 retention time, respectively. GC-MS analysis of *Rhizophora mucronata* leaves reveals that 2- Furancarboxaldehyde, 5-hydroxymethyl was found to be the major constituent with peak area of 91% and retention time of 3.48, followed by 1, 4 – Benzenediol with a peak area of 53% with retention time of 3.86 and Benzenesulfonic acid, 4-hydroxy with a peak area of 40% and retention time of 2.26, respectively in the ethyl acetate extract of *Avicennia marina* leaves seeds, flowers, stems and *Rhizophora mucronata* leaves **(Khattab *et al.,* 2012).**

Generally, the seven most common chemical constituents present are terpenoids (16.25%), tannins (12.5%), steroids (10.0%), alkaloids (9.38%), flavonoids (8.75%), saponins (8.75%), and glycosides (8.13%). Furthermore, mangroves also yielded other compounds namely fatty acid derivative, anthraquinone, amino acid, coumarin, quinine, ester, gum, phenol, terpene quercetin, and anthranoid. However, these compounds are found at low levels and are present in only certain mangrove plants. For example, the presence of fatty acids has been reported only in *Avicennia ilicifolius* and *A. marina* but

not in any other species **(Sadeer *et al.,* 2019). Jia *et al.* (2004)** identified the bioactive compounds from *Avicennia marina* using GC-MS technique. They have reported that *Avicennia marina* contains tannin, phenolic group, alkaloids, xanthoproteins, resins and coumarin and many terpenoids and steroids in the barks, leaves and flowers. A total of 14 flavonoids including flavones, 19 naphthalene derivatives, 5 terpenoids, 7 steroids, 23 tannins 6 fatty acids, 31 glucosides from wide variety of secondary metabolite classes have been listed to date in the genus *Avicennia* **(Sharaf *et al*., 2000).**

Rhizophora mucronata, a popular mangrove species consists of a broad spectrum of chemical constituents such as sugar, tannins, saponins, alkaloids, flavonoids, steroids, terpenoids, glycosides, phenolics **(Gardner, 2016; Sreedhar and Christy, 2015**), gibberellins, lipids, inositols, anthocyanidins, polysaccharides, proteins, minerals, hydrolysable tannins, and polyphenols **(Balasubramanian *et al.,* 2015).** The alkaloid obtained from *Rhizophora mucronata* called as rhizophorine is considered as a major component in the leaf of the plant **(Bandaranayake, 2002**). A study by **Rohini and Das**, **(2010)** revealed the excellent anti-inflammatory activity of the bark extract of *Rhizophora mucronata* with the presence of the phytoconstituents lupeol, quercetin, β-sitosterol and caffeic acid. **Manilal *et al.* (2015)** reported the main constituent, ethanone (1-(2-hydroxy-5-methylphenyl), isolated from the crude extract could play a pivotal role in antibiotic activity of the plant. GC/MS results of *Acanthus ilicifolius* confirmed the presence of alkaloids (acanthicifoline, benzoxazin-3-one), flavonoids, steroids (cholesterol, β-sitosterol), glycosides, saponins, tannins and terpenoids, Ribose derivative isolated from this mangrove species known as 2-benzoxazoline exhibited antiviral and antitumor activities **(Sudirman and Jacob, 2014)**.

Thatoi *et al*. (2016) identified several compounds from the terpenoid class of constituents as taraxerol , taraxerone, betulinic acid, betulin , betulinaldehyde, β-amyrin, rhizophorin A, rhizophorin-B, ent-13S-2,3-seco-14-labden-2,8-olide-3-oic acid, ribenone, ent-16-hydroxy-3-oxo-13-epi-manoyl oxide, ent-15-hydroxy-labda-8,13E-dien-3-one, ent-3α,15-dihydroxylabda-8,13E-diene, excoecarin A , ent-beyerane, rhizophorin-B; nine glycosides compounds as 7-O-trans cinnamoyl-4-epilogenin geniposidic acid, 2′-cinnamoyl-mussaenosidic acid, 10-O-5-phenyl-2,4-pentadienoyl-geniposide, 7-O-cinnamoyl-8-epiloganic acid sodium salt, 8-O-cinnamoylmussaenosidic acid, officinosidic acid, loganin C, iridoid glucoside; five steroids compounds as β-sitosterol, stigmasterol, cholesterol, campesterol, stigmast-7-en-3-ol, four tannins compounds as catechin, chlorogenic acid, gallic acid, ellagic acid, one naphthalene derivative as avicenol C and velutin (flavonoid compound).

ANTIBACTERIAL ACTIVITY

Indian mangroves form Goa such as *Rhizophora mucronata, Sonneratia alba* and *Exoecaria agallocha* were reported for possessing antibacterial activity against human pathogens such as *Staphylococcus aureus*, *Streptococcus* sp., *Salmonella typhi*, *Proteus vulgaris* and *Proteus mirabilis* **(Sahoo *et al.,* 2012)**. *Avicennia marina, Avicennia officinalis, Bruguiera sexangula, Exoecaria agallocha, Lumnitzera racemosa,* and *Rhizophora apiculata* were evaluated against antibiotic resistant pathogenic bacteria **(Abeysinghe *et al.,* 2010)**. *Suaeda maritima* and some mangroves from Odhisha was investigated and reported to have *in vitro* antioxidant as well as antimicrobial activities **(Patra *et al.,* 2011). Rai *et al*. (2010)** examined the antioxidant and antimicrobial

properties of ethanol, methanol and chloroform extracts of *Rhizophora mucronata* leaves. Ethanol, chloroform and methanol extracts of the leaves showed the highest antioxidant potential in superoxide dismutase, erythrocyte haemolysis and free radical scavenging assays respectively.

Suganthy *et al.* (2009) found out that mangrove plant extracts possess great antioxidative ability and potent antibacterial ability against seven food borne pathogens. Among mangrove plants *Sonneratia alba* **(Saad *et al.*, 2012),** *Sonneratia caseolaris* **(Yompakdee *et al.,* 2012)**, *Lumnitzera littorea* **(Saad *et al.,* 2011)**, *Heritiera fomes* **(Wangensteen *et al.,* 2009)** etc are reported from various parts of the world for possessing antimicrobial potential. **Han *et al.* (2008)** reported that abietane diterpenoids synthesized by *Avicennia marina* possess cytotoxic and antimicrobial activities. Phytochemical evaluation of mangroves has been done in *Bruguiera gymnorrhiza* for bruguierols A–C **(Han *et al.,* 2005),** *Avicennia marina* for naphthoquinone derivatives **(Han *et al.,* 2007),** Derris indica for flavonoids **(Koysomboon *et al.,* 2006)** etc., some of which showed effective bioactivity.

Leaf extracts of *Biophytum sensitivum* in different solvents were studied for their antibacterial activity against several human pathogenic bacteria **(Natarajan *et al.,* 2010)** and their activity is quite comparable with that of standard antibiotics. **Vadlapudi *et* al. (2009)** found that methanolic and chloroform extract of mangrove plant *Avicennia alba* is active against different types of gram positive and Gram-negative bacteria including *Streptococcus mutans, Lactobacillus acidophilus, Rhizoctonia solani, Pseudomonas marginales, Erwinia carotovora* and *Acremonium strictum.*

The mangrove plants possess strong antibacterial properties against a broad range of microorganisms. Extracts of *Avicennia* species showed a board spectrum of antimicrobial activity against *Candida albicans, Mycobacterium vaccae, Mycobacterium aurum, Mycobacterium smegmatis, Mycobacterium fortuitum,* and *Staphylococcus aureum* **(Han *et al*., 2007; Jun *et al*., 2008).** Various authors reported that mangrove *Avicennia marina* leaves cures ulcers and also possess antimicrobial activity as well as antitumor property **(Ashihara *et al.,* 2003; Feng *et al*., 2006; Abeysinghe *et al.,* 2006; Tariq *et al.,* 2007; Bobbarala *et al.,* 2009; Manilal *et al.,* 2009; Abeysinghe, 2010).**

Butanol extract exhibited a superior antimicrobial activity against both Gram positive and Gram-negative bacteria as well as effective antifungal activity **(Mahasneh, 2002)**. The leaf extracts of *Avicennia marina* (acetone, chloroform, hexane, methanol, ethanol, ethyl acetate) have been reported to exhibit antibacterial activity selectively against bacteria such as *Agrobacterium tumefaciens, Bacillus cereus, Escherichia faecalis, Escherichia coli, Staphylococcus aureus, Staphylococcus mutans, Klebsiella pneumoniae, Pseudomonas aeruginosa, Bacillus subtilis, Staphylococcus sp., Proteus sp., Pseudomonas sp.* and *Shigella* spp **(Bakshi and Chaudhuri 2014). Ruba *et al.* (2013)** reported various solvent extracts such as petroleum ether, benzene, ethyl acetate and ethanol of *Avicennia marina* stem for their antibacterial activity against *Proteus mirabilis, Salmonella paratyphi, E. coli, Staphylococcus aureus* and *Bacillus subtilis*. **Abeysinghe and Wanigatunge (2006)**, reported the antibacterial activity of *Avicennia marina* leaf extracts in petroleum ether, chloroform, ethyl acetate and ethanol against *Escherichia coli, Pseudomonas* species*, Proteus* species*, Shigella* species and *Staphylococcus species.*

STUDY AREA

Thoothukudi is a port city, a municipal corporation and an industrial of Tamil Nadu, India. The city lies in the Coromandel Coast of Bay of Bengal. Thoothukudi is the capital and headquarters of Thoothukudi district. It is located about 590 kilometres (367 miles) southwest of Chennai, 190 kilometres (118 miles) northeast of Thiruvananthapuram and 580 kilometres (360 miles) southeast of Bengaluru. Thoothukudi consist of 21 islands between Thoothukudi and Rameswaram shores in the Gulf of Mannar are notified as the first Marine Biosphere Reserve of India. About 36,000 species of flora and fauna exist in the region covered with mangroves, sandy shores and sea grass beds. The region around the Thoothukudi shores are home to rare marine flora and fauna. For the present study two locations of Thoothukudi district was chosen namely Hare Island and Punnakayal estuary. Hare Island lies adjoining the V.O. Chidambaram Port Trust in Thoothukudi. Punnakayal village is located in Tiruchendur Tehsil of Thoothukudi district. It is situated 23 km away from sub-district headquarter Tiruchendur and 29 km away from district headquarter Thoothukudi.

PLANT SURVEY

Mangrove plants and mangrove associated plants were collected and photographed in and around the selected study area. The plants were identified with the help of Flora of Presidency of Madras.

MATERIALS

Two plants namely *Avicennia marina* and *Rhizophora mucronata* selected from the study area to evaluate their biochemical composition and antimicrobial potential.

AVICENNIA MARINA (Forssk.)

Small evergreen tree, grow up to 10m high. Stem is erect with fine pale gray scales; Leaves simple leathery, opposite, ovate, petiolate with entire margin and acute tip, shady lustrous green on greater exterior, dull greyish on subordinate exterior by way of excreted salt crystals. Inflorescence cymose, in small terminal or axillary clusters on short stalks, flowers bracteate, scented; calyx lobes 2-4 mm long, obtusish, fine fimbricate marginated; corolla dark yellow, exceeding the calyx with 4 unequal spreading lobes exceeding the tube. Fruit 2-valved capsule, globose, pale green, 1.5-2.5cm long; seeds 2-4 large. As *Avicennia* is growing in a specialized habitat, which is poorly aerated, it is adapted to life in this habitat by the presence of erect leafless outgrowths of the roots called pneumatophores or breathing roots up to 50 cm long, they stick out above water and absorb air, which thought to oxygenate the roots. *Avicennia marina* is also known as gray mangrove or white mangrove **(Behbahani, 2014).**

RHIZOPHORA MUCRONATA Lam.

Evergreen tree grows up to 25 – 30 m high, 70 cm in diameter with numerous branching arching stilt roots. Bark brown or blackish with horizontal fissures. Leaves opposite, elliptical to oblong, 8–15 cm long, 5–10 cm wide, acute, entire, without visible veins, thick and leathery, glabrous, black-dotted beneath. Petiole 3 – 5 cm long. Stipules paired, leaving ring scar. Flower clusters axillary, 2 – 3 times forked, with 3–8 flowers. Bell shaped hypanthium with 4 pale yellow, pointed leathery sepals and 4 cream-colored

petals 9 mm long. Stamens 8, stalkless, anthers 6 – 8 mm long, 4 opposite sepals and 4 opposite petals. Ovary half inferior, conical, 2-celled, with 2 ovules in each cell, 2-lobed style. Berry ovoid or conical, 5–7 cm long, brown, leathery. Seed 1, viviparous, becoming cigar-shaped, to 40 cm long and 2 cm in diameter **(Plate 1).**

SOIL ANALYSIS

The soil samples were collected from Hare Island and Punnakayal. The soil samples were collected near the mangrove roots. It was stored in a polythene bags immediately to determine the physicochemical parameters such as soil pH, electrical conductivity, total nitrogen, phosphorous and Potassium.

PH (Jackson, 1965)

The pH of the soil was determined using digital pH meter. Ten grams of sample was taken in 100 ml distilled water and stirred continuously for 30 minutes with a glass rod. The pH of the suspension was recorded after half an hour of settling by the pH meter pre-calibrated using standard buffers of 4.0, 7.0 and 9.0 pH.

Electrical Conductivity (d S m-1)

About 5 grams of soil was taken separately and dissolved in 10 ml of distilled water and mixed. The solution was used to measure the conductivity with the help of an electrical conductivity meter.

Estimation of Total Nitrogen (Linder, 1944)

5 ml aliquot of digested soil and vermicompost was taken separately in a 50 ml volumetric flask. 2 ml of sodium hydroxide was added to partially neutralize the excess acid and 1ml of 10% sodium silicate was added to prevent turbidity and samples were made up to the volume and were mixed. Then, 5 ml aliquot of sample was taken in a

cuvette and 4 drops of Nessler's reagent was added and thoroughly mixed after the addition of each drop. The blank was also prepared following the same procedure. Absorbance was read at 540 nm in spectrophotometer. Ammonium sulphate was used as standard.

$$\textbf{Percent Nitrogen} = \frac{\textbf{C x ml of digest}}{\textbf{Aliquot taken (ml) x weight of sample (g)}} \textbf{ x 100}$$

C = concentration of N_2 in aliquot as read out from the standard curve.

Estimation of phosphorous (Olsen *et al.,* 1954)

Reagent A

Ammonium molybdate: dissolved 12.0 g of ammonium molybdate in 250 ml of deionized water.

Antimony potassium tartrate: dissolved 0.291 antimony potassium tartarate in 100 ml of deionized water. Added both of the dissolved reagents and made upto 1,000 ml with 5.76 N sulphuric acid.

Reagent B

Ascorbic reagent: Dissolved 1.32 g of ascorbic acid in 250 ml of reagent A and mixed well.

Procedure

30 ml aliquot of digested soil and vermicompost was taken separately in a beaker. 9 ml of deionized water was added followed by 3 ml of reagent B (ascorbic reagent). Absorbance was read at 882 nm using spectrophotometer after 10 minutes of

adding the reagent B. Phosphorous was used as standard. Phosphorus concentration for blank and soil sample was calculated from standard curve using the formula,

$$\text{Soil } PO_4\text{-P mg kg-1} = (PO_4\text{-P mg L-1 in soil - blank}) \times 20$$

Estimation of Potassium (Abul-fadl, 1948)

To 1 ml of digested soil solution 2 ml of sodium cobalt-nitrite reagent was added slowly with constant agitation. After 45 minutes, 2 ml of double distilled water was added, the contents were mixed and centrifuged at 2000 rpm for 15 minutes. The tube was then inverted and immediately drained on filter paper; 2 ml. of water are added down the side of the tube without disturbing the precipitate. The tube was again centrifuged for 5 min., inverted and thoroughly drained. The precipitates were washed with 5 ml 70% ethanol, which was blown into the tube so as to stir up the precipitates. After centrifuging and draining thoroughly, 2 ml. of water was added to the tube, and the tube placed in a boiling water bath until dissolution was complete. While still hot, 1 ml glycine solution (7.5%) and 1 ml Na_2CO_3 solution (25%) were added and thoroughly mixed. 1 ml diluted Folin-Ciocalteu reagent was then added, the contents were mixed again, and the tube was allowed to stand in a water bath at 37^0 C for 10-15 min. After cooling to room temperature, the volume is accurately adjusted to 6 ml and the absorbance was read in a photoelectric colorimeter using red filter. Distilled water was used instead of the sample for the blank.

EXTRACT PREPARATION FOR PHYTOCHEMICAL ANALYSIS

Selected mangrove plants were shade dried and powdered with the help of mixer grinder. The powdered sample was sieved to get uniform size particle and stored in an airtight container. The extract was prepared with the help of different solvents such as

hexane, chloroform, ethyl acetate, methanol, ethanol and water in 1:10 ratio (1gram sample in 10 ml solvent) with the help of soxhlet apparatus.

QUALITATIVE PHYTOCHEMICAL ANALYSIS

Phytochemical constituents were analyzed using hexane, chloroform, ethyl acetate, methanol, ethanol and water extracts of different parts of the selected plant. Standard procedures were followed **(Horbone 1984, Kokate *et al*., 1995, Harborne, 1998)** for the qualitative phytochemical screening.

Test for Alkaloid (Wagner's test):

About 1 ml of extract was taken and few drops of Wagner's reagent was added and the formation of a reddish-brown precipitate indicate the presence of alkaloids.

Test for Flavonoid (Shinoda Test):

About 1 ml of extract was added to pinch of magnesium turnings and 1-2 drops of concentrated hydrochloric acid was added. Formation of pink colour indicated the presence of Flavonoids.

Test for Phenol (Lead acetate test):

About 1 ml of extract was taken and 0.5 ml of 1% lead acetate solution was added and the formation of precipitate indicated the presence of tannins and phenolic compounds.

Test for Tannin (Ferric chloride test):

About 1 ml of extract was taken and 0.5 ml of 5% ferric chloride was added. The development of dark bluish black colour indicated the presence of tannins.

Test for Steroid and Phytosteroid:

About 1 ml of extract was dissolved in 2 ml of chloroform and equal volume of concentrated sulphuric acid was added along the sides of the test tube. Appearance of brown ring indicated the presence of steroids. Appearance of blush brown colour indicated the presence of phytosteroid.

Test for Carbohydrate (Benedict's test):

About 5 ml of Benedict's solution was added to 1 ml of extract and boiled in water bath. The appearance of red or yellow or green precipitate indicated the presence of reducing sugars.

Test for Saponin (Foam test):

About 0.5 mg of extract was diluted with 20 ml distilled water and shaken well in a graduated cylinder for 15 min. The formation of foam to a length of 1cm indicated the presence of saponin.

Test for Glycoside:

About 0.5 ml of extract was dissolved in 1 ml of water and then aqueous NaOH solution was added. Formation of yellow colour indicated the presence of glycosides.

Test for Protein & Amino Acid (Ninhydrin test):

About 0.5 ml of extract was taken and 2 drops of freshly prepared 0.2% ninhydrin reagent was added and heated. The appearance of pink or purple colour indicated the presence of proteins, peptides or amino acids.

Test for Terpenoid:

Five ml of the extract was mixed with 2 ml of chloroform and concentrated sulphuric acid to form a layer. A reddish-brown coloration of the interface showed the presence of terpenoids.

Test for Phlobatannin

Sample was boiled with 1% aqueous hydrochloric acid to produce red precipitate indicated the presence of phlobatannins.

Coumarin:

About 3 ml of 10% NaOH were added to 2 ml of plant extracts. The formation of a yellow colour was an indication for the presence of coumarins.

Cardiacglycoside (Keller-Killani Test):

Two ml of plant extract were treated with 2 ml glacial acetic acid containing a drop of $FeCl_3$. A brown coloured ring or brown-violet under a brown greenish layer indicated the presence of cardiac glycosides.

QUANTITATIVE ESTIMATION OF NUTRIENT COMPOSITION

Total Soluble Protein (Lowry *et al*., 1951)

Requirements:

- Alkaline copper reagent
- Solution A- 20% Sodium carbonate in 0.1 N sodium hydroxide
- Solution B- 1% Sodium potassium tartarate
- Solution C- 0.5% copper sulphate

To prepare 100 ml alkaline copper reagent, 98 ml of solution A, 1ml of solution B and 1 ml of solution C were mixed together freshly.

Folin-ciacalteau reagent (commercial reagent was diluted in the ratio of 1:1 with distilled water at the time of use).

Procedure:

100 mg of sample was homogenized in 10 ml of distilled water and filtered through a muslin cloth and centrifuged at 3000 rpm for 10 minutes. To the supernatant, 10% trichloro acetic acid (TCA) was added in 1:1 ratio and left in an ice bath for 30 minutes to precipitate protein. Then centrifuged at 3000 rpm for 5 minutes and discarded the supernatant. The precipitate was dissolved in 0.1N sodium hydroxide and diluted to a known volume.

To 0.5 ml of protein extract, 5 ml of alkaline copper reagent was added. After thorough mixing, 0.5 ml of folin ciocalteau reagent was added and allowed to stand for 30 minutes; the blue colour appeared and absorbance was measured at 650 nm using UV visible spectrophotometer (Model No: UV 2371). The analysis was performed in triplicates and the amount of protein was calculated and expressed as mg/g DW. Bovine serum albumin (BSA) was used as standard.

Vitamin-C (Ascorbic acid) – Baker and Frank (1968)

Reagent

- 5% of TCA
- Indophenol reagent (20 mg of dichlorophenol indophenol was dissolved in 10 ml of warm distilled water).
- 20 mg of dichlorophenol indophenols ws dissolved in 10ml of warm distilled water

- DT reagent (2 g of 2, 4 dinitrophenyl hydrazine and 1 g of thiourea were dissolved in 100 ml of 9 N sulphuric acid).
- 85% sulphuric acid
- L – ascorbic acid – standard

100 mg of each sample was homogenized with 10 ml of 5% trichloro acetic acid (TCA). The homogenate was centrifuged at 3000 rpm. To 2 ml of protein free supernatant, 1 drop of indophenol reagent and 0.5 ml of DT reagent were added and incubated at 10°C for 1 hour. Then cooled in ice bath and 2.5 ml of 85% sulphuric acid was added. After intermittent shaking for 30 minutes (until red colour appeared), 30 absorbance was measured at 540 nm. L-ascorbic acid was used as standard and the results were expressed as mg/g DW.

Vitamin E (Rosenberg, 1992)

The sample (2.5 g) was homogenized in 50 ml of 0.1 N sulphuric acids and allowed to stand overnight. The content in the flask was shaken vigorously and filtered through Whatman No.1 filter paper. Aliquots of the filtrate was used for estimation. Into stoppered centrifuge tubes, 3 ml of extract and 3 ml of water were pipetted out separately. To both the tubes, 3 ml of ethanol and 3 ml of xylene were added, mixed well and centrifuged. Xylene (2.0 ml) layer was transferred into another stoppered tube. To each tube, 2.0 ml of dipyridyl reagent was added and mixed well. The mixture (3 ml) was pipetted out into a cuvette and the extinction was read at 460 nm. Ferric chloride solution (0.66 ml) was added to all the tubes and mixed well. The red colour developed was read exactly after 15 minutes at 520 nm. Tocopherol was used as standard.

DETERMINATION OF ANTIOXIDANTS

Estimation of Total Phenolic Content (Duan *et al*., 2006)

Requirements:

- 50% Folin – ciocalteau reagent (Folin – phenol)
- 20% Sodium carbonate

Procedure:

100 mg of plant material was homogenized with 10ml of distilled water and filtered through a muslin cloth. 1 ml of the filtrate was added to 1.5ml of de-ionized water and 0.5ml of 50% Folin – ciocalteau reagent and the content were mixed thoroughly. After one minute, 1 ml of 20% sodium carbonate solution was added and mixed. The blank contains all the reagents and solution except the sample. After 30 minutes of incubation at 37ºC, the absorbance was measured at 750nm. Galic acid was used as standard.

Estimation of Total Flavonoid Content (Zhinshen *et al*., 1999)

Requirements:

- 5% Sodium nitrate
- 10% Aluminium chloride
- 1M Sodium hydroxide

Procedure:

100mg of sample was homogenized with 10ml of distilled water and filtered through a muslin cloth. 1ml of extract was added with 4ml of distilled water and mixed. After 5 minutes, 0.3ml of 5% sodium nitrate was added and again after 5 minutes, 0.3ml of aluminium chloride was added. After 5 minutes, 2ml of 1M sodium hydroxide was added and final volume was made up to 10ml with distilled water. A blank was prepared by adding all the reagents except the sample. The absorbance was read at 510nm against blank. Quercetin was used as standard.

Estimation of Total Tannin Content (Julkunen – Titto, 1985)

Requirements:

- 4% Vanillin (prepare with methanol)
- Concentrated hydrochloric acid

Procedure:

100 mg of sample was homogenized with 10 ml of distilled water and filtered through a muslin cloth. 1ml of aliquot of aqueous extract was mixed with 3ml of 4% vanillin (prepare with methanol) and 1.5ml of concentrated HCL was added. The solution was shaken vigorously and left to stand at room temperature for 20 minutes in darkness. A blank was prepared by adding all the reagents except the sample. The absorbance was read at 500 nm. Tannic acid was used as a standard.

FT – IR

Mushroom samples were lyophilized and mixed with KBr pellets and then subjected to FT-IR spectral analysis. The dried pellets were subjected to FT-IR spectroscopy measurement in the spectral range of 4000 – 400 cm with resolution of 4 cm. The results were compared with standard values and the functional groups were identified.

GC-MS ANALYSIS

GC-MS analysis of the mushroom samples were performed using a GC Clarus 500 Perkin-Elmer system comprising a AOC - 20i autosampler and gas chromatograph interfaced to a mass spectrometer (GC-MS) equipped with a Elite-1, fused silica capillary column (330 mm × 0.25 mm ID × 1μm df, composed of 100% Dimethyl polysiloxane). For GC-MS detection, an electron ionization system with ionizing energy of 70 eV was used. Helium gas (99.999%) was used as the carrier gas at constant flow rate of 1ml/minute and an injection volume of 0.5 μl was employed (split ratio of 10:1); Injector temperature 250°C; Ion-source temperature 280°C. The oven temperature was programmed from 110°C (isothermal for 2 minutes), with an increase of 10°C/minute, to 200°C, then 5°C/minute to 280°C, ending with a 9 minutes isothermal at 280°C. Mass spectra were taken at 70 eV; a scan interval of 0.5 seconds and fragments from 40 to 550 Da. Total GC running time was 36 minutes. The relative percentage of each component was calculated by comparing its average peak area to the total areas, software adopted to handle mass spectra and chromatograms was a Turbo mass. Interpretation on mass spectrum of GC-MS was conducted using the database of National Institute of Standard and Technology (NIST) having more than 62,000 patterns. The spectrum of the unknown

components was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained.

DETERMINATION OF ANTIOXIDANT ACTIVITIES

Free Radical Scavenging Assay (Hatano *et al*., 1998)

Requirements:

- DPPH
- Methanol

Procedure:

Free radical scavenging assay was measured by 2-2 Diphenyl, l-picrylhydrazine (DPPH) method proposed by Hatano *et al*. (1998) with slight modifications. 1 ml aliquot of test sample was added to 0.004% DPPH solution prepared in methanol. The mixture was vortexed for 1 minute and kept at room temperature for 30 minutes in darkness. The absorbance was read at 517nm. A low absorbance of the reaction mixture indicated a high free radical scavenging activity. Ascorbic acid is used as a standard.

$$\text{DPPH Scavenging activity (\%)} = \frac{A_{control} - A_{test}}{A_{control}} \times 100$$

A control is the absorbance of the DPPH solution without test solution. A test is the absorbance of DPPH with the test solution. Methanol was used as blank.

HYDROGEN PEROXIDE SCAVENGING ACTIVITY (Chandrika *et al*., 2007)

Requirements:

- Phosphate buffer
- H_2O_2
- Ascorbic acid

Procedure:

Samples were dissolved in 0.5 ml of 0.1 M phosphate buffer (pH 7.4), mixed with 0.5 ml of 20 mM H_2O_2 solution and measured at 230 nm. Ascorbic acid and phosphate buffer were used as positive and negative controls respectively. The activity was calculated according to the following equation:

$$H_2O_2 \text{ scavenging activity } (\%) = (1 - A_1/A_0) \times 100$$

Where A_1 is the absorbance of the test sample and A_0 is the absorbance of negative control.

ANTIBACTERIAL ASSAY

Bacterial cultures of *Bacillus subtilis, Staphylococcus aureus* (Gram positive), *Escherichia coli* and *Vibrio cholera* (Gram negative) were obtained from Microbiology department of St. Mary's College (Autonomous), Thoothukudi and was used for evaluating antibacterial activity.

Preparation of Inoculum: The Gram-positive bacteria (*Bacillus subtilis* and *Staphylococcus aureus*) and Gram-negative bacteria (*Escherichia coli* and

Vibrio cholera) were cultured using mother culture in nutrient broth overnight and incubated at 37ºC.

Disc Diffusion Method (Kirby *et al.,* 1986)

The antibacterial assay was done by standard disc diffusion method. The cultures were spread on to nutrient agar plates using sterile cotton swabs. Sterile paper discs of 5 mm diameter with mushroom extract and standard antibiotic (Amoxicilline) discs were placed over the inoculated plates followed by overnight incubation at 37ºC. The antibacterial activity was assigned by measuring the diameter of the zone of inhibition around the disc.

Medicinal plants are the major source of therapeutic agents to cure human diseases. Recent researchers in medicinal and aromatic plants made the health-care enhancement for the purpose ofmankind. The floral resources of mangrove forest are the best, known for their medicinal properties. Vast studies have been made on mangrove forest plants and their bioactive compounds during these days due to the medical importance. The mangrove herbal extracts have been practiced as a common method for the treatment of health disorders for many countries. The bioactive compounds of mangrove plants are unique in their actions since they possess competence in many bioactive principles against disease producing microbial organisms **(Mizushima and Kobayashi, 1968).**

MANGROVE ASSOCIATES

Plant species that grow in the terrestrial environment and pure halophytes are also found within or in the peripheral areas of mangrove wetlands. These species are called as associate mangroves. The terrestrial species that found associate with mangroves are unable to tolerate highsalts and therefore do not penetrate deep into the mangrove wetland. They normally found in elevated lands present within the mangrove ecosystem. Mangrove associate flora identified in Thoothukudi and Punnakayal are presented in **Plates 2 & 3**. Mangrove associate flora study in Thoothukudi listed a total of 14 plant species which belongs to 10 families. Four families were found to bear 2 associate species (i.e.) Amaranthaceae, Euphorbiaceae,

Malvaceae and Leguminosae, other 6 families were found to have one species each and those families were Chenopodiaceae,Apocyanaceae, Tiliaceae, Poaceae, Boraginaceae, Mimosaceae **(Table 1; Figure 1A).** The growth habit study of the recorded associate species resulted herb (8spp), shrub (4spp) and tree (2spp) **(Figure 1B).** Mangrove associate flora study in Punnakayal listed a total of 16 plant species whichbelongs to 12 families **(Figure 2A).** Nine families were found to have one species each and those families were Aizoceae, Boraginaceae, Asteraceae, Lamiaceae, Euphorbiaceae, Cucurbitaceae, Vitaceae, Pedaliaceae and Poaceae **(Table 2; Figure 2A).** The family Solanaceae was found to have 3 associates and the families Chenopodiaceae and Apocyanaceae was found to have 2 associates **(Figure 2A).** The growth habit study of the recorded associate species resulted herb (11spp), Shrub (2spp) and climber (3spp) **(Figure 2B).**

PHYSICO-CHEMICAL ANALYSIS OF MANGROVE SOIL

The results of soil analysis showed important differences in physico-chemical properties and are presented in **Figure 3.** The pH of the mangrove soil did not show much variations betweenthe locations studied. The pH of the soil was 8.1 and 8.0 collected from unpolluted and polluted site respectively **(Figure 3A)**. From various studies of tropical mangrove forest worldwide, it is observed that mangrove soils may be either acidic or alkaline. Some researchers found soil pH ranging from 2.87 to 6.4 **(Rambok *et al*., 2010; Ferreira *et al*., 2010).** Some other researchers reported that soil pH above 7.0 ranging from 7.4 to 8.22 **(Hossain *et al.,* 2012; Das *et al*., 2012).**

Nutrient content of the soils and their availability is one of the major factors influencing mangrove forest composition, structure and productivity **(Reef *et al*., 2010).**

Many mangrove soilshave extremely low nutrient availability **(Lovelock *et al*., 2005)** but nutrient availability varies between mangroves and also within a mangrove stand **(Feller *et al*., 2003).** The highest values of total nitrogen (166.3 g/acre) and phosphate (45.58 g / acre) were found in soil collected from polluted site and the lowest values of nitrogen and phosphate (118 g/acre and 17.87 g/acre) were found in soil collected from unpolluted site **(Figure 3B).** Mangrove soils are found nutrient limited, particularly in N and P (**Reich and Oleksyn, 2004**; **Lovelock *et al.,* 2007**). Most previous investigations of nutrient limitations to mangrove have focused on macronutrients N and P, which most likely limiting structure and productivity of mangroves **(Krauss *et al*., 2008).** In mangrove soils, N was considered the primary nutrient that affects species composition and structure of forest although more recent analysis found that N and P influence structure and composition in equal proportions **(Elser and Hamilton, 2007).**

There was also an increase in salinity (EC) of soil (6.13 ds/m) collected from unpolluted site compared to the salinity (EC) of soil (5.1 ds/m) collected from polluted site **(Figure 3A).** The potassium content was maximum in soil (153.8 g/acre) collected from unpolluted site and minimum in soil (108.3 g/acre) collected from polluted site (**Figure 3B**). All plants require potassium for maintaining intracellular electric neutrality, osmotic regulations, enzymatic activation, protein synthesis and photosynthetic metabolism. Under high salinity environment, K is also vitally important for osmotic regulation **(Downton, 1982)** and helps to form the electrical potential required to facilitatewater uptake against the strong external salt gradient**.** The availability of K in mangrove soils is variable and there is some evidence for K

limitation in some mangroves affecting forest structure and productivity **(Ukpong,1997).**

QUALITATIVE PHYTOCHEMICAL ANALYSIS

Mangrove plants contain biologically active phytochemicals such as steroids, terpenoids, flavonoids, tannins, saponins, and phenols that are responsible for their bioactivities including theirblood glucose lowering effects, antioxidant, anticancer and antimicrobial properties **(Bandaranayake, 2002).** The phytochemical components of stem and leaf extracts *Avicennia marina* and *Rhizophora mucronata* collected from polluted (Thoothukudi) and unpolluted site (Punnakayal) were examined. Fifteen different phytochemical tests were carried out for twelve different extracts. The results of the phytochemical analysis are presented in **Tables 3 to 5**.

Methanol stem extracts of *Avicennia marina* showed the presence of 13 and 14 phytoconstituents in polluted **(Table 3)** and unpolluted sites **(Table 4)** but methanol leaf extract of *Rhizophora mucronata* collected from unpolluted site showed the presence of 12 phytoconstituents **(Table 5).** The preliminary phytochemical analysis showed the presence of primary and secondary metabolites such as alkaloid, carbohydrate, protein, phenol, tannin, flavonoid, glycoside, saponin,steroid, coumarin, cardioglycoside and terpenoids. The same phytochemical compounds were reported by **Ganesh and Vennila (2011)** in their study. The methanol, ethanol and hexane extracts were found to have a greater number of phytoconstituents than other extracts.

The presence of these chemicals in these plants shows the maximum activity against the various bacterial strains **(Patra *et al*., 2009).** These plants showed the presence of phenolic compounds which are toxic to bacterial pathogens

(Aboaba *et al*., 2006). Similarly, flavonoids present in these plants are preventing the oxidative cell damage and having strong anticancer activity**.** Saponins have the property of cholesterol binding and bitterness**.** Alkaloids found in theseplants are used as basic medicinal agents for analgesic, antispasmodic and antibacterial effect **(Okwu, 2004).** Saponins present in these plants are considered to be antifungal agents and tannins prevent the growth of the microorganisms by precipitating nutritional microbial proteins.

QUANTITATIVE PHYTOCHEMICAL ANALYSIS

The quantitative phytochemical analysis of selected mangrove plant parts for total phenol content, total flavonoid content, total tannin content, protein, vitamins C and E was carried out and reported in **Table 6.** The result of total phenolic content is shown in **Figure 4A**. The TPC of methanolic leaf and stem extracts ranged from 0.36 ± 0.08 mg/g DW to 1.2 ± 0.04 mg/g DW. The phenolic content of leaf extract of *Avicennia marina* collected from unpolluted site was found to be maximum (1.2 ±0.04 mg / g DW) followed by leaf extract of the same plant collected from polluted site (1.1 ± 0.06 mg / g DW). The methanol leaf extract of *Rhizophora mucronata* had minimum amount of total phenolic content (0.36 ± 0.05 mg / g DW) compared to other extracts. The result of total flavonoid content is graphically represented in **Figure 4B.** The stem extracts of *Avicennia marina* collected from polluted and unpolluted site had higher flavonoid content (0.61 ± 0.13 mg / g DW and 0.61± 0.02 mg / g DW) followed by stem extracts of *Rhizophora mucronata* (0.48 ± 0.13 mg / g DW)as compared to the leaf extracts of both the plant. The result of total tannin content is graphicallyrepresented in **Figure 4C.** The tannin content of leaf and

stem extracts of *Rhizophora mucronata* was recorded as 0.15 ± 0.01 mg / g DW and 0.14 ± 0.02 mg / g DW which was higher than leaf and stem extracts of *Avicennia marina* collected from polluted and unpolluted site.

Flavonoids are polyphenols which are only synthesized in plants. They have anti-oxidative and anti-inflammatory actions that work in human body to enhance health and possibly minimize certain effects of ageing. It has been discovered that they can block the action of enzymes and deactivate substances that promote the growth of cancers **(Sharaf *et al.,* 2000; Itoigawa *et al.,* 2001; Sellappan and Akoh, 2002; Khafagi *et al*., 2003; Verena *et al*., 2006).** They have been found to have the ability to inhibit low density lipoprotein oxidation by free radicals (**Rufer and Kulling, 2006).** They have been found to have negative correlation with incidence of coronary heart disease and inhibit platelet aggregation. The bark of Rhizophora species has traditionally been used as powerful astringent in diabetics, hemorrhages and angina. They are also used for traditional fishnet dyeing **(Agoramoorthy *et al.,* 2008).** These uses can be attributed to the tannin contents. Tannins have been extensively studied for their potential anti-bacterial **(Akiyama *et al*., 2001; Lu *et al.,* 2004),** anti-viral **(Cheng *et al.,* 2002; Quideau *et al.,* 2004; Lu *et al*., 2004),** anti-carcinogenic **(Yang *et al.,* 2000; Tanimura *et al.,* 2005)** and anti-AIDS **(Nonaka *et al*., 1990)** effects. Apart from their medical uses and potentials, tannins are important in leather tannery because of their ability to produce different colours with ferric chloride or sulphate. They are also responsible for colour changes in food **(Edeoga *et al*., 2006).** They have also been used as component in industrial particle board adhesive **(Bisanda *et al.,* 2003).** Recent studies have shown that products containing tannins (in

low dosages) in diets can be beneficial **(Schiavone *et al*., 2008).** The incorporation of R. racemosa leaves as feed additives for broiler chicks led to a lineal body weight gain and enhanced performance of breeding cocks **(Wekhe *et al*., 2007).**

Leaves of *Avicennia marina* and *Rhizophora mucronata* collected from polluted and unpolluted site exhibited high vitamin content than stem extracts of the same plants **(Table 6; Figure 5A).** The maximum Vitamin C was recorded (13.38 ± 0.58mg/g DW) in leaf extract of collected from polluted site followed by leaf extract of the same plant (12.4 ± 0.46mg/g DW) collected from unpolluted site. Vitamin E content of *Rhizophora mucronata* leaf was found to be 12.16 ± 0.53 mg /g DW which is higher than other extracts **(Table 6; Figure 5B).** The crude protein content of the leaf sample of *Rhizophora mucronata* was found to be 4.9 ± 0.4 mg/g DW which is higher than other extracts followed by leaf sample of *Avicennia marina* (4.2 ± 0.4 mg/g DW) collected from unpolluted site **(Table 6; Figure 5C**).

ANTIOXIDANT ACTIVITY

Oxidative injury now appears the fundamental mechanism underlying a number of human disorders such as inflammation, viral infections, autoimmune pathologies, diabetes, cancer etc. **(Aruoma, 2003).** So, it is quite possible that antioxidants of natural origin can play a pivotal role in management of oxidative stress mediated complications. The antioxidant potential of plant extracts can be evaluated by *in vitro* antioxidant methods. In the present study,the antioxidant activities of the stem and leaf extracts of *Avicennia marina* and *Rhizophora mucronata* were evaluated by employing DPPH and hydrogen peroxide scavenging assays and results are presented in **Table 7**.

DPPH antioxidant activity test is a direct and dependable method for determining the radical scavenging action. An odd electron from the DPPH radical is responsible for the absorbance at 517 nm and also for a noticeable deep purple colour. When DPPH accepts an electron from an antioxidant compound, the DPPH becomes colourless, which can be quantitatively measured from the changes in absorbance. The present study showed that both stem and leaf extracts of *Avicennia marina* and *Rhizophora mucronata* could scavenge DPPH radical. The stem extract scavenging potential varied between 34.3% to 44.3%. The leaf extracts potential for scavenging DPPH radical varied between 16.9 % to 26.2% **(Figure 6A).** In the present study, better antioxidant potential was observed in the stem of the selected mangrove plants than the leaves of the same. The present results agree with the findings of **Gawali and Jadhav (2011); Nusrat *et al.,* (2008).**

Hydrogen peroxide plays an important role in cell communication at low levels but duringpathological conditions, the high amount of hydrogen peroxide was generated in biological systemreact with naturally occurring iron complexes in *in vivo* to generate extremely reactive hydroxyl radicals and this may be the beginning of many of its toxic effects **(Miller *et al.,* 2000).** As shownin **Figure 6B**, the percentage inhibition of H_2O_2 by the methanolic leaf extract of *Avicennia marina* collected from polluted site was found to be 75.9% followed by the same leaf extract of *Avicenniamarina* collected from unpolluted site (72.3%). The percentage inhibition of H_2O_2 was found to be minimum in stem extract of *Avicennia marina* collected from unpolluted site (10.72%). Mangrove usually grow in estuarine swamps and have unique adaptations to combat environmental stress conditions e.g. high salinity, high temperature, excessive radiation

and low nutrient. An inevitable consequence of this process results in the production of ROS and accordingly the antioxidant enzymes were up regulated due to altered expression of these antioxidant genes **(Naskar *et al*., 1995).**

FTIR

FTIR is an important tool which enable us to understand the involvement of functional groups in the interactions between biomolecules **(Janakiraman *et al.,* 2011).** The FT-IR analysis was performed to determine the presence of functional group in the methanolic extract of leaves of *Avicennia marina* and *Rhizophora mucronata* collected from polluted and unpolluted site. The FTIR analysis of selected mangrove plants with frequency range, functional groups and their compounds are presented in **Tables 8 to 10** and the FTIR spectrum is shown in **Figures 7 to 9**. The result of FTIR analysis of methanolic leaf extract of *Avicennia marina* collected from polluted site showed 18 peaks from 620.67 cm^{-1} to 3422.45 cm^{-1} which were assigned to O – H of alcoholand phenol, C – H and C = C of alkanes, N – H of amines, C = O of saturated esters and C – Br of alkyl halides respectively **(Table 8; Figure 7).** The FTIR spectrum of methanolic leaf extract of *Avicennia marina* collected from unpolluted site is presented in **(Table 9; Figure 8).** The leaf extract exhibited 14 peaks at 516, 619, 1021, 1114, 1168, 1252, 1320, 1441, 1528, 1636, 737, 2360, 2884 and 3436 cm^{-1} for C – Br of alkyl halides, C – O of aromatic ester, C – N of aliphatic and aromatic amine, O - H of alcohol and phenol, C – H and C = C of alkanes and N – O of nitro compound functional groups **(Jyoti *et al*., 2016; Syed Ali *et al*., 2018; Ibrahim, 2015).** The FTIR spectrum of methanolic leaf extract of *Rhizophora mucronata* collected from unpolluted site is presented in

Figure 9. The peak at 3442 cm^{-1} is assigned to the O – H of H – bonded alcohols and phenols. The peak at 2884 cm^{-1} isattributed to C – H stretching of alkanes. The peak at 1644 cm^{-1} corresponds to C = C stretching of alkenes. The peak at 1537 cm^{-1} is assigned to N – O asymmetric stretch of nitro compound and in the region of 515 to 667 cm^{-1} are corresponding to the C – Br stretching of alkylhalides **(Table10).**

GC-MS ANALYSIS

Phytochemicals are responsible for medicinal activities of the plants. Nowadays, the organic compounds from plants have been studied and their activity has increased. The combination of GC and MS, which are best separation technique and best identification technique respectively made GC-MS as an ideal technique for volatile and semi volatile bioactive compound's qualitative analysis **(Grover and Patni, 2013).** The GC-MS analysis supports the presence of important bioactive compounds. The relative concentrations of various compounds were calculated by the use of gas chromatogram which gives many peaks. The height of the peakcorresponds to the relative concentration of compound. The compounds which are eluted at different timings through gas chromatogram are picked up by the mass analyzer and produce particular fragmentation pattern. This fragmentation pattern is compared to the compounds present in the reference library (NIST) in which the structure of compounds is determined. This provides the unique chemical fingerprint that shows the importance of plant under study.

The results of GC-MS analysis of methanol leaf extract of *Avicennia marina* and *Rhizophora mucronata* are given in **Tables 11 – 13.** In the GC-MS analysis of leaf

of *Avicennia marina* collected from polluted site, cyclotrisilxane hexamethyl, 5-methyl-2-phenylindolizine and trimethyl [4-(2-methyl-4-oxo-2-pentyl) phenoxy] silane was found to be major constituents with a peak area of 94% and 16.497 retention time followed by 1,4-bis (trimethylsilyl) benzene with a peak area of 5.96% and 16.572 retention time **(Table 11; Figure 10).** GC-MS analysis of leaf of *Avicennia marina* collected from unpolluted site revealed the presence of 6 different compounds such as benzo [h] quinoline, 2,4-dimethyl, trimethyl [4-(2-methyl-4-oxo-2-pentyl) phenoxy] silane, 2-ethylacridine, cyclotrisilxane hexamethyl, Tris (tert-butyldimethyl silyloxy) arsane and silicic acid, diethyl bis (trimethylsilyl) ester with peak areas between 41.61% to 94.04% and 16.92 to 17.168 retention time respectively **(Table 12; Figure 11).**GC-MS chromatogram of methanol leaf extract of *Rhizophora mucronata* collected from unpolluted site is presented in **Figure 13.** In the present study, 12 phytochemical constituents have been identified from methanol leaf extract of *Rhizophora mucronata* by GC-MS analysis **(Table13).** The result showed 1,4-bis (trimethylsilyl) benzene was the chemical compound with highest retention time (17.149) and peak area of 33.49% and trimethyl [4-(2-methyl-4-oxo-2-pentyl) phenoxy] silane was the chemical compound with lowest retention time (16.771) and peak area of35.45%. The compounds 2-ethylacridine and indole-2-one,2,3-dihydro-N-hydroxy-4-methoxy-3- 3-dimethyl showed antimicrobial and antitumor activities as reported by **(Vijayakumar and Leon Stephan Raj, 2019),** silicic acid, diethyl bis (trimethylsilyl) ester showed antibacterial activity **(Hema *et al*., 2011),** thieno [2,3-c] furan-3-carbonitrile, 2-amino-4,6- dihydro-4,4,6,6-tetramethyl showed analgesic, antianginal, antiarthritic, antihypersensitive **(Brintha *et al.,* 2017),** cyclotrisilxane

hexamethyl showed antibacterial and antioxidant activities **(Juliet *et al*., 2018).**

ANTIBACTERIAL ACTIVITY

The antibacterial activity test was done by paper disc diffusion method. In the present study, the antibacterial activity of the extracts of leaf and stem of selected mangrove plants in different solvents were tested against four human pathogenic bacteria. The bacteria used in this study are *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis* and *Vibrio cholerae*. The inhibition zone around each disc was measured in millimeter. The results of antibacterial activity of selected mangrove plant parts are presented in **Tables 14 to 17; Figures 13 to 18 and Plate 4 to 7**. Different solvents leaf extractsof *Avicennia marina* (**Figure 13 and 14)** and *Rhizophora mucronate* (**Figure 17)** showed more antibacterial activity against *Escherichia coli* compared to the stem extracts of *Avicennia marina* (**Figure 15 and 16; Plate 4)** and *Rhizophora mucronate* **(Figure 18; Table 14)**. Methanol leaf extract of *Avicennia marina* collected from unpolluted site showed better antibacterial activity (18 mm) against *Escherichia coli* **(Figure 14)** and ethanol leaf extract of the same plant collected from polluted site showed more antibacterial activity (19 mm) against the same bacteria **(Figure 13; Table 14).** Ethyl acetate leaf extract of *Avicennia marina* collected from unpolluted site showed larger inhibition zone (18mm) than the positive control (10 mm) against *Bacillus subtilis* **(Figure 14; Table 15; Plate 5).** Similarly, alcoholic extracts of the same expressed good growth inhibition activity against *Staphylococcus aureus* **(Figure 14; Table 16; Plate 6)** compared to positive control (10 mm). The results of antibacterial activity revealed that alcoholic leaf extracts of *Avicennia marina* have significant activity against *Escherichia coli*. Similar result was described by **Tambekar and Dahikor (2011).** Chloroform leaf

extract of *Avicennia marina* collected from unpolluted site showed maximum antibacterial activity (10 mm) compared to other solvent extracts against *Vibrio cholerae* (**Figure 14; Table 17; Plate 7**). In the present investigation leaf extract of selected mangrove plants showed good antibacterial activity against all tested bacteria compared to stem extracts. **Inabo and Obanibi (2006)** reported the same result with this result.

India has a great diversity of plants used in folk medicine and only few of those have beenstudied for antibacterial activity **(Ravikumar *et al.,* 2005).** The antibacterial activity exhibited by the mangrove plant parts could be due to the presence of phytochemical like alkaloids, tannins, flavonoids and sugar present in the plant extracts. The bark, leaves and fruits of *Avicennia marina*have reported as antibacterial, antifungal, antiviral agents and also possess antciancer, antitumor, antiulcer properties **(Packia Lincy *et al*., 2013; Dhayanthi *et al.,* 2012; Ravikumar *et al*., 2011).** In this present investigation, *Escherichia coli* was the most susceptible strain whereas *Vibrio cholerae* was the most resistant one **(Table 14 and 17).** Relative resistance of *Vibrio cholerae* to the plant extracts may be due to some kinds of resistance mechanism such as enzymatic inactivation of some bioactive compounds, target site modification and decrease intracellular inhibitory accumulation **(Schwarz and Noble, 1999).** From these results, it could be concluded that extracts by solvents of both the plants supreme the growth of the tested strain to varying degrees indicating the presencebroad spectrum of inhibitory principles **(Pimpliskar *et al.,* 2011)** and both the leaves contain antibacterial compounds. In our study, it was confirmed by GC-MS analysis.

The mangrove plant tissues contained various bioactive compounds such as alkaloid, phenol, flavonoid, saponin, steroid, protein, vitamin and tannin. The presence of these metabolites is an indication of their potentials as medicinal plants. The results obtained from this study can be valuable for further studies on *Avicennia marina* and *Rhizophora mucronata* and the recognition of their medicinal and bioactive values can enhance conservation plans for mangroves in Thoothukudi and Punnakayal. However, the chemical constituents present in these plant leaves which are responsible for various activities such as antioxidant, antimicrobial, antitumor, antiarthritic as they are rich in a wide variety of secondary metabolites and are to be investigated further. The adaptability of the mangrove trees to salinity could be optimized genetically as to isolate alien genes, which will be transgressed into other crops vulnerable to this ecological problem

Plate 1

HABIT OF MANGROVE PLANTS SELECTED FOR THE PRESENT STUDY

***Avicennia marina* (Forssk.)**

***Rhizophora mucronata* Lam.**

PLATE: 2A MANGROVE ASSOCIATES IN POLLUTED SITE

(THOOTHUKUDI)

Suaeda maritima

Calotropis gigantea

Euphorbia hirta

Phyllanthus amarus

Thespesia populneoides

PLATE: 2B MANGROVE ASSOCIATES IN POLLUTED SITE

(THOOTHUKUDI)

Corchorus aestuans

Abutilon indicum

Dactyloctenium aegyptium

Alternanthera sessilis

Crotalaria retusa

PLATE: 2C MANGROVE ASSOCIATES IN POLLUTED SITE (THOOTHUKUDI)

Tephrosia purpurea

Acacia nilotica

Heliotropium currassavicum

Achyranthes aspera

PLATE: 3A MANGROVE ASSOCIATES IN UNPOLLUTED SITE

(PUNNAKAYAL)

Salicornia brachiata

Sueda maritima

Sesuvium portulacastrum

Calotropis gigantea

Heliotropium currssavicum

PLATE: 3B MANGROVE ASSOCIATES IN UNPOLLUTED SITE

(PUNNAKAYAL)

Tridax procumbens

Leucas aspera

Phyllanthus amaras

Lycopersicon esculentum

Cissus quarangularis

PLATE: 3C MANGROVE ASSOCIATES IN UNPOLLUTED SITE

(PUNNAKAYAL)

Solanum suratensis

Pedalium murex

Percularia daemia

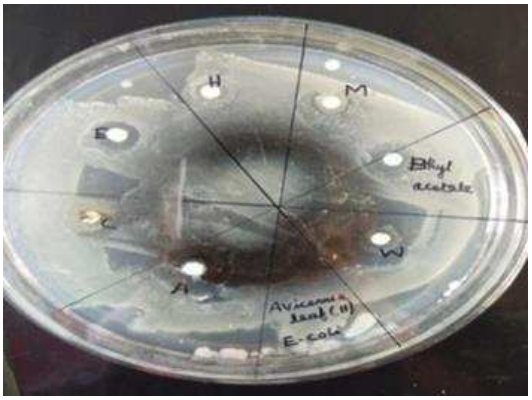
Physalis angulata

Chloris barbata

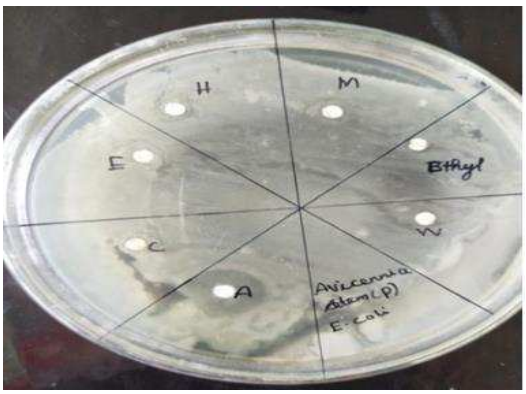
Plate 4

ANTIBACTERIAL ACTIVITY OF SELECTED MANGROVE PLANTS AGAINST ESCHERICHIA COLI

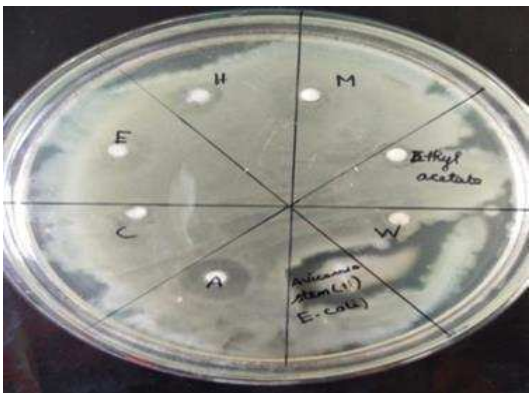
Avicennia marina leaf Punnakayal



Avicennia marina leaf Thoothukudi

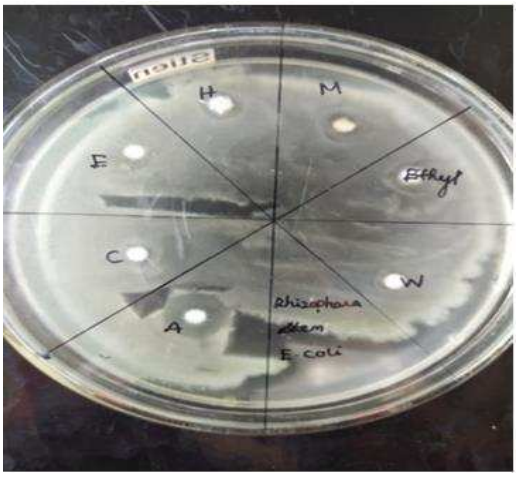


Avicennia marina stem Punnakayal



Avicennia marina leaf Thoothukudi

Rhizophora mucronata leaf Punnakayal



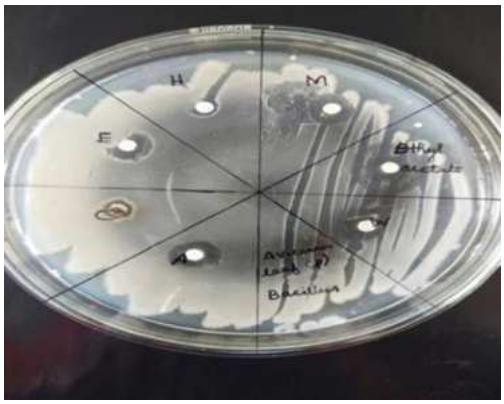
Rhizophora mucronata stem Thoothukudi

A – Acetone C – Chloroform E- Ethanol

H – Hexane ET- Ethyl Acetate W – Water

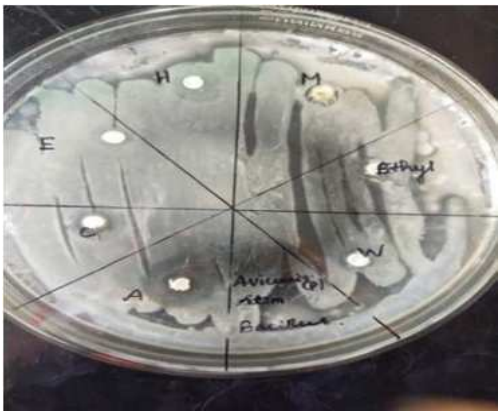
Plate 5

ANTIBACTERIAL ACTIVITY OF SELECTED MANGROVE PLANTS AGAINST BACILLUS SUBTILIS

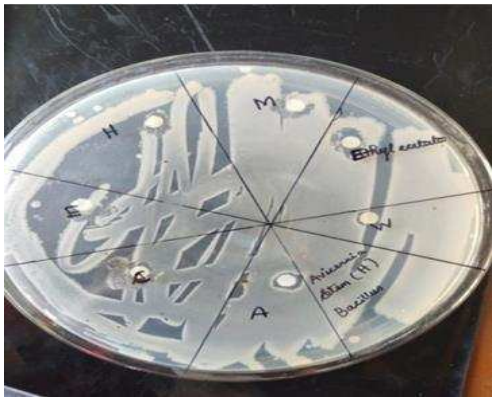


Avicennia marina leaf Punnakayal

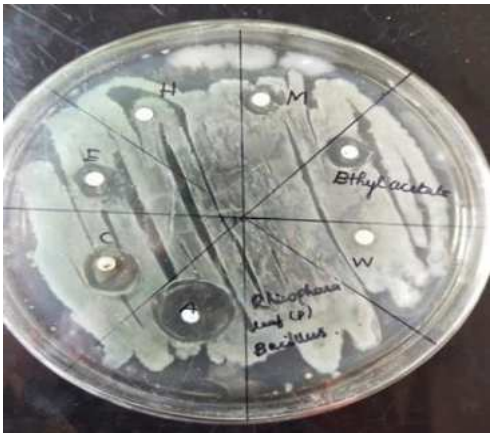
Avicennia marina leaf Thoothukudi



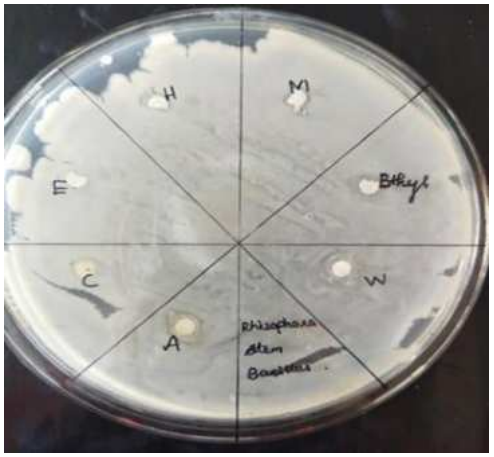
Avicennia marina stem Punnakayal



Avicennia marina leaf Thoothukudi



Rhizophora mucronata leaf Punnakayal



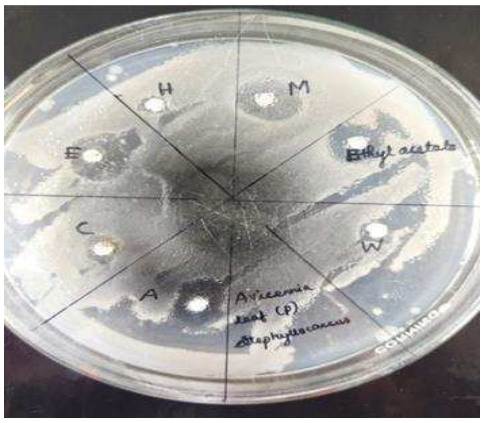
Rhizophora mucronata stem Thoothukudi

A – Acetone C – Chloroform E- Ethanol

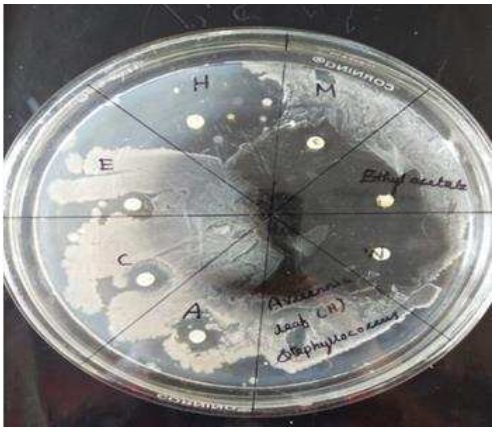
H – Hexane ET- Ethyl Acetate W – Water

Plate 6

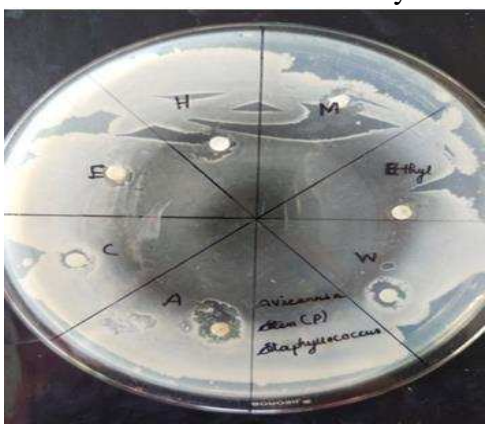
ANTIBACTERIAL ACTIVITY OF SELECTED MANGROVE PLANTS AGAINST STAPHYLLOCOCCUS AUREUS



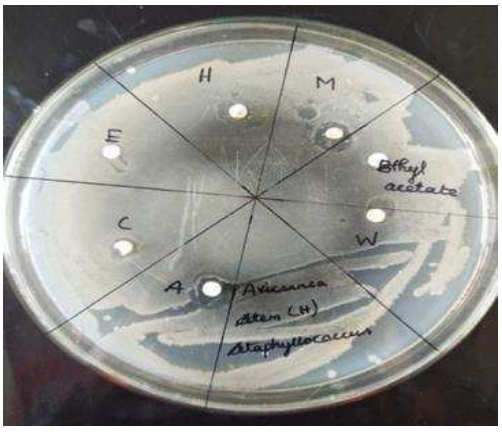
Avicennia marina leaf Punnakayal



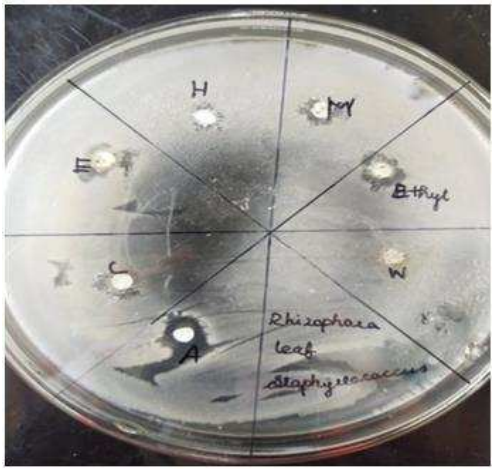
Avicennia marina leaf Thoothukudi



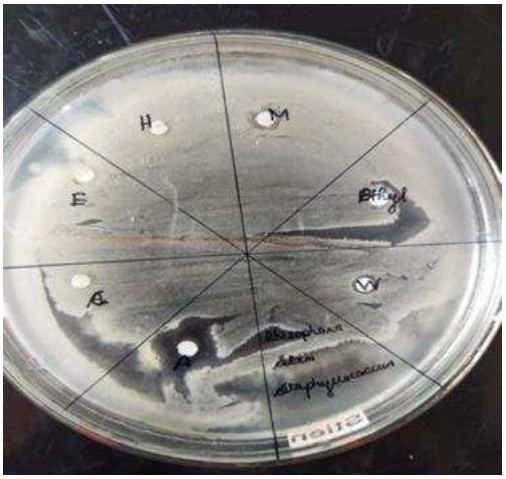
Avicennia marina stem Punnakayal



Avicennia marina leaf Thoothukudi



Rhizophora mucronata leaf Punnakayal



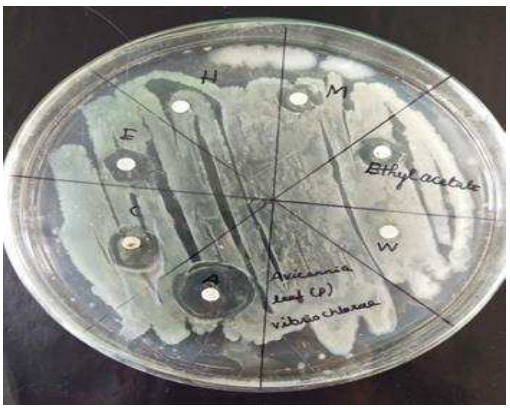
Rhizophora mucronata stem Thoothukudi

A – Acetone C – Chloroform E- Ethanol

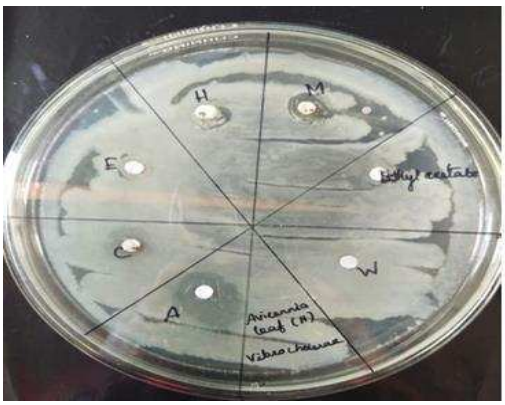
H – Hexane ET- Ethyl Acetate W – Water

Plate 7

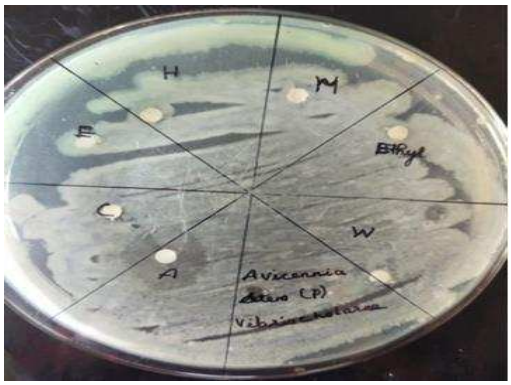
ANTIBACTERIAL ACTIVITY OF SELECTED MANGROVE PLANTS AGAINST VIBRIO CHOLERAE



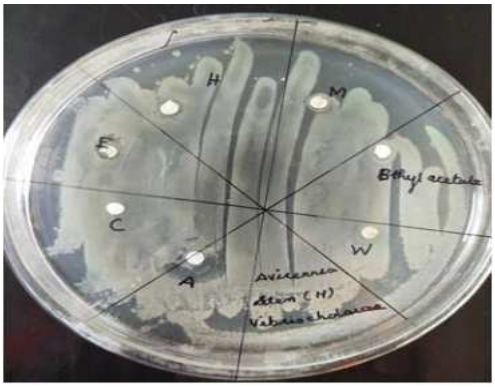
Avicennia marina leaf Punnakayal



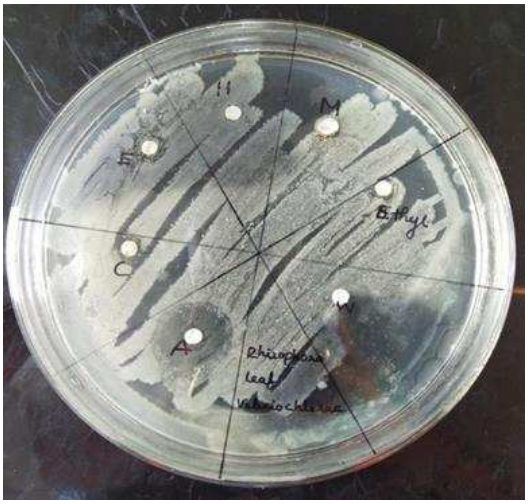
Avicennia marina leaf Thoothukudi



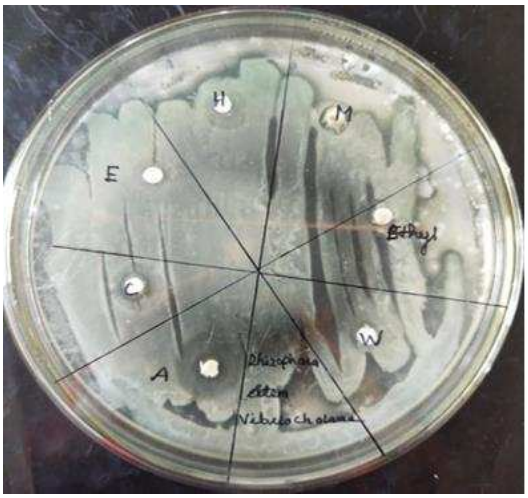
Avicennia marina stem Punnakayal



Avicennia marina leaf Thoothukudi



Rhizophora mucronata leaf Punnakayal



Rhizophora mucronata stem Thoothukudi

A – Acetone C – Chloroform E- Ethanol

H – Hexane ET- Ethyl Acetate W – Water

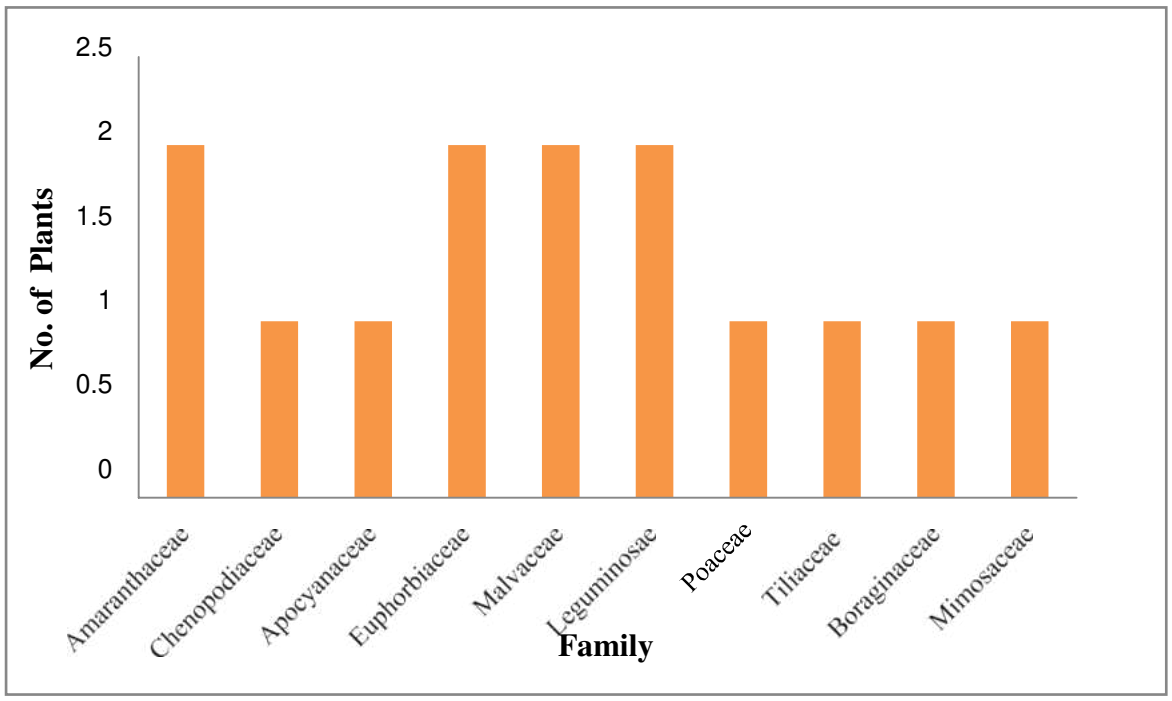


Figure 1A:Familywise Distribution of Mangrove Associatesin Polluted Site(Thoothukudi)

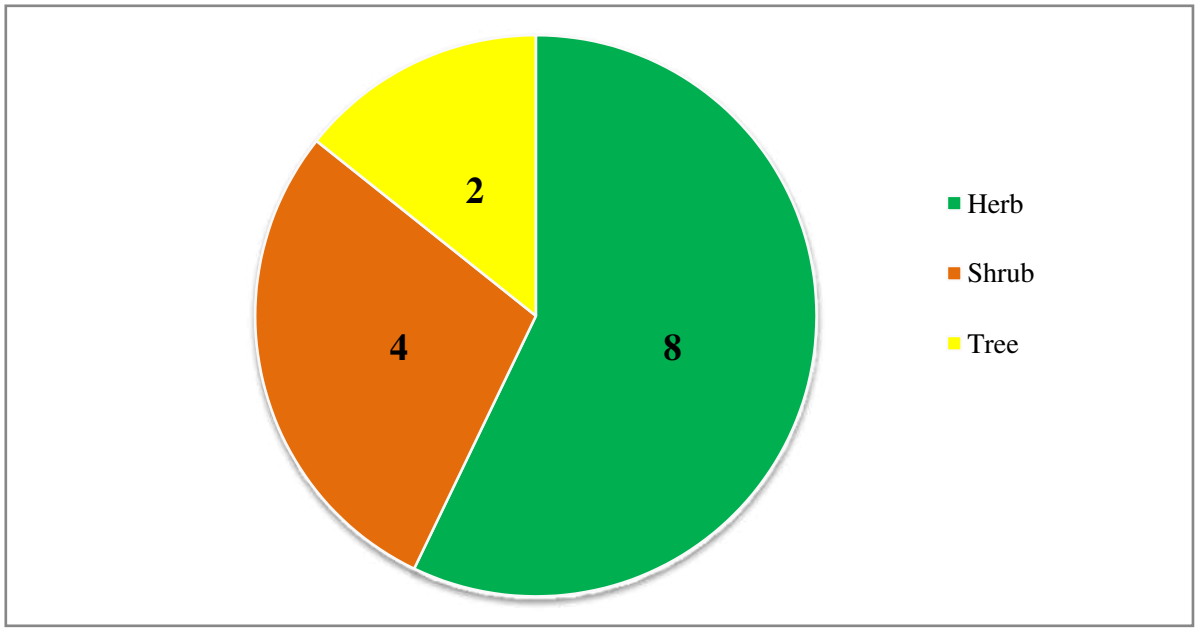


Figure 1B: Habitwise Distribution of Mangrove Associates in Polluted Site(Thoothukudi)

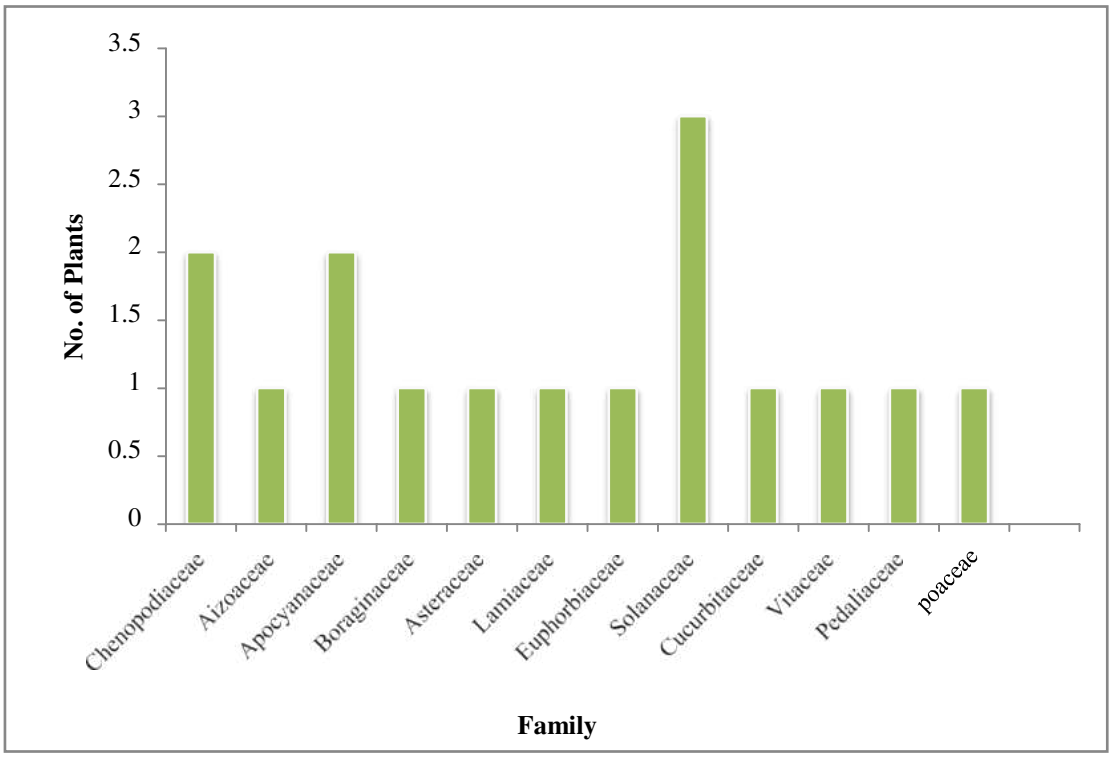


Figure 2A:Familywise Distribution of Mangrove Associates in unpolluted site (Punnakayal)

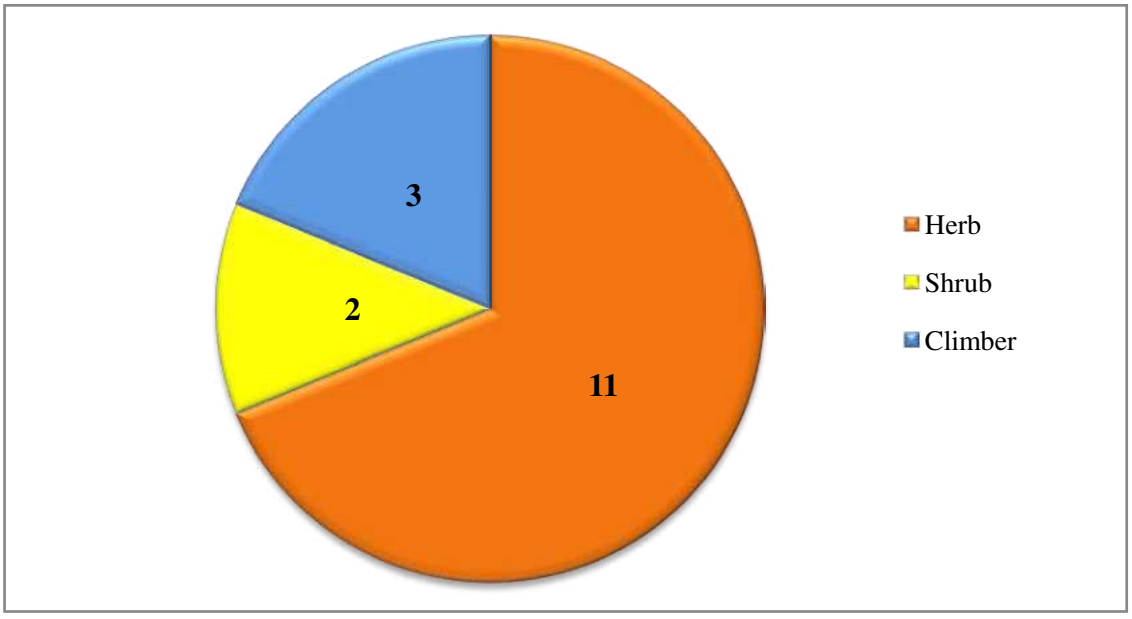


Figure 2B: Habitwise Distribution of Mangrove Associates in Unpolluted Site (Punnakayal)

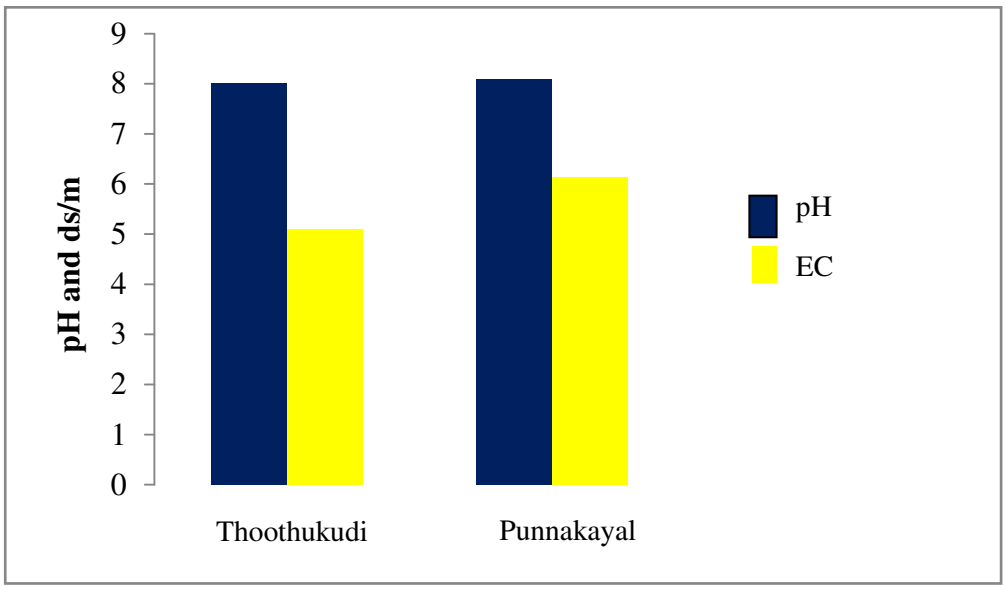


Figure3A: pH and Electrical Conductivity of Mangrove Soil Collected from Polluted Site(Thoothukudi) and Unpolluted Site (Punnakayal)

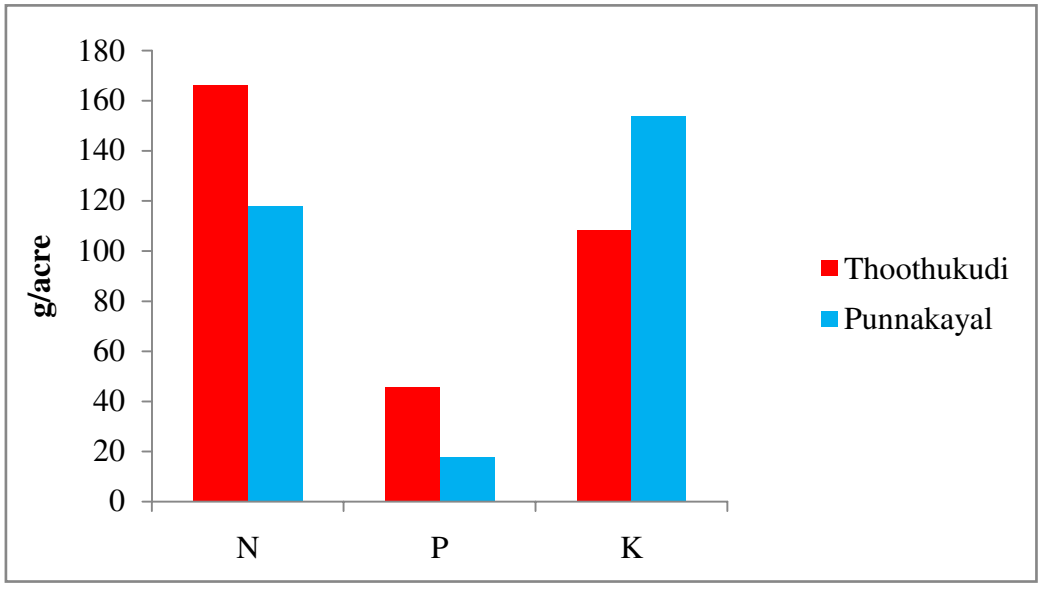


Figure 3B: Macronutrient Content of Mangrove Soil Collected fromPolluted Site(Thoothukudi) and Unpolluted Site (Punnakayal)

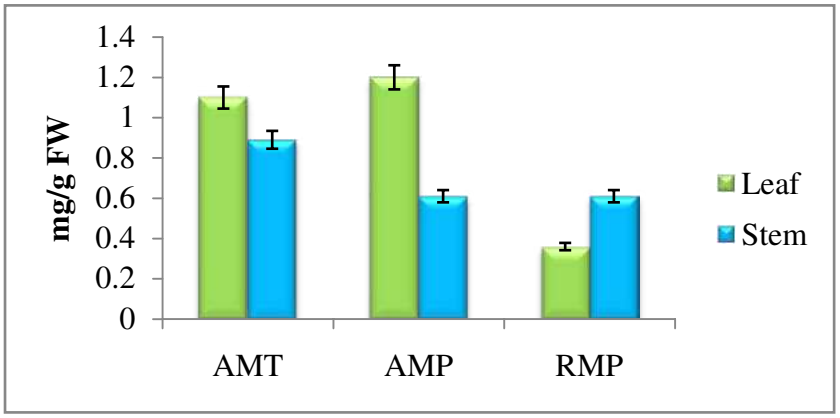


Figure 4 A: Phenol Content of SelectedMangrove Plants

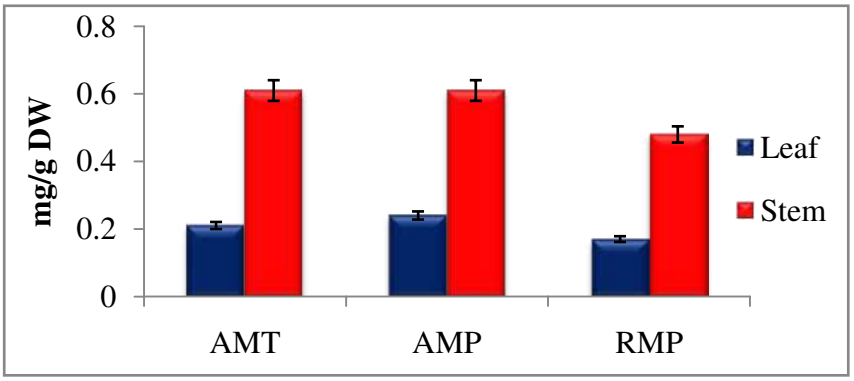


Figure 4 B: Flavonoid Content of Selected Mangrove Plants

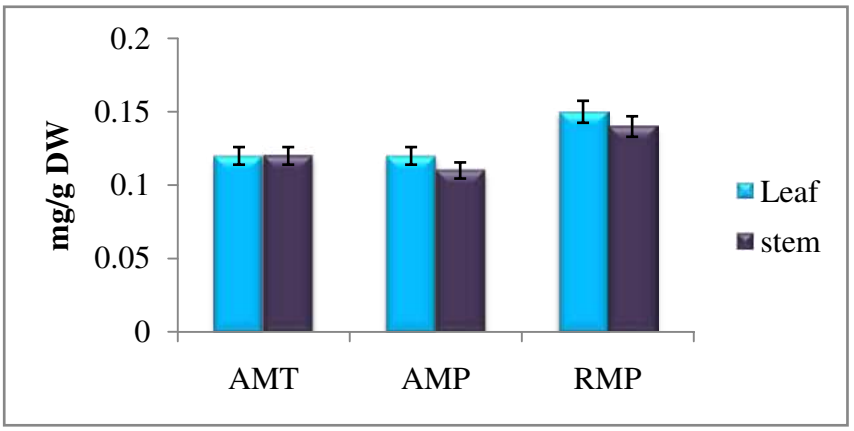


Figure4 C: Tannin Content of Selected Mangrove Plants

AMT-*Avicennia marina* Thoothukudi

AMP – *Avicennia marina* Punnakayal

RMP - *Rhizophora mucoranata* Punnakayal

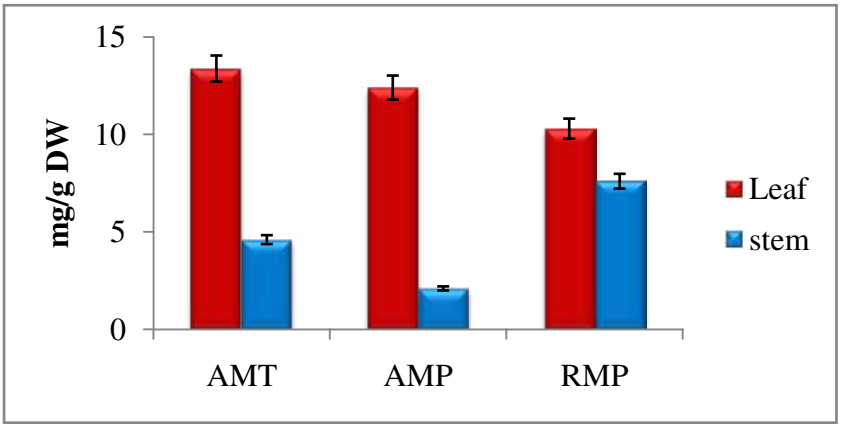


Figure 5A: Vitamin C content of Selected Mangrove Plant

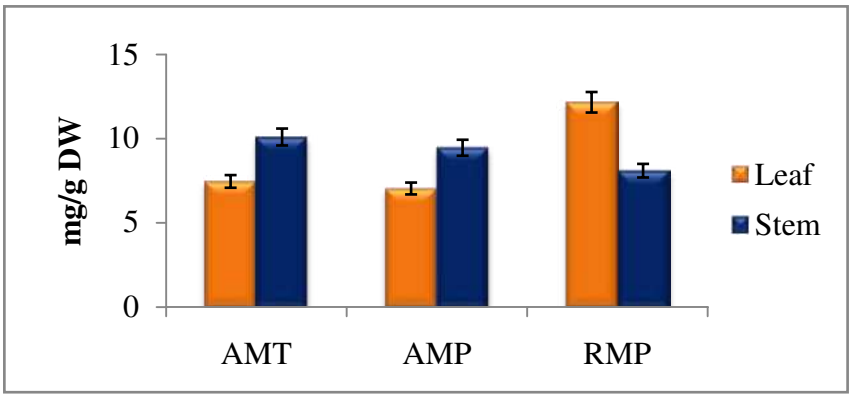


Figure 5B: Vitamin E Content of Selected Mangrove Plant

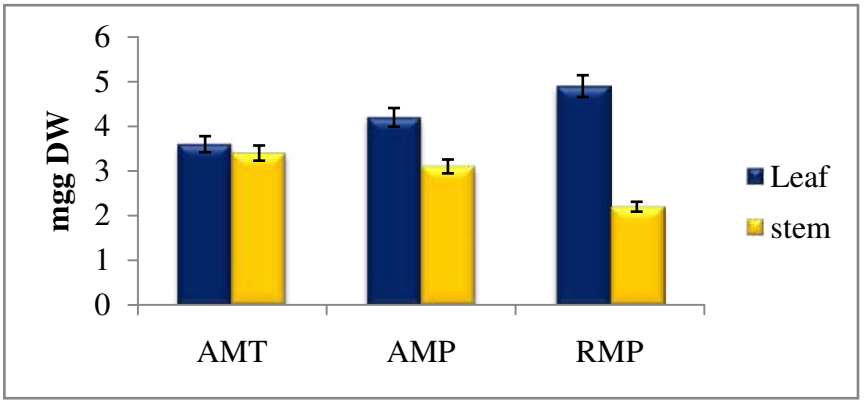


Figure 5C: Protein Content of Selected Mangrove Plant

AMT-*Avicennia marina* Thoothukudi

AMP – *Avicennia marina* Punnakayal

RMP - *Rhizophora mucoranata* Punnakayal

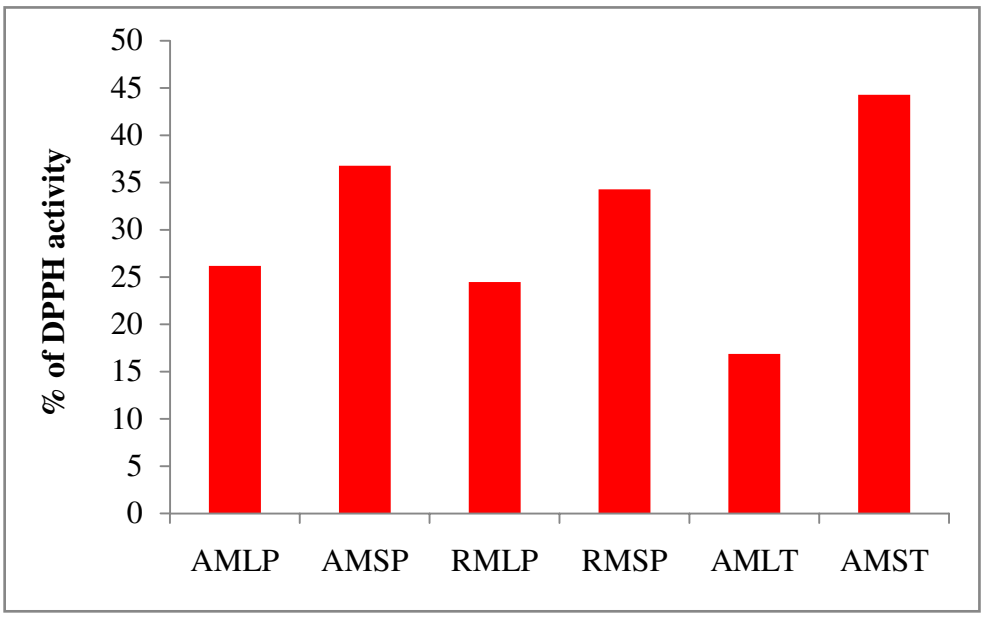


Figure 6A: DPPH radical scavenging activity of selected mangrove plant

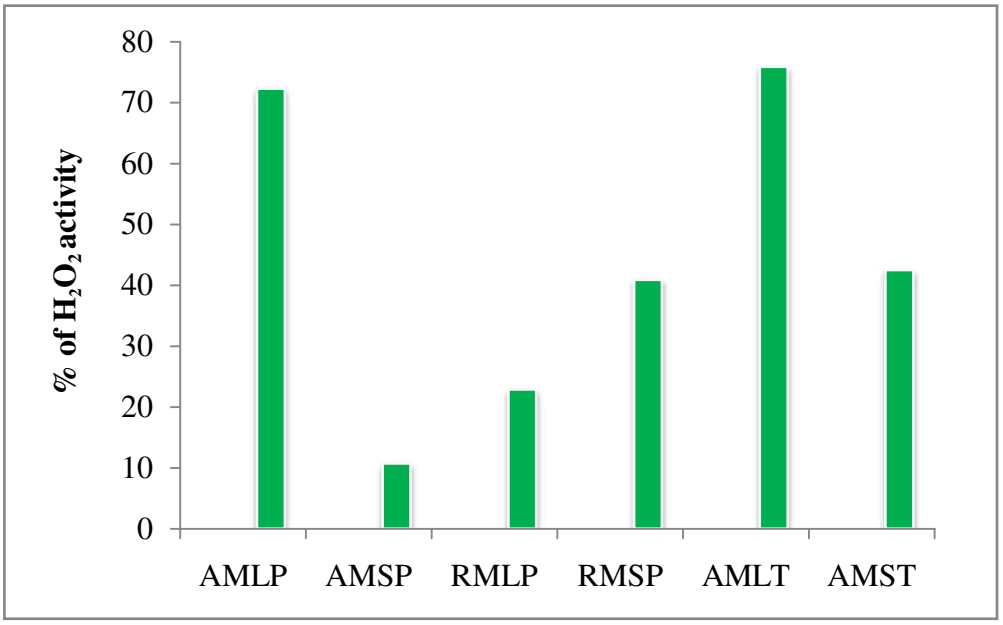


Figure 6B: H_2O_2scavenging activityof selected mangrove plant

AMLP – *Avicennia marina* leaf Punnakayal

AMSP - *Avicennia marina* stem Punnakayal

RMLP - *Rhizophora mucoranata* leaf Punnakayal

RMSP - *Rhizophora mucoranata*stem Punnakayal

AMLT-*Avicennia marina* leaf Thoothukudi

AMST-*Avicennia marina* stem Thoothukudi

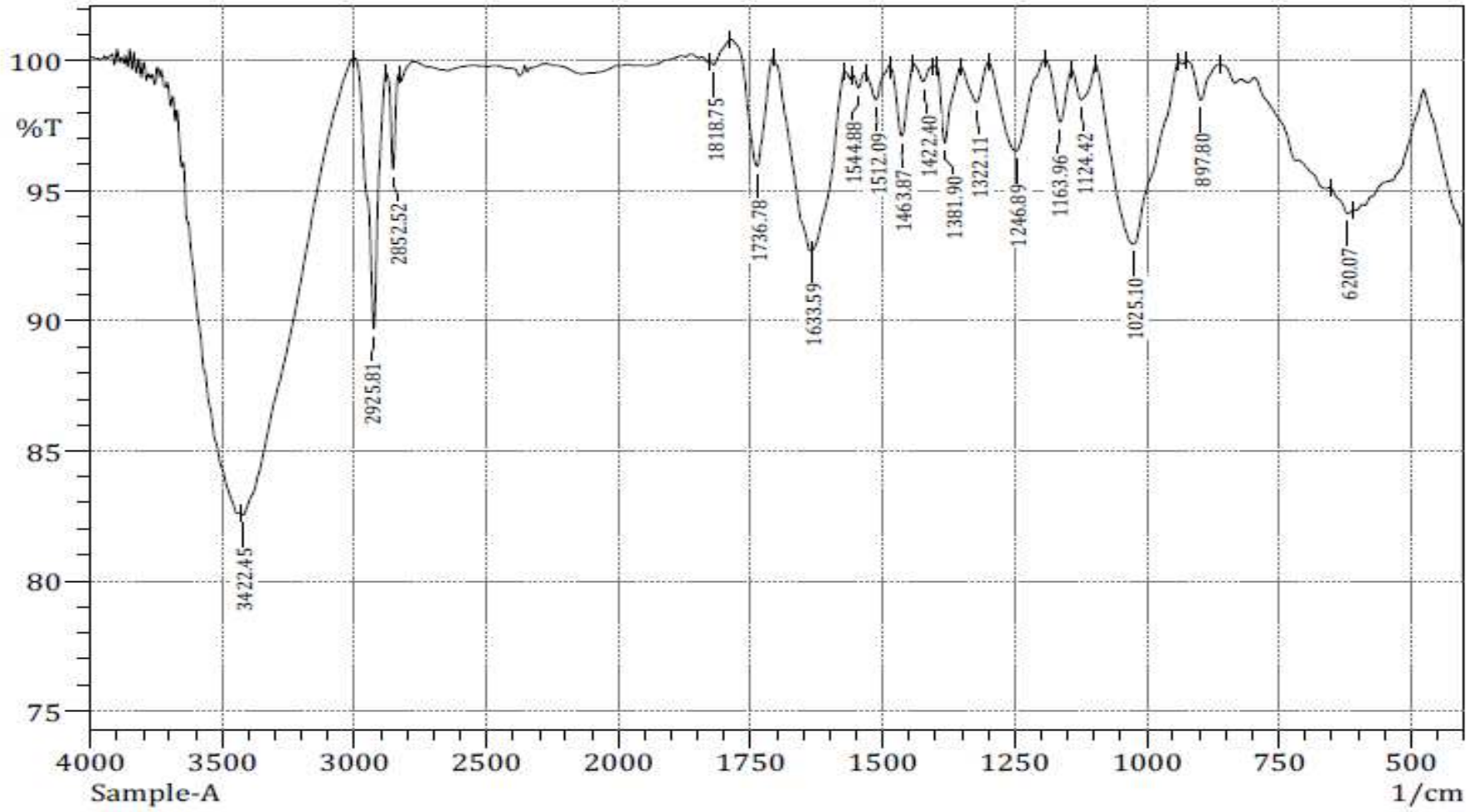


FIGURE 7: FTIR SPECTRUM OF AVICENNIA MARINA LEAF COLLECTED FROM POLLUTED SITE (THOOTHUKUDI)

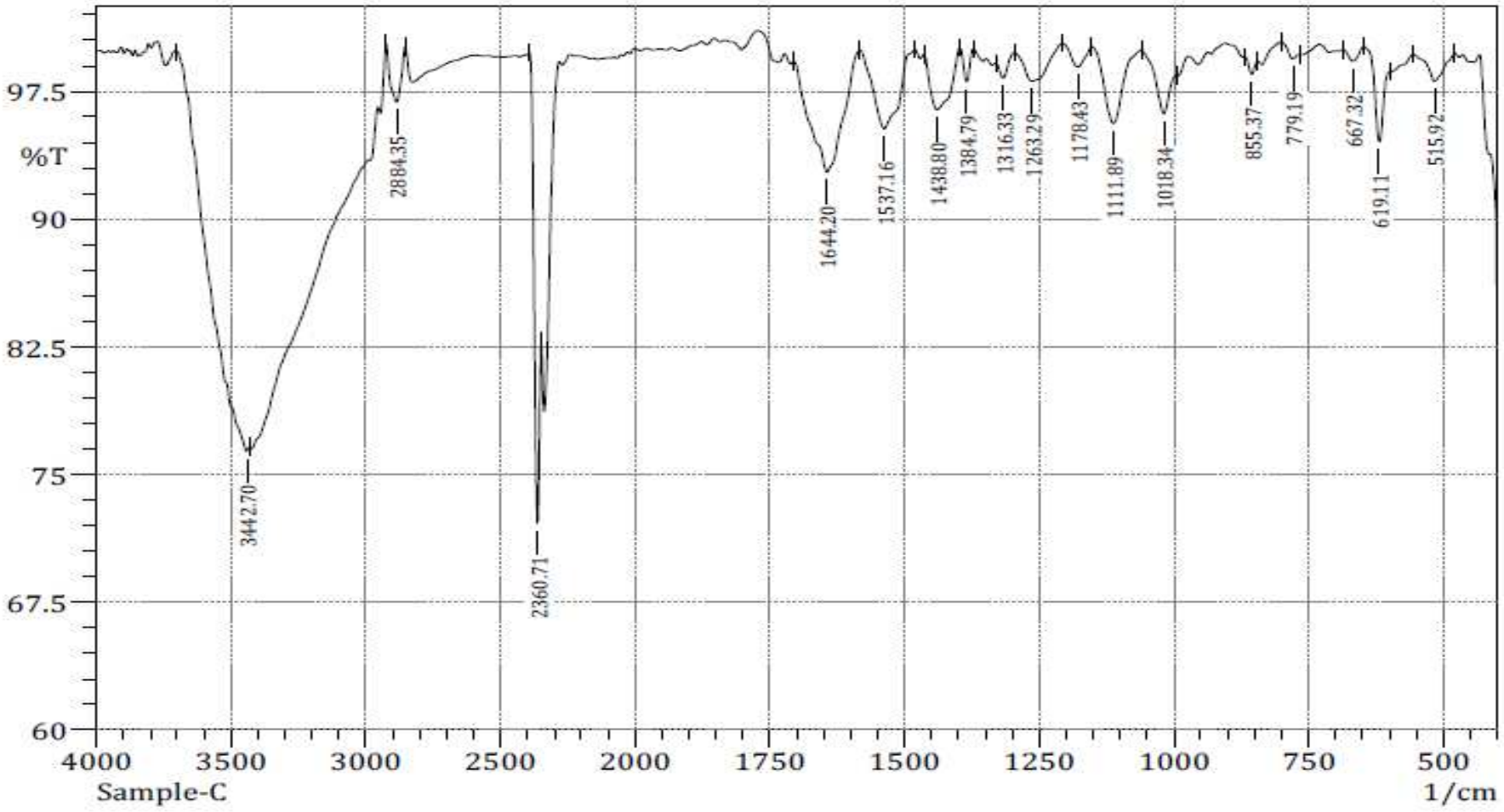


FIGURE 8: FTIR SPECTRUM OF AVICENNIA MARINA LEAF COLLECTED FROM UNPOLLUTED SITE (PUNNAKAYAL)

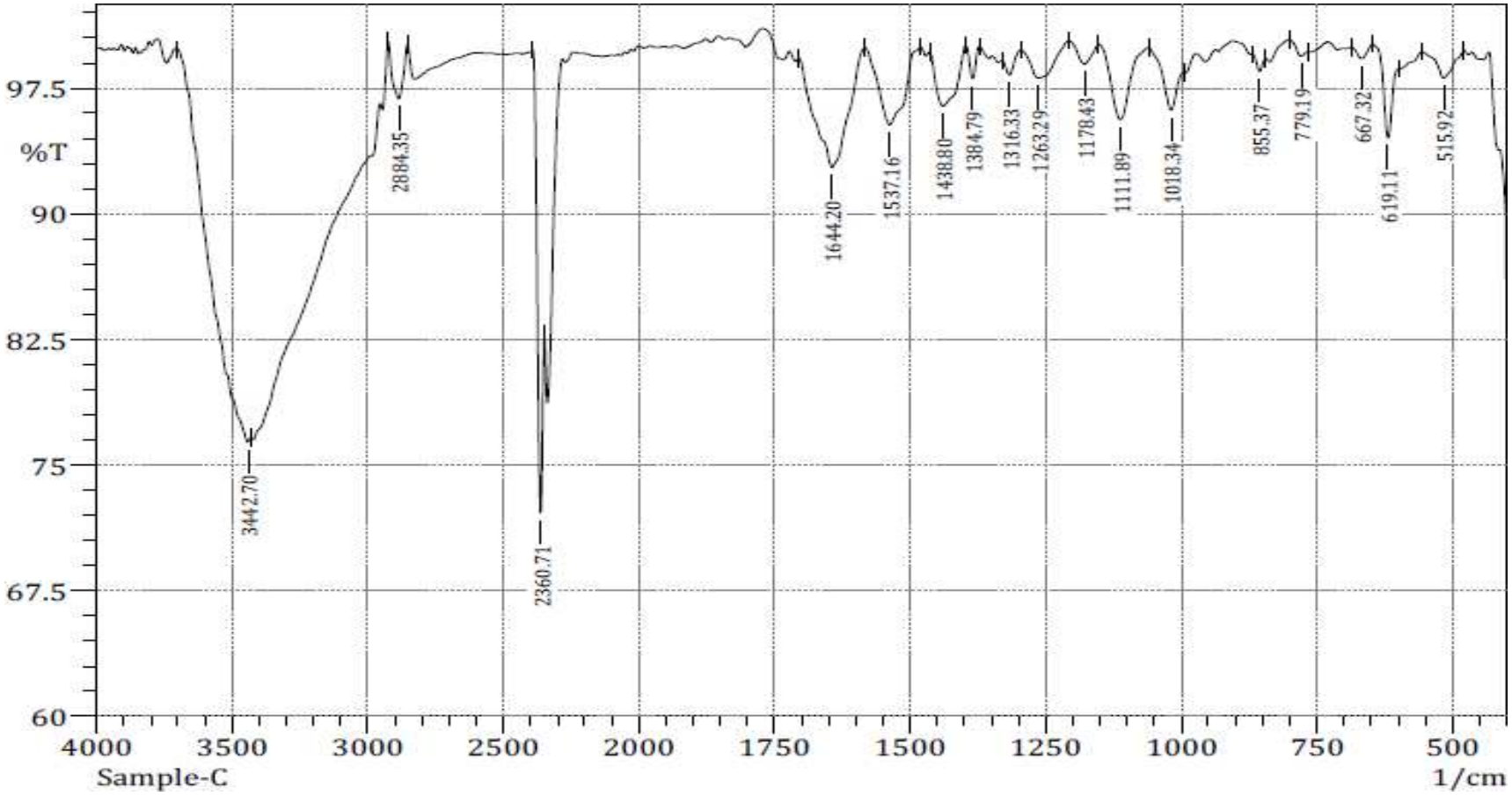


FIGURE 9: FTIR SPECTRUM OF RHIZOPHORA MUCRONATALEAF COLLECTED FROM UNPOLLUTED SITE (PUNNAKAYAL)

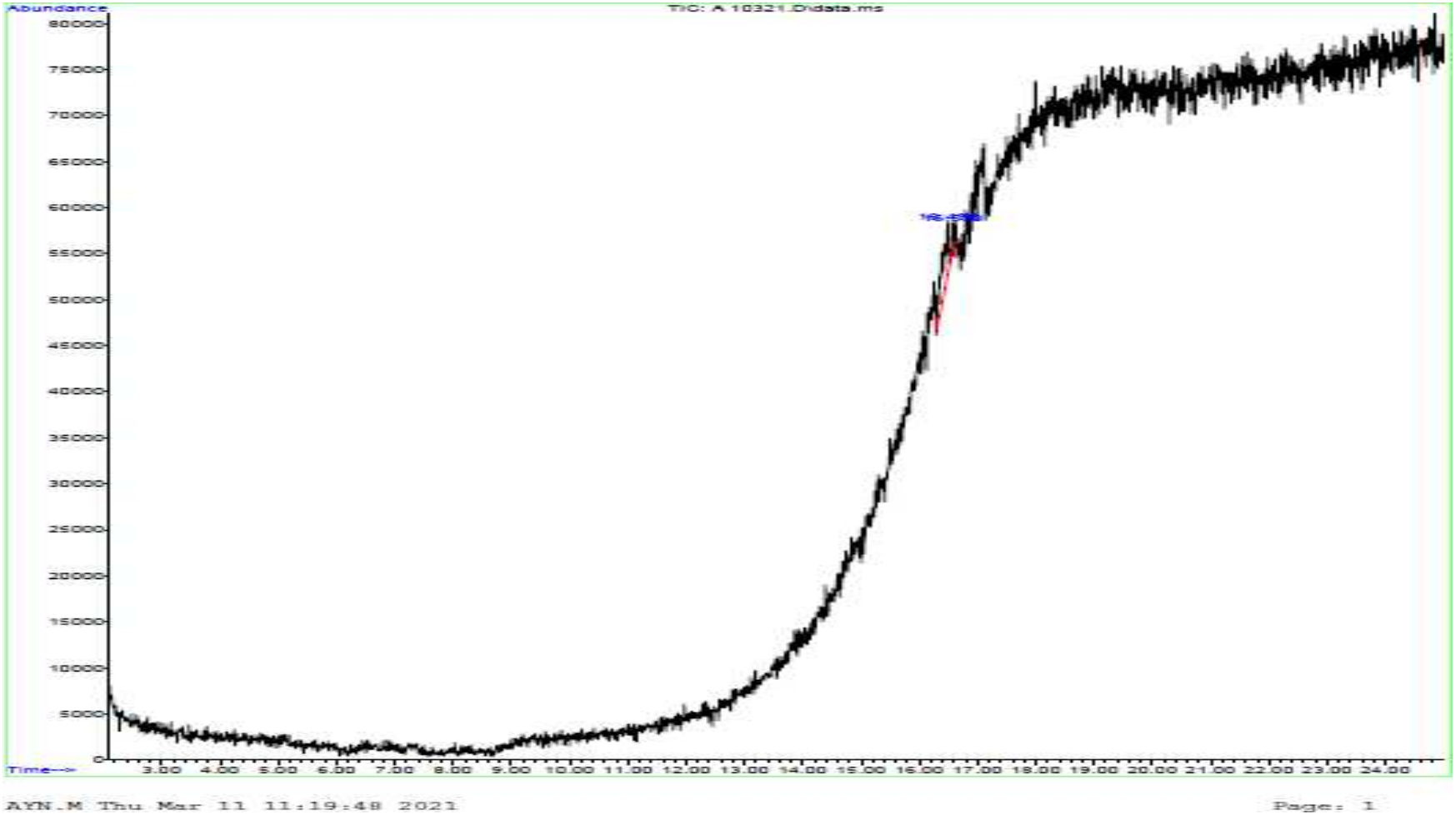


FIGURE10: GC-MS SPECTRUM OF AVICENNIA MARINA LEAF COLLECTED FROM POLLUTED SITE(THOOTHUKUDI)

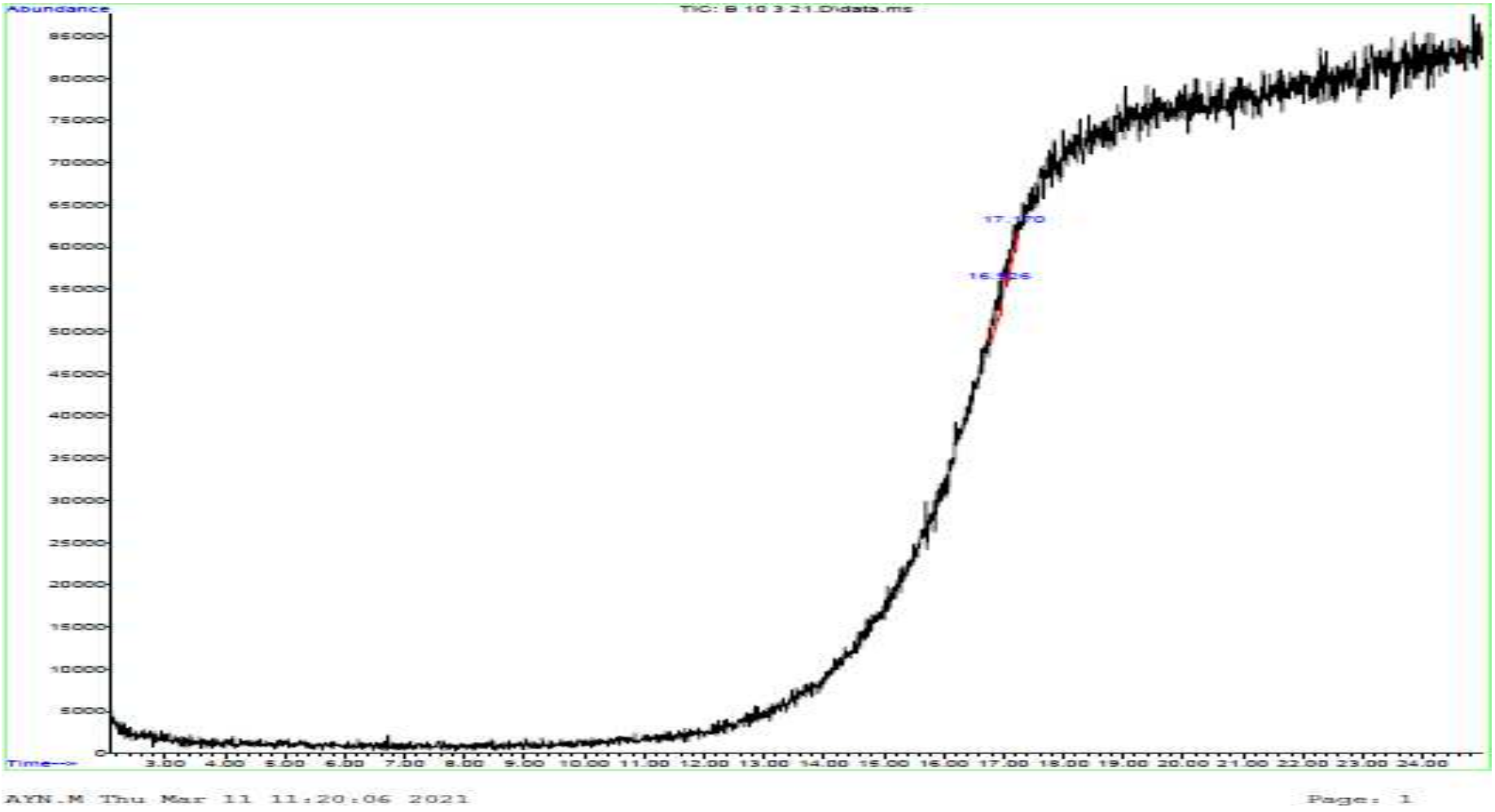


FIGURE11: GC-MS SPECTRUM *OF* AVICENNIA MARINALEAF COLLECTED FROM UNPOLLUTED SITE (PUNNAKAYAL)

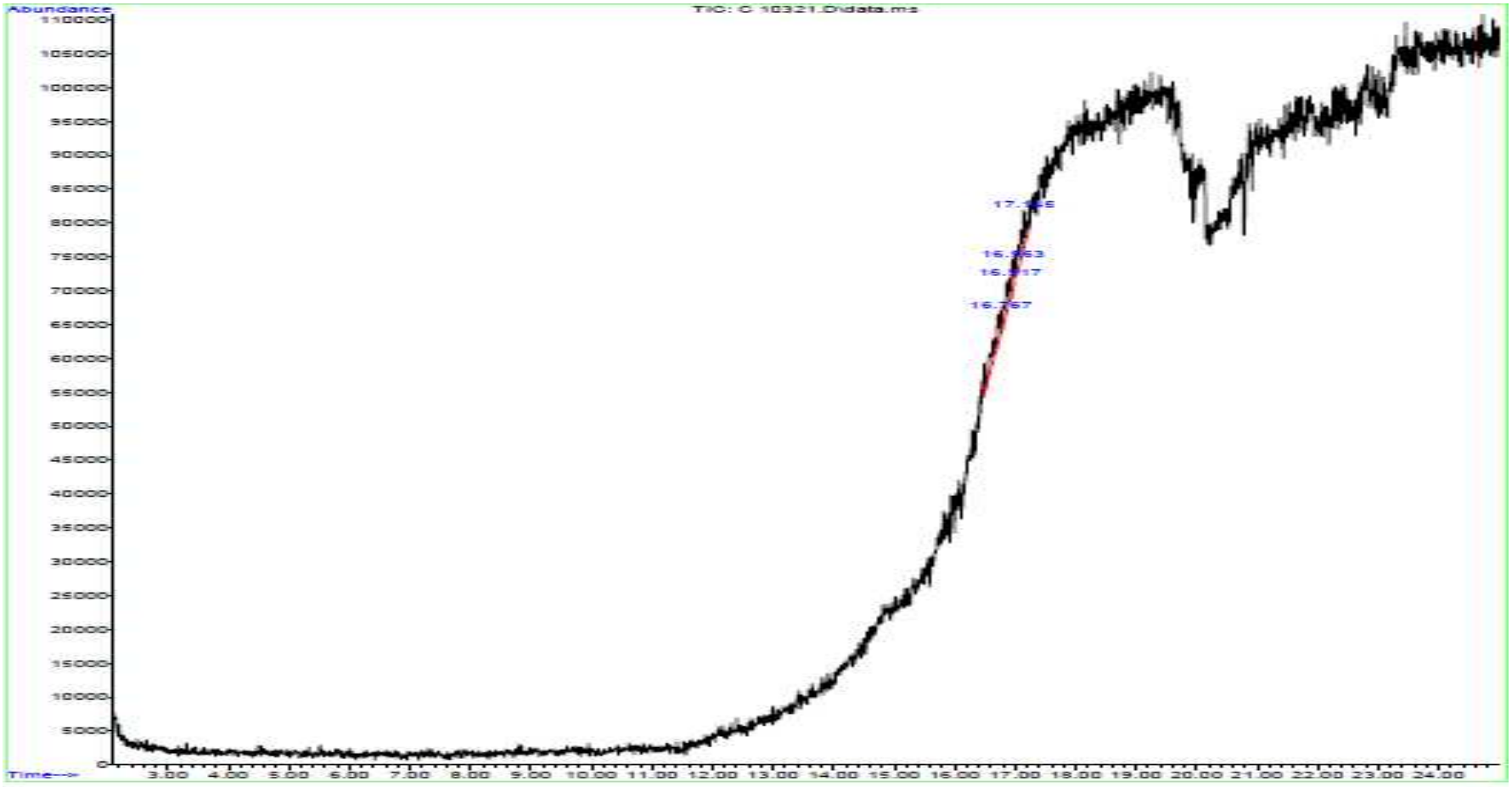


FIGURE 12: GC-MS SPECTRUM OF RHIZOPHORA MUCRONATALEAF COLLECTED FROM UNPOLLUTED SITE (PUNNAKAYAL)

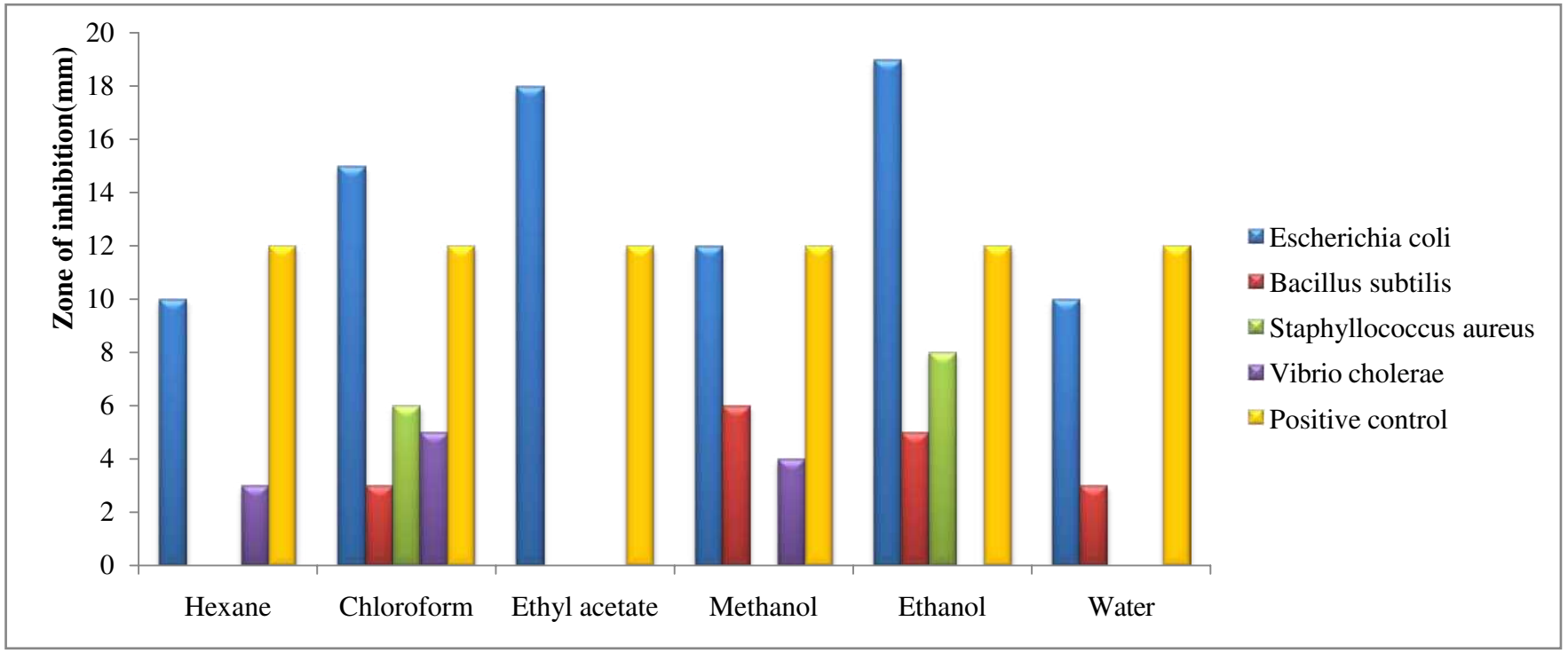


FIGURE 13: ANTIBACTERIAL ACTIVITY OF AVICENNIA MARINA LEAF COLLECTED FROM POLLUTED SITE (THOOTHUKUDI)

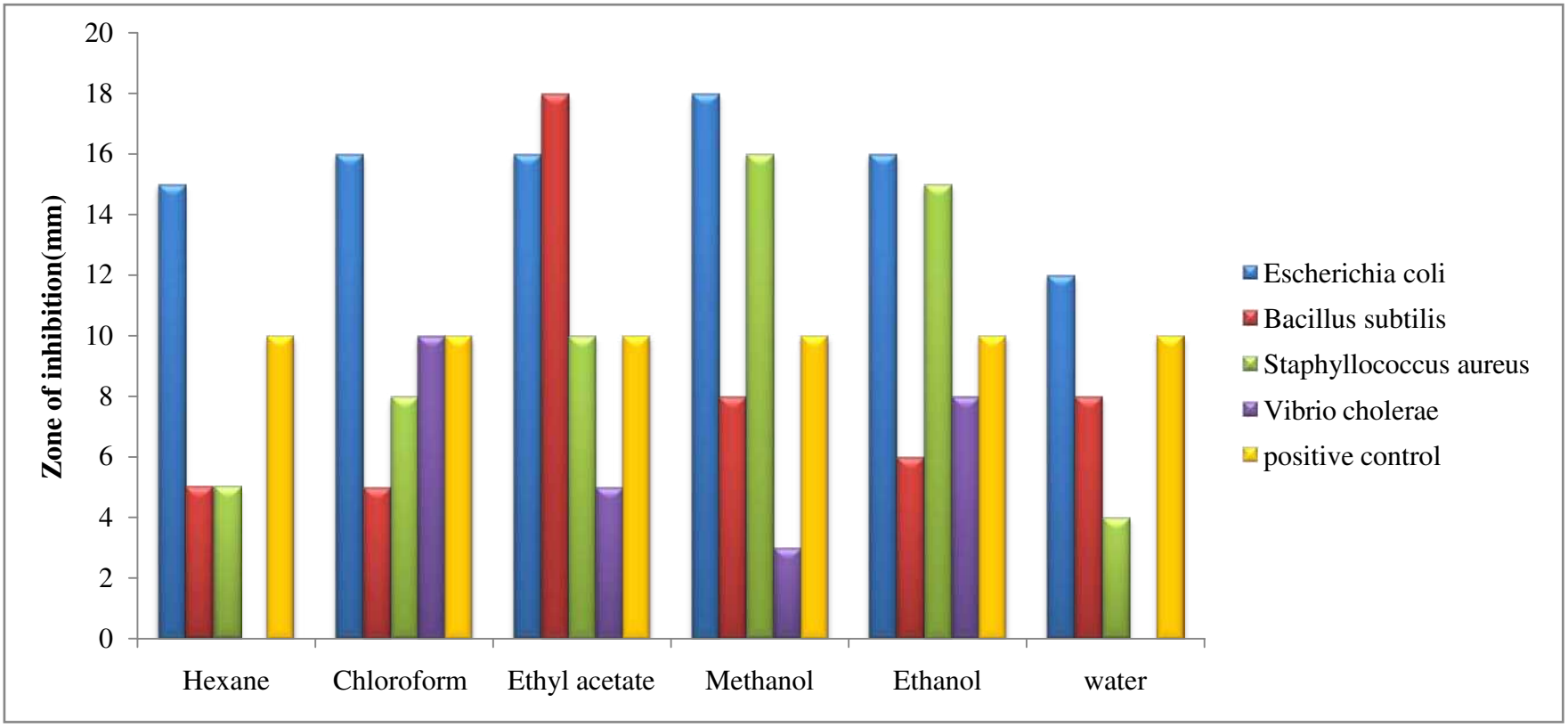


FIGURE 14: ANTIBACTERIAL ACTIVITY OF AVICENNIA MARINA LEAF COLLECTED FROM UNPOLLUTED SITE (PUNNAKAYAL)

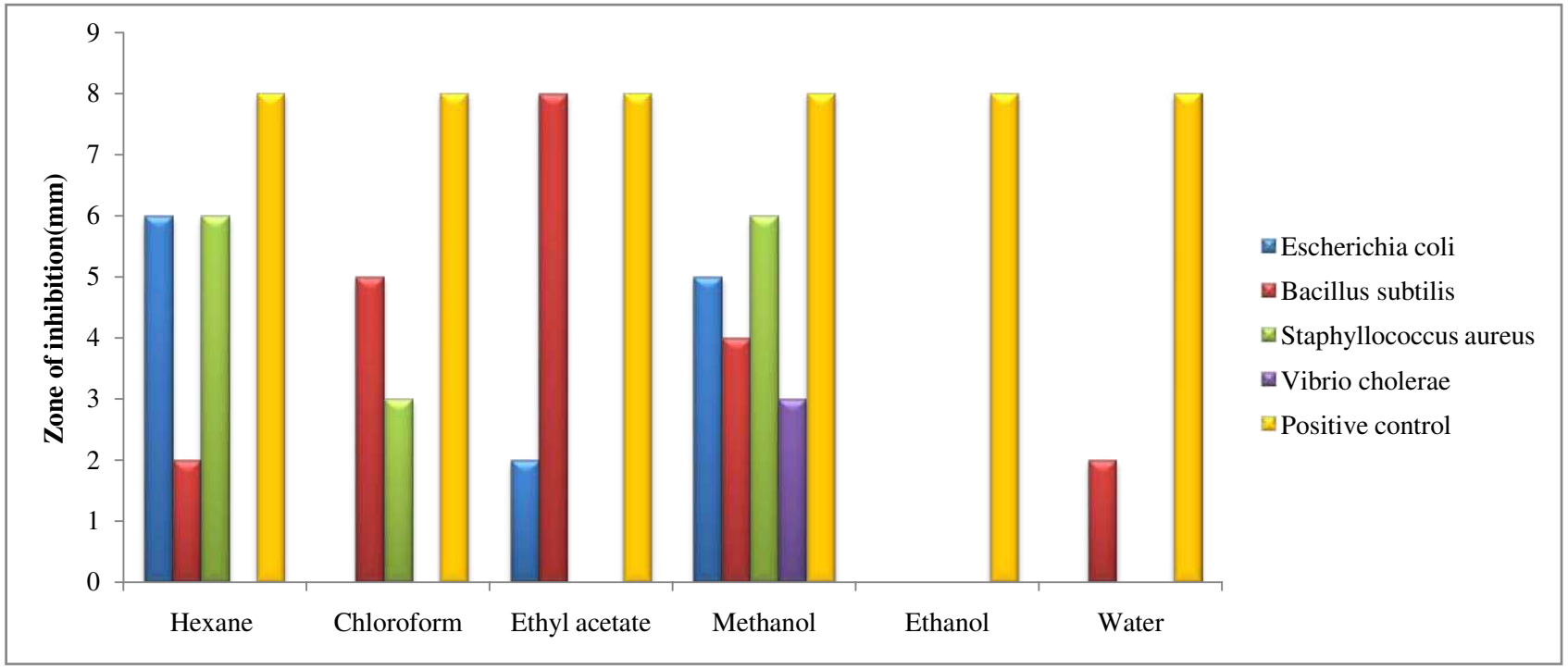


FIGURE15: ANTIBACTERIAL ACTIVITY OF AVICENNIA MARINA STEM COLLECTED FROM POLLUTED SITE (THOOTHUKUDI)

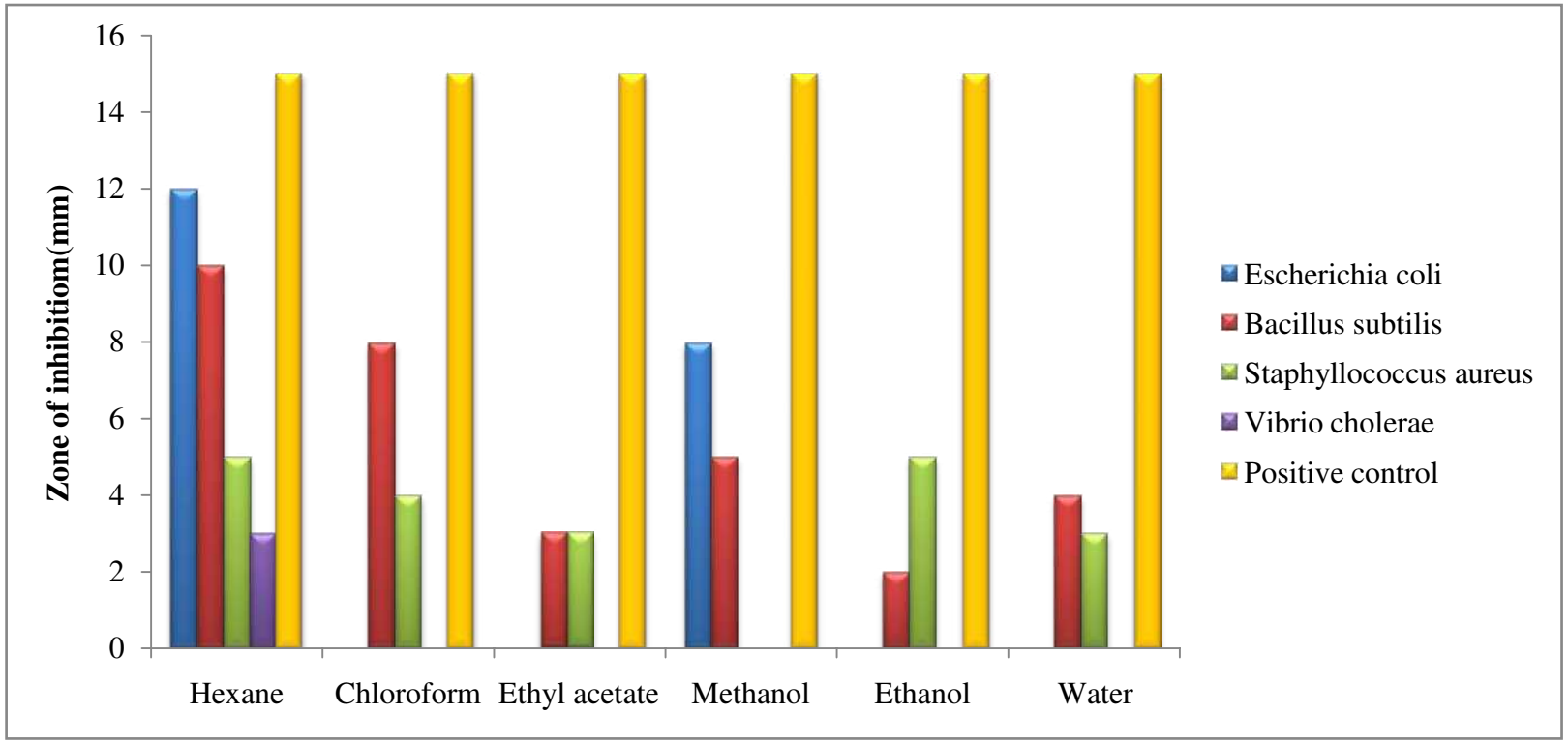


FIGURE 16: ANTIBACTERIAL ACTIVITY OF AVICENNIA MARINA STEM COLLECTED FROM UNPOLLUTED SITE (PUNNAKAYAL)

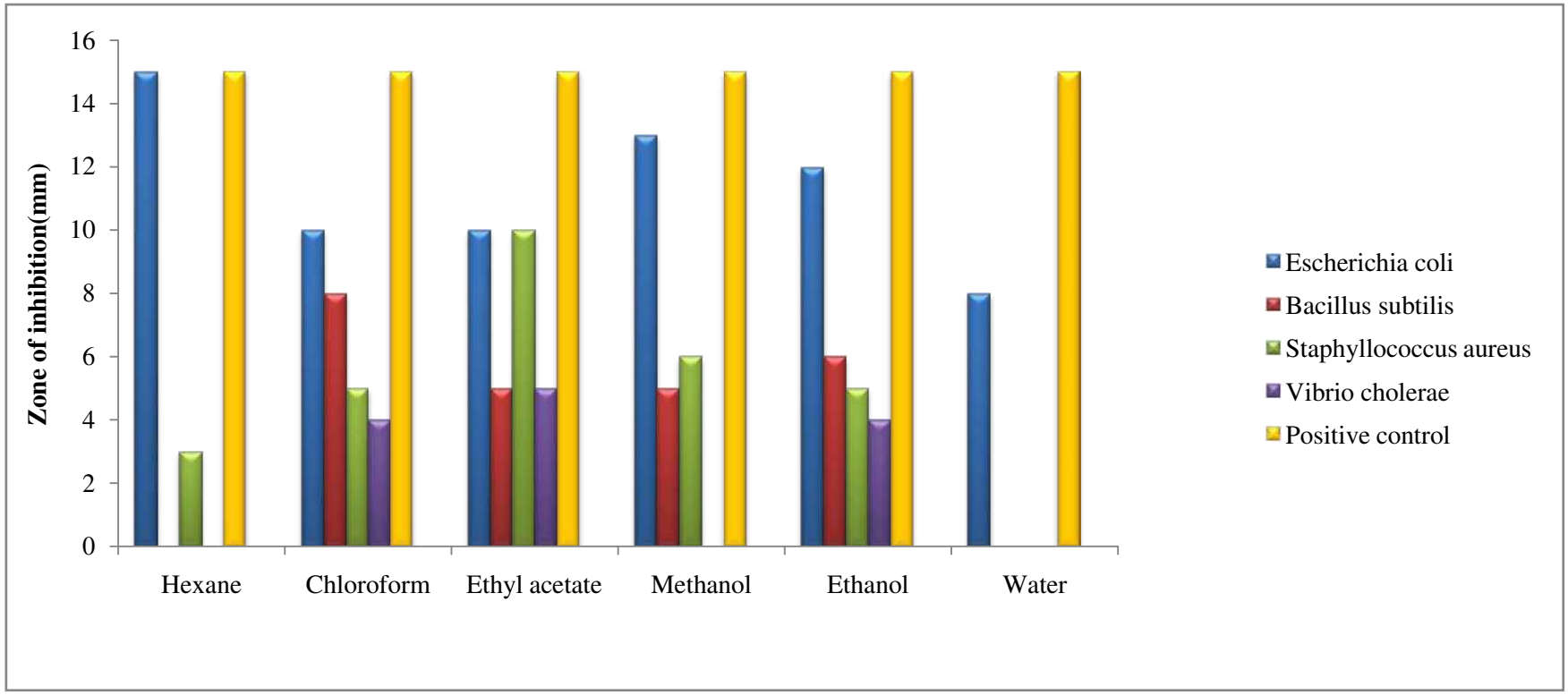


FIGURE 17: ANTIBACTERIAL ACTIVITY OF RHIZOPHORA MUCRONATA LEAF COLLECTED FROM UNPOLLUTED SITE (PUNNAKAYAL)

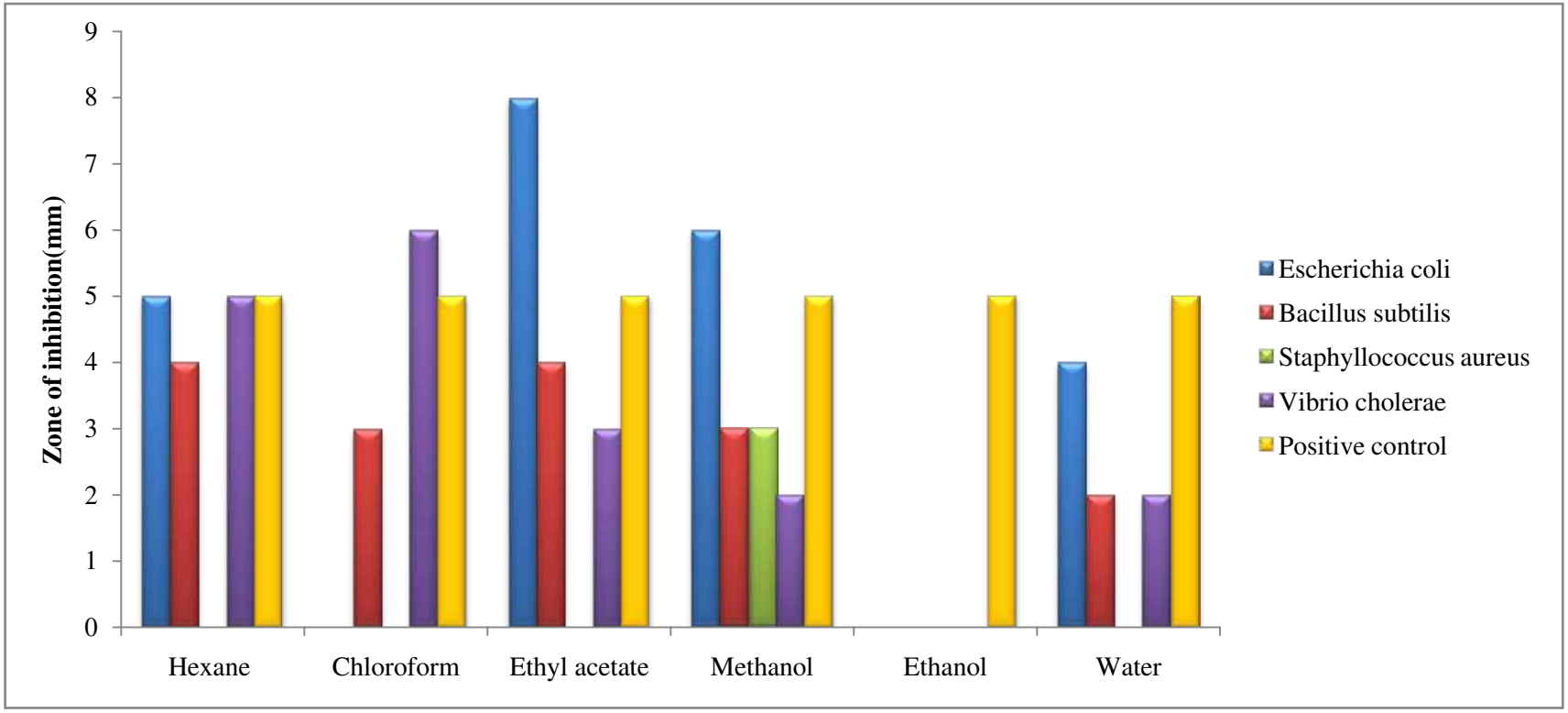


FIGURE 18: ANTIBACTERIAL ACTIVITY OF RHIZOPHORA MUCRONATA STEM COLLECTED FROM UNPOLLUTED SITE (PUNNAKAYAL)

TABLE 1: DISTRIBUTION OF MANGROVE ASSOCIATES IN POLLUTED SITE (THOOTHUKUDI)

S. No.	Botanical Name	Family	Vernacular Name	Habit	Medicinal Uses
1.	*Suaeda maritima*	Chenopodiaceae	Umairi	Shrub	The leaves of plant have been used as medicine for hepatitis locally used as a food
2.	*Calotropis gigantea*	Apocynaceae	Erukku	Shrub	The root bark of this plant is used as medicine in the treatment of leprosy, piles, wounds, tumors, parasitic infections, and dysentery
3.	*Euphorbia hirta*	Euphorbiaceae	Ammaan pachcharisi	Herb	It is often used traditionally for female disorders, respiratory ailments (cough, coryza, bronchitis, and asthma), and worm infestations in children, dysentery, jaundice, pimples, gonorrhea, digestive problems, and tumors.

4.	*Phyllanthus amaras*	Euphorbiaceae	Keezha nelli	Herb	It is an important plant of Indian Ayurvedic system of medicine which is used in the problems of stomach, genitourinary system, liver, kidney and spleen. It is bitter, astringent, stomachic, diuretic, febrifuge and antiseptic. The whole plant is used in gonorrhoea, menorrhagia and other genitalaffections
5.	*Corchorus aestuans*	Tiliaceae	East Indian Mallow	Herb	Used as a stomachic, anti-inflammatory and as a treatment for pneumonia
6.	*Abutilon indicum*	Malvaceae	Thuthi	Shrub	The juice of the leaves is used for demulcent and diuretic. A decoction of the leaves is used to treat fever, colic, and for cleaning wounds and ulcers
7.	*Dactyloctenium aegyptium*	Poaceae	Duck grass	Herb	Decoction of seeds used to relieve pains in the region of the kidney; stems and leaves applied externally fortreatment of ulcers.

8.	*Alternanthera sessilis*	Amaranthaceae	Ponnanganni	Herb	The plant is said to be abortifacient, cholagogue, febrifuge and galactagogue. It is eaten by nursing mothers who wish to increase their milk flow. An infusion of the entire plant is used as a remedy against intestinal cramps, fever, diarrhoea and dysentery.
9.	*Crotalaria retusa*	Leguminosae	Kilukiluppai	Herb	The roots are used against coughing up blood. The plant is used as a treatment for complaints such as cough, dyspepsia, fever, cardiac disorders, stomatitis,diarrhoea, scabies and impetigo
10.	*Tephrosia purpurea*	Leguminosae	Kolunchi	Shrub	Plant is used to cure external wounds, gastroduodenal disorders, tightness of chest and cough. Root decoction is helpful in enlargement and obstruction of liver, kidney and spleen.
11.	*Heliotropiumcurrssavicum*	Boraginaceae	Sirudhel koduku	Herb	The dried roots are ground to powder and applied to sores and wounds. A decoction of the plant is taken as a remedy for leucorrhoea

12.	*Acacia nilotica*	Mimosaceae	Karuvelai	Tree	The dried, powdered bark is used as a disinfectant in healing wounds. In Senegal the powdered bark is used as an anthelmintic and is dusted on to skin ailments. The stem is used to treat asthma. Seeds are taken to treat diarrhoea.
13.	*Thespesia populneoides*	Malvaceae	Poovarasu	Tree	Ground up bark is used to treat skin diseases, dysentery and haemorrhoids. Leaves are applied to inflamed and swollen joints.
14.	*Achyranthus aspera*	Amaranthaceae	Nayuruvi	Herb	It is used in the treatment of dropsy, rheumatism, stomach problems, cholera, skin diseases and rabies.

TABLE 2: DISTRIBUTION OF MANGROVE ASSOCIATES IN UNPOLLUTED SITE (PUNNAKAYAL)

S. No	Plant name	Family	Vernacular name	Habit	Medicinal value
1	*Salicornia brachita*	Chenopodiaceae	Pavazhappoondu	Herb	Used as sea vegetable.
2	*Suaeda maritima*	Chenopodiaceae	Umari	Shrub	The leaves of plant have been used as medicine for hepatitis locally used as a food
3	*Sesuvium portulacastrum*	Aizoaceae	Vangaravaasi	Perennial herb	The plant is used as a haemostatic. A decoction of the plant is considered the best antidote for stings of venomous fish; it should be applied externally for a long time. The leaves are said to be antiscorbutic
4	*Calotropis gigantea*	Apocynaceae	Erukku	Shrub	The root bark of this plant is used as medicine in the treatment of leprosy, piles, wounds, tumours, parasitic infections, and dysentery

5	*Heliotropium curassavicum*	Boraginaceae	Sirudhel koduku	Herb	The dried roots are ground to powder and applied to sores and wounds. Decoction of the plant is taken as a remedy for leucorrhoea
6	*Traidax procumbens*	Asteraceae	kinarruppacan, vettukkayappuntu	Herb	The plant is used as a wound healing and as an anticoagulant, antifungal, insect repellent
7	*Leucas aspera*	Lamiaceae	Thumbai	Herb	The plant is used traditionally as an antipyretic and insecticide. Medicinally, it has been proven to possess various pharmacological activities like antifungal, antioxidant, antimicrobial, antinociceptive and cytotoxic activity
8	*Phyllanthus amaras*	Euphorbiaceae	Keezha nelli	Herb	It is used in the problems of stomach, genitourinary system, liver, kidney and spleen. It is bitter, astringent, stomachic, diuretic, febrifuge and antiseptic. The whole plant is used in gonorrhoea, menorrhagia and other genital affections
9	*Lycopersicon esculentum*	Solanaceae	Takkali	Herb	The skin of tomato fruits is a good source of

					lycopine, a substance that has been shown to protect people from heart attacks. Lycopine is used to treat enlarge prostate and the difficulties in urination that accompany this disorder.
10	*Solanum suratensis*	Solanaceae	Kandankattiri	Herb	The herb is useful for bronchial asthma, chest pain, cough, cure vomiting, hair fall, dropsy, scabies, itching and skin diseases. It is useful for healing wounds and cardiac diseases associated with edema. The herb is also found to be helpful for lowering headache and migraine.
11	*Cucurbita maxima*	Cucurbitaceae	Carkkaraipparani, Parangikayi, Pushinikkayi	Climber	Seeds help to treat intestinal infections, kidney problems and to expel tapeworms. Flowers are used topically to soothe minor injuries.
12	*Cissus quandrangularis*	Vitaceae	Pirandai	Woody climber	It is commonly used for bone health and weight loss. It is used to treat diabetes, high cholesterol, haemorrhoids.

13	*Pedalium murex*	Pedaliaceae	Yanai Nerunjil	Herb	Traditionally used for the treatment of puerperal diseases, digestive tonics, ulcers, fevers, wounds, other ailments and general debility.
14	*Percularia daemia*	Apocynaceae	Aadu thinna paalai	Climber	Used to prevent seizures, increase sexual desire, boost the immune system, and start menstruation. Used to treat snakebite, intestinal pain, gallbladder pain, arthritis, gout, achy joints (rheumatism), eczema, weight loss and wounds
15	*Physalis angulata*	Solanaceae	Sodaku thakkali	Herb	Used for the treatment of malaria, ulcer, pains and other diverse ailments.
16	*Chloris barbata*	Poaceae	Kottaippul	Herb	Used externally for skin disorders leaves juice used in fever, diarrhea and diabetes.

TABLE: 6 QUANTITATIVE PHYTOCHEMICAL ANALYSIS OF SELECTED MANGROVE PLANTS

S. No.	Location	Plant Part	Protein (mg/g DW)	Phenols (mg/g DW)	Flavonoids (mg/g DW)	Tannins (mg/g DW)	Vitamin C (mg/g DW)	Vitamin E (mg/g DW)
1.	**Punnakayal**	*Avicennia marina* leaf	4.2±0.4	1.2 ± 0.04	0.24±0.01	0.12±0.01	12.4±0.46	7.04±0.36
2.		*Avicennia marina* stem	3.1±0.2	0.61±0.13	0.61±0.13	0.11±0.01	2.1±0.08	9.46±0.28
3.		*Rhizophora mucronata* leaf	4.9±0.1	0.36±0.05	0.17±0.03	0.15±0.01	10.3±0.4	12.16±0.53
4.		*Rhizophora mucronata* stem	2.2±0.3	0.61±0.08	0.48±0.01	0.14±0.02	7.6±0.51	8.09±0.98
5.	**Thoohukudi**	*Avicennia marina* leaf	3.6±0.05	1.1±0.06	0.21±0.07	0.12±0.02	13.38±0.58	7.46±0.71
6.		*Avicennia marina* stem	3.4±0.1	0.89±0.1	0.61±0.02	0.12±0.04	4.6±0.91	10.09±0.25

TABLE: 11 PHYTOCOMPOUNDS IDENTIFIED BY GC-MS ANALYSIS OF AVICENNIA MARINA LEAF COLLECTED FROM POLLUTED SITE (THOOTHUKUDI)

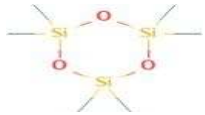
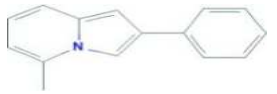
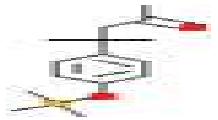
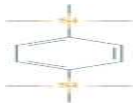
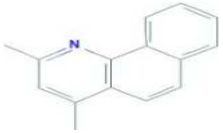
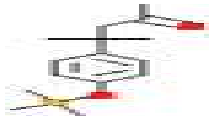
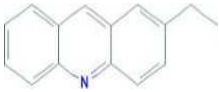
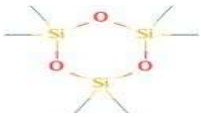
S. No.	RT	Name of the Compound	Molecular Formula	Molecular Weight (g/mol)	Peak (Area %)	Molecular Structure	Reported Bioactivity
1	16.497	Cyclotrisiloxane, hexamethyl	$C_6H_{18}O_3Si_3$	222.46	94.04		Antioxidant activity **(Alok Prakash and Suneetha, 2014)**
2	16.497	5-Methyl-2-phenylindolizine	$C_{15}H_{13}N$	207.27	94.04		Unknown
3	16.572	Trimethyl [4-(2-methyl-4-oxo-2-pentyl) phenoxy]silane	$C_{15}H_{24}O_2Si$	264.43	94.04		Vitamin D, rickets and Antioxidants
4	16.572	1,4-Bis(trimethylsilyl) benzene	$C_{12}H_{22}Si_2$	222.47	5.96		Unknown

TABLE: 12 PHYTOCOMPOUNDS IDENTIFIED BY GC-MS ANALYSIS OF AVICENNIA MARINA LEAF COLLECTED FROM UNPOLLUTED SITE (PUNNAKAYAL)

S. No.	RT	Name of the Compound	Molecular Formula	Molecular Weight (g/mol)	Peak (Area %)	Molecular Structure	Reported Bioactivity
1.	16.922	Benzo[h]quinoline, 2,4-dimethyl	$C_{15}H_{13}N$	207.27	58.39		Unknown
2.	16.922	Trimethyl [4-(2-methyl-4-oxo-2-pentyl) phenoxy]silane	$C_{15}H_{24}O_2Si$	264.43	94.04		Vitamin D, rickets and Antioxidants
3.	16.922	2-Ethylacridine	$C_{15}H_{13}N$	207.27	58.39		Antimicrobial and Antitumor **(J.Vijayakumari and T.Leon Stephan Raj, 2019)**
4.	17.168	Cyclotrisiloxane, hexamethyl	$C_6H_{18}O_3Si_3$	222.46	94.04		Antioxidant activity **(Alok Prakash and Suneetha, 2014)**

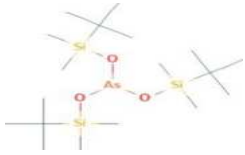
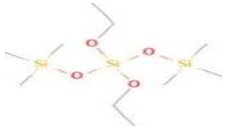
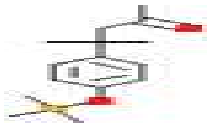
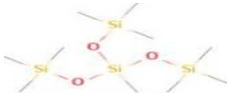
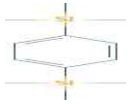
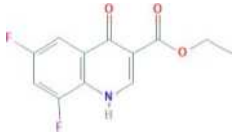
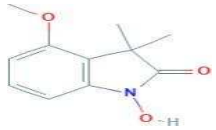
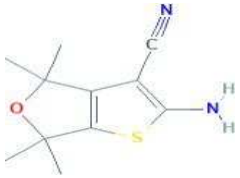
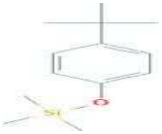
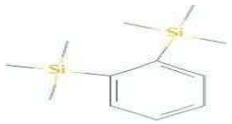
5.	17.168	Tris (tert-butyldimethyl silyloxy) arsane	$C_{18}H_{45}AsO_3Si_3$	468.7	41.61		Unknown
6.	17.168	Silicic acid, diethyl bis(trimethylsilyl) ester	$C_{10}H_{28}O_4Si_3$	296.58	41.61		Antibacterial activity **(Hema *et al.*, 2011).**

TABLE: 13 PHYTOCOMPOUNDS IDENTIFIED BY GC-MS ANALYSIS OF RHIZOPHORA MUCRONATA LEAF COLLECTED FROM UNPOLLUTED SITE (PUNNAKAYAL)

S. No.	RT	Name of the Compound	Molecular Formula	Molecular Weight (g/mol)	Peak (Area%)	Molecular Structure	Reported Bioactivity
1.	16.771	Trimethyl [4-(2-methyl-4-oxo-2-pentyl) phenoxy] silane	$C_{15}H_{24}O_2Si$	264.43	35.45		Vitamin D, rickets and Antioxidants
2.	16.771	Methyltris(trimethylsiloxy) silane	$C_{10}H_{30}O_3Si_4$	310.68	35.45		Unknown
3.	16.771	1,4-Bis(trimethylsilyl) benzene	$C_{12}H_{22}Si_2$	222.47	35.45		Unknown
4.	16.913	3-Quinolinecarboxylic acid, 6,8-difluoro-4-hydroxy-, ethyl ester	$C_{12}H_9F_2NO_3$	253.2	21.83		Unknown

5.	16.913	Indole-2-one, 2,3-dihydro-N-hydroxy-4-methoxy-3,3-dimethyl-	$C_{11}H_{13}NO_3$	2207.23	21.83		Anti microbial **(J.Vijayakumari and T.Leon Stephan Raj, 2019)**
6.	16.913	Thieno[2,3-c]furan-3-carbonitrile, 2-amino-4,6-dihydro-4,4,6,6-tetramethyl-	$C_{11}H_{14}N_2OS$	222.31	21.83		Analgesic, Antianginal, Analgesic, non-opioid, Antihypertensive, Antiarthritic, Dementia treatment, Neurotransmitter uptake inhibitor **(Brintha *et al*., 2017)**
7	16.960	Trimethyl(4-tert-butylphenoxy)silane	$C_{13}H_{22}OSi$	222.4	9.22		Unknown
8	16.960	1,2-Bis(trimethylsilyl)benzene	$C_{12}H_{22}Si_2$	222.47	9.22		antioxidant, antimicrobial, anticanserous and antitumerous activity **(Alok prakash and Suneetha, 2014)**

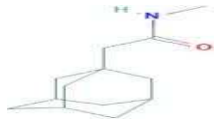
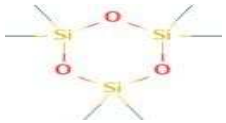
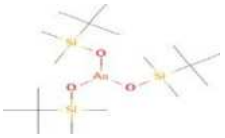
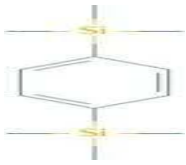
9	16.960	N-Methyl-1-adamantaneacetamide	$C_{13}H_{21}NO$	207.31	9.22		Unknown
10	17.149	Cyclotrisiloxane, hexamethyl-	$C_6H_{18}O_3Si_3$	222.46	33.49		Antibacterial activity, antioxidant **(Papitha *et al.*, 2017, Juliet *et al.*, 2018)**
11	17.149	Tris(tert-butyldimethylsilyloxy)arsane	$C_{18}H_{45}AsO_3Si_3$	468.7	33.49		Unknown
12	17.149	1,4-Bis (trimethylsilyl) benzene	$C_{12}H_{22}Si_2$	222.47	33.49		Unknown

TABLE: 14 ANTIBACTERAL ACTIVITY OF SELECTED MANGROVE PLANTS AGAINST ESCHERICHIA COLI

Location	Plant	Zone of inhibition (mm)						
		Positive Control	**Hexane**	**Chloroform**	**Ethyl acetate**	**Methanol**	**Ethanol**	**Water**
Unpolluted Site (Punnakayal)	*Avicennia marina* leaf	10	15	16	16	18	16	12
	Avicennia marina stem	15	12	-	-	8	-	-
	Rhizophora mucoranata leaf	15	15	10	10	13	12	8
	Rhizophora mucoranata stem	5	5	-	8	6	-	4
Polluted Site (Thoohukudi)	*Avicennia marina* leaf	12	10	15	18	12	19	10
	Avicennia marina stem	8	6	-	2	5	-	-

TABLE: 15 ANTIBACTERAL ACTIVITY OF SELECTED MANGROVE PLANTS AGAINST BACILLUS SUBTILIS

Location	Plant	Zone of inhibition (mm)						
		Positive control	**Hexane**	**Chloroform**	**Ethyl acetate**	**Methanol**	**Ethanol**	**Water**
Unpolluted Site (Punnakayal)	*Avicennia marina* leaf	10	5	5	18	8	6	8
	Avicennia marina stem	15	10	8	3	5	2	4
	Rhizophora mucoranata leaf	15	-	8	5	5	6	-
	Rhizophora mucoranata stem	5	4	3	4	3	-	2
Polluted Site (Thoohukudi)	*Avicennia marina* leaf	12	-	3	-	6	5	3
	Avicennia marina stem	8	2	5	8	4	-	2

TABLE: 16 ANTIBACTERAL ACTIVITY OF SELECTED MANGROVE PLANTS AGAINST STAPHYLOCOCCUS AUREUS

Location	Plant	Zone of inhibition (mm)						
		Positive control	**Hexane**	**Chloroform**	**Ethyl acetate**	**Methanol**	**Ethanol**	**Water**
Unpolluted Site (Punnakayal)	*Avicennia marina* leaf	10	5	8	10	16	15	4
	Avicennia marina stem	15	5	4	3	-	5	3
	Rhizophora mucoranata leaf	15	3	5	10	6	5	-
	Rhizophora mucoranata stem	5	-	-	-	3	-	-
Polluted Site (Thoohukudi)	*Avicennia marina* leaf	12	-	6	-	-	8	-
	Avicennia marina stem	8	6	3	-	6	-	-

TABLE: 17 ANTIBACTERAL ACTIVITY OF SELECTED MANGROVE PLANTS AGAINST VIBRIO CHOLERAE

Location	Plant	Zone of inhibition (mm)						
		Positive control	**Hexane**	**Chloroform**	**Ethyl acetate**	**Methanol**	**Ethanol**	**Water**
Unpolluted Site (Punnakayal)	*Avicennia marina* leaf	10	-	10	5	3	8	-
	Avicennia marina stem	15	3	-	-	-	-	-
	Rhizophora mucoranata leaf	15	-	4	5	-	4	-
	Rhizophora mucoranata stem	5	5	6	3	2	-	2
Polluted Site (Thoohukudi)	*Avicennia marina* leaf	12	3	5	-	4	-	-
	Avicennia marina stem	8	-	-	-	3	-	-

TABLE: 3 Qualitative Phytochemical Analysis of *Avicennia marina* Collected from Polluted Site (Thoothukudi)

Phytochemical Test	Hexane		Chloroform		Ethyl acetate		Ethanol		Methanol		Water	
	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem
Alkaloid	+	+	+	+	-	+	+	+	+	+	+	+
Protein	-	-	-	-	-	-	-	-	+	+	-	-
Carbohydrate	-	+	+	-	-	-	+	-	+	+	+	-
Glycoside	+	+	+	-	+	-	+	-	-	+	+	-
Saponin	+	+	-	+	+	+	-	-	-	+	-	-
Phenol	+	-	+	-	+	-	+	+	+	+	+	-
Tannin	-	+	+	+	+	+	-	+	+	+	-	-
Flavonoid	-	+	+	+	-	+	-	+	+	+	-	+
Steroid	+	-	+	+	+	-	+	+	+	+	+	-
Phytosterol	+	-	+	-	+	-	+	-	-	-	-	-
Coumarin	-	-	-	-	-	-	+	+	-	+	-	-
Terpinoid	-	+	+	+	-	+	-	+	+	+	-	-
Cardioglycoside	-	+	-	-	-	-	+	+	-	+	-	-
Coumarin	-	+	-	-	-	-	+	+	+	+	-	-
Phlobatannin	-	-	-	-	-	-	-	-	-	-	-	-

'+' indicates presence '-' indicates absence

TABLE: 4 Qualitative Phytochemical Analysis of *Avicennia marina* Collected from Unpolluted Site (Punnakayal)

Phytochemical Test	Hexane		Chloroform		Ethyl acetate		Ethanol		Methanol		Water	
	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem
Alkaloid	+	+	+	+	+	+	+	+	+	+	-	+
Protein	-	-	-	-	-	-	-	-	+	+	-	-
Carbohydrate	+	+	-	-	-	-	+	+	+	+	-	-
Glycoside	+	+	+	-	+	-	+	-	+	+	+	-
Saponin	+	+	-	+	+	+	+	-	-	+	-	-
Phenol	+	+	+	-	+	-	+	+	+	+	+	-
Tannin	+	+	+	+	+	+	+	+	+	+	-	-
Flavonoid	+	+	+	+	-	-	+	+	+	+	-	-
Steroid	+	+	+	+	+	+	+	+	+	+	+	-
Phytosterol	+	+	+	+	+	+	-	-	-	+	-	-
Coumarin	+	-	-	-	-	-	+	+	+	+	-	-
Terpinoid	+	+	+	-	+	-	+	+	+	+	-	-
Cardioglycoside	-	+	-	-	-	+	+	+	-	+	-	-
Coumarin	+	+	-	-	-	+	+	+	+	+	-	-
Phlobatannin	-	-	-	-	-	-	-	-	-	-	-	-

'+' indicates presence '-' indicates absence

TABLE: 5 Qualitative Phytochemical Analysis of *Rhizophora mucronata* Collected from Unpolluted Site (Punnakayal)

Phytochemical Test	Hexane		Chloroform		Ethyl acetate		Ethanol		Methanol		Water	
	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem
Alkaloid	+	+	+	+	+	+	+	+	+	+	-	-
Protein	+	+	-	-	-	-	+	-	+	+	-	-
Carbohydrate	+	-	+	-	-	-	+	+	+	+	-	-
Glycoside	-	-	-	-	-	-	+	-	+	-	-	-
Saponin	+	+	+	+	+	-	-	-	+	-	-	-
Phenol	+	+	+	+	+	+	+	+	+	+	-	+
Tannin	+	+	+	+	+	+	+	+	+	+	+	+
Flavonoid	+	+	+	+	+	+	+	+	+	+	+	+
Steroid	-	+	-	-	-	-	-	-	+	-	-	-
Phytosterol	-	+	-	+	-	-	+	-	+	-	-	-
Coumarin	-	-	-	-	-	-	-	+	-	+	-	-
Terpinoid	+	+	+	+	+	+	-	+	+	-	+	-
Cardioglycoside	+	+	+	-	-	+	+	-	-	+	-	-
Coumarin	-	-	-	-	-	-	-	+	-	+	-	-
Phlobatannin	-	-	-	-	-	-	+	-	+	-	-	-

'+' indicates presence '-' indicates absence

TABLE: 7 ANTIOXIDANT ACTIVITIES OF SELECTED MANGROVE PLANTS

Location	Plant	% of Inhibition	
		DPPH	H_2O_2
Unpolluted site (Punnakayal)	*Avicennia marina* leaf	26.2	72.3
	Avicennia marina stem	36.8	10.72
	Rhizophora mucoranata leaf	24.5	22.9
	Rhizophora mucoranata stem	34.3	40.9
Polluted site (Thoothukudi)	*Avicennia marina* leaf	16.9	75.9
	Avicennia marina stem	44.3	42.5

TABLE: 8 FTIR ANALYSIS OF AVICENNIA MARINA LEAF

COLLECTED FROM POLLUTED SITE (THOOTHUKUDI)

S.No	Frequency (1/cm)	Functional group	Compound
1.	620.07	C-Br stretching	Alkyl halides
2.	897.8	C=C bending	Alkene
3.	1025.1	C-N stretch	Aliphatic amines
4.	1124.42	C-N stretch	Aliphatic amines
5.	1163.96	C-N stretch	Aliphatic amines
6.	1246.89	C-H wag	Alkyl halides
7.	1322.11	C-N stretch	Aromatic amine
8.	1381.9	none	None
9.	1422.4	C-C stretch	Aromatics
10.	1463.87	C-H bend	Alkanes
11.	1512.09	N-O asymmetric stretch	Nitro compounds
12.	1544.88	N-O asymmetric stretch	Nitro compounds
13.	1633.59	N-H bend	Amines
14.	1736.78	C=O stretch	Esters, saturated aliphatic
15.	1818.75	none	None
16.	2852.52	C-H stretch	Alkanes
17.	2925.81	C-H stretch	Alkanes
18.	3422.45	O-H stretch, H-bonded	Alcohols, phenols

TABLE: 9 FTIR ANALYSIS OF AVICENNIA MARINA LEAF COLLECTED FROM UNPOLLUTED SITE (PUNNAKAYAL)

S. No	Frequency (1/cm)	Group	Compound
1.	516.89	C-Br stretch	Alkyl halides
2.	619.11	C-Br stretch	Alkyl halides
3.	1021.24	C-N stretch	Aliphatic amines
4.	1114.78	C-O stretch	Alcohols, carboxylic acids, esters, ethers
5.	1168.78	C-H wag ($-CH_2X$)	Alkyl halides
6.	1252.68	C-N stretch	Aromatic amines
7.	1320.18	C-O stretch	Alcohols, carboxylic acids, esters, ethers
8.	1441.69	C-C stretch	Aromatics
9.	1528.48	N-O asymmetric stretch	Nitro compounds
10.	1636.49	C=C Stretch	Alkanes
11.	1737.74	C=O stretch	Aldehydes, saturated aliphatic
12.	2360.71	none	none
13.	2884.35	C-H stretching	Alkanes
14.	3436.91	O-H stretch, H-bonded	Alcohols, phenols

TABLE: 10 FTIR ANALYSIS OF RHIZOPHORA MUCRONATA

COLLECTEDFROM PUNNAKAYAL

S.No.	Frequency range (1/cm)	Group	Compound class
1	515.92	C-Br stretch	Alkyl halides
2	619.11	C-Br stretch	Alkyl halides
3	667.32	C-Br stretch	Alkyl halides
4	779.19	C-Cl stretch	Alkyl halides
5	855.37	=C-H bend	Alkanes
6	1018.34	C-O Stretch	Alcohols, carboxylic acids, esters, ethers
7	1111.89	C-O stretch	Alcohols, carboxylic acids, esters, ethers
8	1178.43	C-N stretch	Aliphatic amines
9	1263.29	C-N stretch	Aromatic amines
10	1316.33	N-O symmetric stretch	Nitro compounds
11	1384.79	none	none
12	1438.8	C-C stretch	Aromatics
13	1537.16	N-O asymmetric stretch	nitro compound
14	1644.2	C=C- Stretch	Alkanes
15	2360.71	none	none
16	2884.35	C-H stretch	alkane
17	3442.7	O-H Stretch, H-bonded	Alcohols, phenols

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BIOPOTENTIALS OF SEAWEEDS COLLECTED FROM SOUTH EAST COAST OF INDIA

A short term project work submitted to

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Affiliated to

MANONMANIAM SUNDARANAR UNIVERSITY, THIRUNELVELI

in partial fulfilment of the requirement for the degree of

MASTER OF SCIENCE IN BOTANY

Submitted by

J.N. MONISHA

Reg. No. 19APBO10

Under the guidance of

Dr. R. MARY SANTHI

DEPARTMENT OF BOTANY

ST.MARY'S COLLEGE (Autonomous)

THOOTHUKUDI - 628001

April – 2021

CERTIFICATE

This is to certify that this dissertation entitled **"BIOPOTENTIALS OF SEAWEEDS COLLECTED FROM SOUTH EAST COAST OF INDIA"** is submitted to **St. Mary's college (Autonomous), Thoothukudi** affiliated to **MANONMANIAM SUNDARANARUNIVERSITY,THIRUNELVELI** in partial fulfilment of the award of the degree of **Master of science in Botany**, and is a record of work done in the Department of Botany, St. Mary's College (Autonomous), Thoothukudi during the year 2020–2021 by **J.N. MONISHA (Reg No. 19APBO10)**

M Sam 16/04/21

SIGNATURE OF THE GUIDE

M. Glory

HEAD OF THE DEPARTMENT

HOD of Botany
St. Mary's College (Autonomous)
Thoothukudi- 628 001.

16/04/2021

EXAMINER

Lucia Rose

SIGNATURE OF THE PRINCIPAL

St. Mary's College (Autonomous)
Thoothukudi - 628 001.

DECLARATION

I do here by declare that this dissertation entitled **"BIOPOTENTIALS OF SEAWEEDS COLLECTED FROM SOUTH EAST COAST OF INDIA"** Submitted by me in partial fulfillment for the award of the degree of **'Master of Science in Botany'**, in the result of my original and independent work carried out under theguidance of **Dr.R. MARY SANTHI, M.Sc., M.Phil., Ph.D.,** Assistant professor of Botany, St. Mary's College (Autonomous), Thoothukudiand it has not been submittedelsewhere for the award of any other degree.

Station: Thoothukudi - 628001

J.N.Monisha

J.N.MONISHA

Date: 15-04-2021

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CONTENTS

INTRODUCTION

1. INTRODUCTION

About seventy one percent of the surface of this planet is covered by salt water. Beneath this surface, the water depth averages 3.8 km, giving a volume of $1370x10^6$ km^3. Since life exists throughout this immense volume, the oceans constitute the single largest repository of organisms on the planet. Algae are an extremely diverse group of chlorophyll containing organisms that make up the lower phylogenetic echelons of the plant kingdom (Bold and Wynne, 1985). Marine algae are one of the largest producers of biomass in the marine environment (Bhadury and Wright, 2004).

Seaweeds are the eukaryotic organisms that live in salty water and recognized as a potential source for bioactive natural products. In the sea, three types of plants occur and they are phytoplanktons, seaweeds or marine algae and seagrasses. Phytoplanktons are microscopic and free-floating forms; they are the primary producers of the sea (Kolanjinathan *et al.,* 2014). Marine algae are macroscopic, attached or freely floating plants. They form one of the important marine living renewable resources. They are primitive plants without any true root, stem and leaves. Marine algae are classified into four groups namely Chlorophyceae (green algae), Phaeophyceae (brown algae), Rhodophyceae (red algae) and Cyanophyceae (blue-green algae) based on the type of pigments, morphological, anatomical and reproductive structures (Kolanjinathan *et al.,* 2014).

Seaweeds occur in the intertidal shallow and deep waters of the sea upto 180 m depth in estuaries and backwaters. They grow on dead corals, rocks, stones, pebbles, other substrates and as epiphytes on seagrasses. Several species of green, brown and red algae with luxuriant growth occur along the Southern Tamilnadu coast from Rameswaram to Kanyakumari covering 21 islands of Gulf of Mannar (Kolanjinathan *et al.,* 2014). The

seaweed flora of India is highly diversified and comprises mostly of tropical species in all 271 genera and 1153 species of marine algae which includes forms and varieties (Anonymous, 2005).

Marine ecosystems are among the richest and most complex ones in terms of biodiversity. Original chemical and physical conditions in such an environment favours the production of quite specific and potent active molecules. Among other reasons, marine organisms have been found to produce bioactive substances because, they are living in an exigent, competitive and aggressive environment (Aneiros and Garateix, 2004; Rocha *et al.,* 2011). These characteristics render marine organisms as ideal candidates for novel sources of both pre-existing and unrecognized high value-added biomolecules with potential for providing sustainable economic and human benefits. Marine algae produces a cocktail of metabolites with potential commercial value. Secondary metabolites also contribute to growth, reproduction and defense, thus play primary role in organism integrity (Cabrita *et al.,* 2010).

Seaweed fertilizers are having nitrogen, phosphorus, potash trace elements and metabolites similar to plant growth regulators. Liquid fertilizers derived from seaweeds are found to be superior to chemical fertilizers due to high level of beneficial compounds that useful for plant growth and development. Seaweed fertilizer is a natural bioactive material; water soluble derived from marine macro algae. Seaweed extract is a new generation of natural organic fertilizers containing highly effective nutrients, increased yield, promotes faster generation of seeds and resistant ability of many crops. Seaweed fertilizer could be absorbed by plant. (Tensingh Baliah, 2017). The seaweed extract which contains plant growth hormones, regulators, promoters, auxins, gibberellins and vitamins consequently improves their yield and quality. (Erulan, 2009). The Dried or fresh seaweeds and liquid

extracts have been increasingly employed by horticulturists, gardeners, farmers, and orchadistsas a fertilizer (Zodape, 2001).

The seaweed extracts (SWEs) contain plant growth hormones, regulators, growth promoters, carbohydrates, amino acids, antibiotics and vitamins which consequently enhance the yield and quality of crops seed germination crop resistance to frost, fungal, and insect attacks(Kamarajan, 2012). Seaweed extract is natural organic fertilizer which promotes faster seed germination and is highly nutritious to plants (Sasikala, 2016). The seaweed extract contains regulators, plant growth hormones, carbohydrates, auxins, gibberellins and vitamins and helped to maintain soil fertility (Erulan, 2009). It is cost effective and eco-friendly for sustainable agriculture. The fertilizer obtained from seaweed extract are biodegradable, non-polluting, non-polluting, non-toxic and non-hazardous to humans, animals and birds (Sasikala, 2016). Liquid extracts obtained from seaweed are successfully used as for seed germination, foliar sprays for several crops (Kamarajan, 2012).

Sea weed extract are used as nutrient supplements, biostimulants or biofertilizers in agriculture and horticuluture to increase plant growth and yield (Rosalba *et al.,* 2013). Numerous studies have revealed a wide range of beneficial effects of seaweed on plants, such as early seed germination and establishment, better crop performance and yield , inducing resistance to biotic and abiotic stress and many more (Mahima Begum, 2018). Seaweed extract is effective for ripening of fruits, increasing shelf-life of the produce, improves the quality of produce, and serves as an excellent soil conditioner (Zodape, 2001). The sea weed extract was found effective in increasing the biomass growth of roots and shoots, number of leaves, flower and fruits, maturity time and yield (Dhivya *et al.,* 2015). Crop yield can increase in two ways related to vigourous seeds: (1) maximum density made by higher seedling percentage even under abiotic stress conditions and (2) higher emergence rate and increased growth (Ghasemi- Golezani *et al.,* 2010).

Brown algae are the basic raw material for the production of sodium alginate. Sodium alginate is one of the important chemical uses in the reactive printing of fabric. China is the largest seaweed producing country and exporting its derivative. From last decay the consumption of seaweed in food sector is rapidly increasing year by year and the shortage have to face to the alginate industry which lift up the price of sodium alginate. From the start of February to the end of the July the pieces of naturally grown seaweed comes on seashore. The amount of the naturally grown algae is seriously depends on the weather condition, tides and lowers, sea level and daytime. More than 25 percent demand of sodium alginate can be fulfilled by utilizing natural source and also decrease the import volume. The price of the sodium alginate can be managed by minimizing the import (Javed, 2015).

Artificial seeds technology allows to produce in large quantities in vitro somatic embryos for seedless plant that share some similar properties of natural embryos, and can be used for in field propagation of selected plant species that have commercial value. Somatic embryo are the primary plant material for the production of synthetic seeds. This technology contributes to supply genetically homogenous seeds, unlike traditional hybrid seeds, obtained from a gametic process which are known to produce plants that are different from the characteristics of the parent plants. They simply encapsulate the somatic embryos with a mixture of nutrient gel that contains carbon, organic and inorganic salts, vitamins, hormones, antimicrobials to protect embryos from damage. It also allows the growth and germination to happen without unwanted differences (Shallal, 2020).

OBJECTIVES

2. OBJECTIVES OF THE STUDY

The objective of the present study is to increases the soil fertility using algal extract as a biofertilizer and also to improve the seed germination, growth as well as quality products for better production process.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Marine algae are the primitive algae without root, stem and leaves. They belongs to the non-flowering group called cryptogam. The marine algae are of two types microalgae and macroalgae. Based on the colour the algae are divided into many classes some of them are chlorophyceae, Phaeophyceae, Rhodophyceae, and cyanophyceae. The macroalgae grow well in rocks, corals and soil substrates in the intertidal, shallow and deep water of the sea upto 150 meters. These algae are also seen in estuaries and backwaters. Seaweeds are well used in food, medicine, cosmetics and fertilizers.

Distribution and resources

The southern coastal region of Tamil Nadu was rich in algal flora during early seventies. The region was having more than 200 species (John Peter Paul and Patric Raj, 2011). Economic uses of sea weeds particularly for food, feed, phycocolloids and agro-based products, are well known for centuries and are now being extensively investigated for their application in biofuel, nutraceutical, medicinal, personal-care and food additive industries (Hafting *et al.,* 2015, Kim *et al.,* 2017).

Sea weeds as a liquid fertilizer

The extract obtained from the seaweed are called as Liquid seaweed fertilizer. The extract is a natural organic fertilizer which promotes faster seed germination and is highly nutritious to plants. The sea weed extract contains regulators, plant growth hormone, carbohydrates, auxins, gibberellins and vitamins and helps to maintain soil fertility. It is cost effective and eco-friendly for sustainable agriculture. The fertilizer obtained from seaweed extract is biodegradable, non-polluting, nontoxic and non-hazardous to human, animals and birds (Sasikala *et al.,* 2016). Many beneficial effects have been reported on the use of

seaweed extracts, positive responses include improved germination, root development, leaf quality, general plant vigor, and resistance to pathogens (Khan *et al.,* 2009).

Sea weed extract has its wide applications as soil amendment in pest control and in plant disease management. The application of seaweed extracts in plants as foliar spray, seed treatment or soil treatment are mainly focused in many research aspects. Treatment with sea weeds extract increases nutrient uptake of soil and makes them resistant to environmental stress. The most promising and advantageous of seaweed-derived-plants resistant to climate and pests (Sasikala *et al*., 2016). The seaweed extract are marketed as liquid biostimulants because chemical analysis of seaweeds and their extracts has revealed the presence of a wide variety of plant growth-promoting substances such as auxins , cytokinins, and betaines (Khan *et al.*, 2009).

The Liquid seaweed extract can be used by following methods. It can be treated by seed treatment, soil treatment or by foliar spray application. Most of the research works that has done so far concentrated on above methods either in one mode of application or any of the two methods in combination. (Sasikala*. et al*., 2016). The growing agricultural practices need more fertilizers for higher yield to satisfy the need of food for human beings. Developed countries utilize growth hormones in the culitivation of crops. In India utilization of sea weeds and their extracts will be useful for achieving higher agricultural production (Chitra and Sreeja, 2013).

Liquid fertilizers derived from seaweeds are found to be superior to chemical fertilizer due to high level of organic matter, micro and macro element, vitamins, fatty acids and growth regulators (Booth 1969). The excessive use of chemical fertilizer cause serious health hazards as well as pollute the environment. Therefore, in recent years many plat extracts have been used in agriculture. Out of these, use of natural seaweeds as fertilizers has allowed the

gradual substitution of conventional synthesis fertilizers (Hong *et al*., 2007) These natural fertilizers are biodegradable, non-toxic in nature , non-polluting and non-hazardous to human, animals and birds.(Dhargalkar and Pereira, 2005). The dominating sea weed producing states are Tamil Nadu, Gujarat, Maharashtra, Goa, Lakshadweep, Andhra Pradesh and Karnataka, Anadaman and Nicobar islands, few species are also found in West Bengal and Orissa (Tandel *et al*., 2016)

Fertilizers differ from plant growth regulators which differ from fertilizers in several ways. While the growth regulators alter cell division, root and shoot elongation, initiation of flowering and other metabolic function, the fertilizer simply supply minerals needed for the nutrition and normal growth of the plant. Therefore, seaweed liquid fertilizer (SLF), a blend of both plant growth regulators and organic nutrient input is ecofriendly promoting sustainable productivity and maintaining the soil health.(Mohanty, 2013).

Seaweed as a food

In East Asian countries such as Japan, China, Korea, Taiwan and in South East Asian countries such as Thailand, Indonesia, Malaysia and Philippines, seaweeds are commonly used as food (Stengel and Walker, 2015). People who is live in coastal areas in France, Scandinavia, South West England, Ireland, and Scotland also consume seaweeds, albeit to much more limited extent (McHugh, 2003; Stengel and Walker, 2015).

In China, marine algae bears the name sea-vegetable and it is not uncommon for coastal inhabitants of Japan, Malaya, China and Phillippines to be seen on the seashore during low tide collecting these sea-vegetables. Korea and Japan are the two countries where algae are farmed, but only in Japan extensive seaweed cultivation is done (Krishnamurthy, 1981)

Green seaweed

The green seaweeds *Ulva* spp., *Enteromorpha* spp., *Monostroma* spp., *Caulerpa* spp., *Codium* spp., are commonly known as source of food. In Asia countries especially Japan, dried fronds of edible *Monostroma* spp. *Enteromorpha* spp. are being known as 'aonori-green laver-ele ele-lulua- lumi boso'. These algae are eaten by human as edible raw, dried, or cooked. They used in preparation of 'nori-jam' (Lobban and Harrison, 1994; Novaczek. 2001).

Brown seaweed

Laminaria spp., kombu, *Undaria* spp. wakame, *Hizikia fusifirme* 'hiziki' is edible and an improtant resource Asia countries especially China and Japan. They are consumed raw, boiled or dried material with sweetened green beans, jelly, Crushed ice, and coconut milk in southern Vietnam (Tsutsui *et al.,* 2005. *Sargassum* spp. is known as horsetail and it is eaten as soup or dressed with soyabean sauce or after being processed in Korea (Madlener, 1997) and in Hawaii (Novaczek, 2001).

Red seaweed

Gelidium, Gracilaria, Pterocladis and other many red algae are used in the manufacture of agar which are used widely as a growth medium for microorganism and other biotechnological and food applications. Another important red seaweed alga is *Eucheuma* used in the production of Carrageenan, an important product used in cosmetics, food processing and industrial uses, as well as food source. Some of the most significant carageenen species include *Betaphycus gelatinae, Eucheuma denticulatum*, and several species of the genus *Kappaphycus* including *Kappaphycus alvarezii* (Lobban and Harrison, 1994).

Seaweeds have been used as a part of human diet in China, Japan, Thailand and South Korea for many years (Mabeau and Fleurence, 1993; Wong and Cheung, 2000). Some seaweed is generally suitable for making cool, gelatinous dishes or concoctions (Ito and Hori, 1989; Manivannan *et al*., 2009). In general, seaweeds are considered as low calorie food item, but rich in vitamins, minerals and dietary fibre (Ito and Hori, 1989). Seaweeds are also utilized as animal feed ingredient, raw material for fertilizer and as well as in various industrial applications (Mabeau and Fleurence, 1993; Fleurence, 1999; Rupérez, 2002).

Marine algae have been utilized in Japan as raw materials in the manufacture of many seaweed food products, such as jam, cheese, wine, tea, soup and noodles and in the western countries, mainly as a source of polysaccharides for food and pharmaceutical applications. Human consumption of green algae, brown algae and red algae were 5, 66.5 and 33 percent respectively and it was high in Asia mainly in Japan, China and Korea (Dawes and Mathieson, 2003).

Use of seaweeds as food has been traced back to the fourth century in Japan and the sixth century in China. Today those two countries and the Republic of Korea is the largest consumer of seaweeds as food. Japanese have been consuming sea vegetables for more than 10,000 years. In ancient Chinese culture sea vegetables was noted as a delicacy. Some species of seaweeds like nori (*Porphyra* sp), wakame (*Undaria pinnatifida*) were used in every day cookery in Japan. The most important food species in Japan are nori, Kombu (*Laminaria japonica*) and wakame (Chaturvedi, 2010).

Low income families who reside near tidal areas may rely exclusively on ocean resources and eat seaweeds as part of their regular diet (Ostraff, 2006). Red algae like *Porphyra* and nori are used in soups and salads. There are many types of seaweeds available commercially in the Malaysian market. Only four types are commonly used for food preparation in the Japanese restaurants in Malaysia namely nori (*Porphyra* sp.), kumbu

(*Laminaria japonica*), wakame (*Undaria pinnatifida*) and hijiki (*Sargassum fusiforme*). Nori has the highest amount of protein compared to the other types. Wakame has the highest amount of calcium. Sea lettuce (*Ulva lactuca*) is very high in iron. Hijiki (*Sargassum fusiforme*) is high in calcium and fiber. Dulse (*Rhodymenia palmata*) is rich in vitamins B6 and B12. Almost all types contain iodine, sodium, potassium, magnesium, copper, zinc, and vitamins A, B, C, E and K. (Ismail and Hong, 2002).

MacArtain *et al.* (2007) presented information on the nutritional aspects of seaweeds in terms of fiber, mineral contents, fats and lipids, vitamin contents, and components that have a confirmed and investigated nutritional effect.

Alginate, agar, carrageenan and gelatinous substances are collectively known as hydrocolloids or phycocolloids and have attained commercial significance especially in food production. Most of the carrageenan is extracted from *Kappaphycus alvarezii* and *Euchema denticulatum*. The original source of carrageenan was *Chondrus crispus*. (Buck *et al*., 2006). Carrageenan was a family of linear sulphated polysaccharide extracted from red seaweeds. The name is derived from a type of seaweed that is abundant along the Irish coast line (Buck *et al*., 2006). Carrageenan and agar are used as thickening and gelling agents in food, pastry, yoghurts, chocolate, milk and as growth medium for microorganisms (Chandini *et al*., 2008). In India however seaweed consumption is negligible except in the preparation of porridge from *Gracilaria spp.* and *Acanthophora spp.* in coastal status of Kerala and Tamil Nadu (Dhargalkar and Pereira, 2005).

The seaweeds in India also are used to certain extent as food, and in coastal areas of Tamil Nadu a particular type of seaweed (*Gracilaria edulis*) is being used since decades for making gruel. A few recipes like Ulva jam, agar jelly, some food products requiring agar, seaweed salad, vegetable curry and porridge and some other practical uses to which these seaweeds can added (Krishnamurthy, 1981).

Brown seaweeds (known also as macroalgae) are abundant in many coastlines and in some of them they represent an almost unexploited but very valuable marine resource (Magdalena, 2019). The majority of them are alginates and / or heteroglycans rich in sulphated L- fucoses and called fucans or fucoidans (Rinaudo 2007) These natural polysaccharides are widely used in various industries like textile, agri-food, paper, cosmetic, biomedical and pharmaceutical because of their rheological properties such as gelling, viscosifying and stabilization of dispersions (Draget *et al.,* 2006). Alginates, extracted from brown seaweed with an acid and an alkali, are used in a wide range of applications, particularly in the food, industrial, and pharmaceutical fields because of their water holding and gel forming capacities and ability to form and stabilize emulsions (Pilnic and Rombouts, 1955)

Synthetic seeds have potential for a considerable level of cost lowering (Kok-Siong *et al.,* 2012; Roy and Mandal, 2008) with rapid multiplication of plant with genetic uniformity (Saiprasad, 2001). Artificial seeds are described as artificially encapsulated somatic embryos, which represent any vegetative part of plants (Rihan *et al.,* 2017). Because they are obtained from somatic cells, it is possible to be used for clonal propagation. Artificial seeds have different implementation in plant biotechnology - for instance in clonal propagation, germplasm conservation, plant breeding where propagation via normal seeds is not probable, easy storage, genetic uniformity etc. For some ornamental plants, propagation through somatic embryogenesis and synthetic seeds have been regarded as the only way out. Encapsulation technology is a fast-growing research domain in biotechnology and broadly studied. Artificial seeds are convenient for conservation and delivery of tissue-cultured plants, which is why many types of plants, fruits or cereal have been grown from artificial seeds (Shallal, 2020).

The artificial or synthetic seeds are obtained mainly by deriving somatic embryos from plant tissue cultures and encapsulating them with the help of a hydrogel. Production technology of synthetic seeds in horticultural plants offered new techniques for preparing seed analogues from the micropropagules, like protocorm-like body (PLB) formation and plant regeneration (Bapat and Rao, 1988). Micropropagules are encased in productive coatings of gelling agents such as alginate, agar, carrageenan, gellan gum (gelrite), sodium pectate, and carboxyl methyl cellulose (Kok-Siong *et al.,* 2012).

Micro shoots encapsulation and somatic embryos as well as retrieval of plantlets have been reported in a number of plant species, such as: cauliflower (Kok-Siong *et al.,* 2012), sandalwood (Bapat and Rao, 1988), and other plants (Falcinelli *et al.,* 1997). Seeds production by utilizing somatic embryos and other kinds of explants is possibly fruitful for the huge scale propagation of superior hybrids of important species. The synthetic seed technology may only be successful with competent. Different micropropagules have been regarded for purpose of producing artificial seeds (Stanica, 1999); consequently, somatic embryos and axillary shoot buds have been favoured.

Tamil Nadu coastal environment played an important role in country's economy by natural resources and rich biodiversity (Muthukrishnan and Aruchamy, 2012). pH is a most important physical properties of soil. It having great effects on solute concentration and absorption in soil (Akpoveta *et al.,* 2010).At low pH values solubility of micronutrients is high while at high pH solubility and availability of micronutrient to plant is declined (Brady *et al.,* 2002). Bulk density values are required for converting gravimetric soil water content to volumetric and to calculate soil porosity which is the amount pore space in the soil (Blake and Hartge, 1986).

Soil organic carbon is the basis of soil fertility. It release nutrient for plant growth, promotes the structure, biological and physical health of soil, and is buffer against harmful substances. Increasing soil organic carbon has two benefits- as well as helping to mitigate climate change, it improves soil health and fertility. Many management practices that increase soil organic carbon also improve crop and pasture yields (Kamlesh *et al.,* 2017)

The presence of higher content of organic matter in the soil can be another reason for lowering of the pH (Hodes *et al.,* 1996). Organic matter commonly increases water content at field capacity, increases available water content in sandy soil and increases both air and water flows rates through fine textured soil (Ramulu *et al.,* 2001).

MATERIALS AND METHODS

4. MATERIALS AND METHODS

4.1. Study area

India (08^{o} 04'-37^{o} 06'N and 68^{o} 07'-97^{o} 25'E) has a coastline of 7516 Km including 1256 islands with 2 million Km^{2} Exclusive Economic Zone (EEZ) (Rao, 2000). India with a vast coastline supporting rich standing crop of marine algae wherever rocky or coral formations occur along the coast (Ramachandrudu and Kaliaperumal, 2016). The state of Tamil Nadu on the Eastern part of the Southern most tip of Peninsular India, between 8°05'-13°34' N and 76°14'-80°21' E covering about 1,30,058 Km^{2} geographical area, constitutes about 4 % of the country's total area. The state is bounded by the Bay of Bengal to the east, Indian Ocean to South and the Arabian sea on to the South West. Seaweeds were collected from coastal areas of Hare Island.

4.2. Algal sample collection

Marine algae along the coastal areas of Hare Island, Tamil Nadu were studied during Samples of the marine algae were collected randomly by hand picking or by using a metal scrapper during low tides. The collected samples were washed thoroughly in seawater to remove epiphytes and sand particles. They were labelled and transported to the laboratory in polythene bags containing sea water from the same locality for identification. Herbarium of the algal species (SMC 10089 to 10094) selected for further studies were deposited in the Department of Botany, St. Mary's College (Autonomous), Thoothukudi, Tamil Nadu, India.

4.3. Identification of Macroalgae

Macroalgae were identified based on standard keys (Srinivasan, 1969; 1973; Desikachary *et al.,* 1990; 1998; Krishnamurthy, 2000; Baluswami, 2007; Krishnamurthy and Baluswami, 2009).

4.4. Selection and Surface Sterilization of Seeds

Vigna radiata (L.) R. Wilczek. is one of the common pulses and cultivated since ancient times in India. Green gram is properly indigenous to India. It is grown in almost all the states in India. Alfalfa (*Medicago sativa* L.) is a perennial herbaceous legume. Due to its high nutritional quality, high yields and high adaptability, alfalfa is one of the most important legume forages of the world. Therefore, *Vigna radiata* (L.) R. Wilczek. and *Medicago sativa* L. was selected in the present study. About 10 seeds the test plants were immersed in a beaker of water. The seeds which floated on the surface of water were removed. The seeds which sunk to the bottom of the beaker were selected for the study. The selected seeds were washed in running tap water for 5 minutes and rinsed with distilled water for 5 minutes. After washing, the seeds were sterilized by keeping in 0.1% mercuric chloride for 5 minutes. The surface sterilized seeds were washed in distilled water and rinsed 5 times for 5 minutes each (Idu *et al.,* 2003). The surface sterilized and rinsed seeds were employed for the present study.

4.5. Preparation of Seaweed Liquid Fertilizer (SLF):

Air dried plant sample was finely ground with mortar and pestle and 10 g was weighed on electronic balance. 100 mL distilled water was added. The mixture was incubated for two days (48h). Thereafter, the extract was filtered through What-man No.1 filter paper. Now, the extract was made up into 100 mL with distilled water (10%). From this, various concentrations of extract were prepared using distilled water in the following manner,

S. No.	Percentage of Conc.	Extracts (mL)	Distilled water (mL)
1.	Control	-	100
2.	2%	20	80
3.	4%	40	60
4.	6%	60	40
5.	8%	80	20
6.	10%	100	-

4.5. Germination and growth study:

The germination study was conducted during January, 2021. The seeds were washed with distilled water. Seeds (10) were soaked in different concentration of the extract for 24 h. The growth parameters like percentage of germination, root length, shoot length, seedling length, number of leaves, fresh weight and dry weight were observed and recorded. Shoot length was measured from collar region to the tip of the shoot of the plants. Similarly root length was measured from collar region to the tip of the primary root. Uprooted plants were washed with distilled water and it was blotted with blotting paper to check the fresh weight of the plant. It was then shade dried to obtain the dry weight of the sample. The control seeds were soaked in different water for the same period. Triplicates were maintained for each treatment. The treated seeds were kept under observation for 7 days.

4.5.1. Vigour index (Rinku, 2018):

Seed vigour is an important quality parameter which needs to be determined in addition to germination. Vigour index of seeds calculated by using Abdul Baki and Anderson (1973) formula was:

Vigour index = Germination (%) x seedling length (cm).

4.6. Phytochemical screening (Horbone 1984; Kokate *et al,* 1995 and Horborne 1998**)**

4.6.1. Test for alkaloids:

Take 1 mL of extract add a few drops of Wagner's reagent, a reddish brown precipitate is formed.

4.6.2. Test for flavanoid (Shinoda test):

Take 1 mL of the extract add a pinch of magnesium turnings and 1-2 drops of concentrated Hydrochloric acid formation of pink colour indicates the presents of flavanoid.

4.6.3. Test for phenol:

Take 1 mL of extract add 0.5 mL of lead acetate solution, formation of precipitate.

4.6.4. Test for Tannins:

1 mL of extract in 20 mL of water in a test and then filtrated. A few drops of 0.1% ferric chloride was added and observed green or blue – black colouration which confirms the presence of tannin.

4.6.5. Test for steroid:

Take 1 mL of the extract and 2 mL of chloroform and equal volume of concentrated sulphuric acid. If the brown ring occurs, it indicates the presence of steroids.

4.6.5. Test for phytosteriod:

Take 1 mL of the extract and 2 mL of chloroform and equal volume of concentrated sulphuric acid. If bluish brown colour appear it indicates the presence of phytosteriods.

4.6.6. Test for carbohydrates:

Take 5 mL of Benedict solution in 1 mL of the extract and boiled in a water bath. Appearance of red or yellow or green precipitate indicate the presence of reducing sugar.

3.6.7. Test for Terpenoids

Take 5 mL of the extract and add 2 mL of chloroform and concentrated sulphuric acid to form a layer. Appearance of reddish brown colour indicates the presence of terpenoids.

4.6.8. Test for Glycoside

0.5 mL of the extract was dissolved in 1 mL of water and then aqueous NaoH solution was added. Appearance of yellow colour indicates the presence of glycoside.

4.6.9. Test for Protein (Ninhydrin test)

0.5 mL of extract was taken and 2 drops of freshly prepared 0.2% ninhyddrin reagent was added and heated. The appearance of pink or purple colour indicates the presence of protein.

4.6.10. Test for coumarin

Take 3 mL of 10% NaoH and add 2 mL of the plant extract in it. Formation of yellow colour indicates the presence of coumarin.

4.7. Seaweed Recipes:

4.7.1. *Ulva* jam (Krishnamurthy, 1981)

Ulva lactuca were collected and washed in running tap water, air dried and powdered. Syrup is prepared and in the boiling condition the powder is added and left to boil for about 30 minutes. When the material is boiled well, colour and essence are added. The vessel is removed from the heater. The jam is cooled and bottled immediately. This can be used just like any other jam, along with bread, chappathi, etc.

4.7.2 Agar jelly (Krishnamurthy, 1981)

Fresh *Gracilaria corticata* plants are collected and are washed several times in soft water to remove sand, lime, sea salts and other adhering matter and spread in the sun till dry. The seaweed is ground in a stone mortar adding water and placed in a spacious tray or basin with soft water for 24 h in a cool, airy place. The pulp is dried thoroughly in the sun and

pulverized in a coffee grinder. 100 mL of soft water is boiled in an enamel or stainless steel vessel. To the boiling water 10 g of sea weed meal is added and stirred from time to time, and then it is filtered through an organdy cloth, essence and colouring matter are added to the extract and again it is filtered in a tray.

4.8. Extraction of sodium alginate (Le-Gloahec-Herter method, 2019)

25 g of seaweed powder was taken and mixed with 2% 200 mL Hcl for thirty minutes in order to dissolve alkaline earth salt remnants. In addition, washing with clean water was carried out until the pH was neutral. In the next stage of extraction by seaweed the slurry was blended with 200 mL of 4% Na_2CO_3 solution then heated in a 90^o C water bath for two hours while periodically being stirred. This process was carried out until all cellulose became fine particles and homogeneous paste was produced. Then the dilution was carried out by adding distilled water and 4% Na_2CO_3 in a ratio of 3:7. Then the resulting solution was filtered using a filter cloth to obtain the filtrate. The filtrate was then heated to a temperature of 40^o C and then coagulated using $CaCl_2$ 10% and filtrate in a ratio of 1:5 and stirred for fifteen minutes to obtain calcium alginate clots. The remaining filtrate was coagulated with $CaCl_2$ 5% and filtrate solution in a ratio 1:5 to obtain a lump of alginate calcium. Then, the calcium alginate obtained was acidified with 5% Hcl until the pH of the calcium alginate 2 - 3 was obtained, then washed with 95% alcohol and alginate in a ratio of 1:1 by soaking while stirring periodically for 20 minutes and filtered. After that, Na incorporation was carried out using 1% Na2CO3 and calcium alginate solution in a ratio of 1:1.5 for one hour while stirring periodically, followed by rewashing using 95% alcohol two times. The last stage was drying at temperature of 70-75 ^oC for eight hours in a cabinet dryer. The final product obtained was dry sodium alginate. After obtaining dried sodium alginate, it was blended and sieved 80 mesh.

3.8.1. Artificial seed

The Pomegranate (*Punica granatum*) is selected and it is dipped in 3% sodium alginate and are exposure to 100 mM $Cacl_2.2H_2o$ solution for 30 minutes. It has produced optimal beads with firm, clear, round and uniform size and suitable for handling. Then it is placed in a test tube containing nutrient medium.

3.9. Soil Analysis:

The soil samples were collected from Hare Island, Tuticorin, during low tide. It is stored in a polythene bags immediately to determine the physic-chemical parameters like soil moisture, bulk density, soil porosity, soil pH and organic matter).

RESULT

5. RESULT

During December 2020, January to March 2021, seaweeds were collected along the coastal areas of Hare Island, Tamilnadu, India are documented in Table 5.1, 5.2 and 5.3. A total of 26 marine algae were collected along the study areas of among which ten members are from Chlorophyceae, ten members from Phaeophyceae and six members from Rhodophyceae.

The Seaweed Liquid Fertilizer of *Hpnea musciformis* and *Gracilaria corticata* were used as base for *Vigna radiata* (L.) R. Wilczek. and *Medicago sativa* L (Figure 5.1, 5.1.1, 5.1.2, 5.1.3, 5.1.4, Table 5.4 and 5.5). Germination of seed was observed on 4^{th} day and frequency of germination was found to be 100% in control and all the treatments. The experiment results showed the stimulation both in shoot and root growth. The lowest germination percentage was recorded at 10% extract of *Gracilaria corticata*. The highest germination (100%) of the green gram was observed with 6% and 8% in *Gracilaria corticata* and 2% and 4% in *Hypnea musciformis* extract tested of green gram. In alfalfa the highest germination of green gram is 2%, 6% and 8% and in *Gracilaria corticata* 2%, 4% and 8%. Maximum root length 2.34 cm of the *Vigna radiata* was observed at 6% concentration of *Gracilaria corticata* treatment. The lowest root length (1.43 cm) was observed at seaweed extract level (10%) of *Hypnea musciformis* in green gram. Maximum root length (2.94 cm) of the *Vigna radiata* was observed at 65 concentration of *Gracilaria corticata* treatment. The lowest root length (1.02 cm) was observed at seaweed extract level (10%) of *Hypnea musciformis* in alfalfa. The maximum shoot length of the green gram was observed at 6% concentration of *Gracilaria corticata* treatment among the six seaweeds tested (8.4 cm). The lowest shoot length (3.72 cm) was observed in *Hypnea musciformis.*

Table 5.1: List of green algae collected along the coastal areas of Hare Island

S. No.	Order	Family	Name of the alga	Available status
1.	Ulvales	Ulvaceae	*Ulva lactuca* Linn.	++
2.	Ulvales	Ulvaceae	*Ulva reticulata* Frost.	+++
3.	Ulvales	Ulvaceae	*Ulva fasciata* Delile	++
4.	Cladophorales	Cladophoraceae	*Chaetomorpa antennina* Borry de Saint- Vincent	+
5.	Siphonales	Caulerpaceae	*Caulerpa scalpelliformis* (R.Br.) Weber-Van- Bosse	+
6.	Siphonales	Caulerpaceae	*Caulerpa racemosa* (Forsk.) Weber-Van-Bosse	+
7.	Siphonales	Caulerpaceae	*Caulerpa taxifolia* (Vahl) Ag.	+
8.	Siphonales	Caulerpaceae	*Caulerpa peltata* (Turn.) Lamour	+
9.	Siphonales	Codiaceae	*Codium tomentosum* (Hudson) Stack.	+
10.	Siphonales	Valoniaceae	*Valoniopsis pachynema* (Martens) Boergs.	+

Table 5.2: List of brown algae collected along the coastal areas of Hare Island

S. No.	Order	Family	Name of the alga	Available status
1.	Scytosiphonales	Chnoosporaceae	*Chnoospora fastigiata* J. Ag. var. pacifica J.Ag.	+
2.	Dictyotales	Dictyotaceae	*Padina tetrastromatica* Hauck	+
3.	Dictyotales	Dictyotaceae	*Padina pavonica* (L.) Lamour	++
4.	Dictyotales	Dictyotaceae	*Stoechospermum marginatum* (Ag.) Kütz.	+
5.	Fucales	Sargassaceae	*Sargassum duplicatum* J. Ag.	++
6.	Fucales	Sargassaceae	*Sargassum wightii* (Grev.) J. Ag.	++
7.	Fucales	Sargassaceae	*Sargassum polycystum* C. A. Agardh	+
8.	Fucales	Sargassaceae	*Sargassum tenerrimum* J. G. Agardh	+
9.	Fucales	Sargassaceae	*Turbinaria ornata* J. Ag.	+
10.	Fucales	Sargassaceae	*Turbinaria conoides* Kützing	+

Table 5.3: List of red algae collected along the coastal areas of Hare Island

S. No.	Order	Family	Name of the alga	Available status
1.	Gigartinales	Gracilariaceae	*Gracilaria corticata* J. Ag.	+++
2.	Gigartinales	Gracilariaceae	*Gracilaria verrucosa* (Huds.) Papenfuss	+
3.	Gigartinales	Gracilariaceae	*Gracilaria edulis* (S.G.Gmelin)	+
4.	Gigartinales	Hypneaceae	*Hypea musciformis* (Wulfen) J. V. Lamouroux	+++
5.	Gigartinales	Hypneaceae	*Hypnea valentiae* (Turner) Mont.	+
6.	Ceramiales	Rhodomelaceae	*Acanthophora spicifera* (M.Vahl) Borgesen	+

Figure 5.1: Effect of SLF on seed germination of *Vigna radiata* (L.) R. Wilczek. and *Medicago sativa* L

Table 5.4: Effect of Seaweed Liquid Fertilizer of *Gracilaria corticata* and *Hypnea musciformis* on shoot and root length of *Vigna radiata* (L.) R. Wilczek.

Algae used	Concentration	No. of seeds germinated(%)	Seed vigour index	Root length	Shoot length	Seedling length	Number of leaves	Fresh weight	Dry weight
Gracilaria corticata	Control	83	533	1.53	4.901	6.431	1.03	0.137	0.0117
	2	96	894	1.73	7.597	9.327	1.6	0.536	0.0164
	4	96	957	2.01	7.963	9.973	1.23	0.685	0.0168
	6	100	1045	2.34	8.11	10.45	1.6	0.799	0.0170
	8	100	977	2.10	7.67	9.77	1.73	0.635	0.0163
	10	70	557	1.61	6.36	7.97	1.0	0.512	0.0163
Hypnea musciformis	Control	83	533	1.53	4.901	6.431	1.03	0.137	0.0117
	2	100	640	149	4.915	6.405	1.62	0.212	0.0120
	4	100	657	1.56	5.017	6.577	1.10	0.325	0.0127
	6	90	601	1.59	5.122	6.682	1.10	0.336	0.0123
	8	96	580	1.51	4.54	6.05	1.0	0.191	0.0121
	10	83	452	1.43	4.021	5.451	1.23	0.098	0.0087

Figure 5.1.1: Effect on SLF from *Gracilaria corticata* on *Vigna radiata*

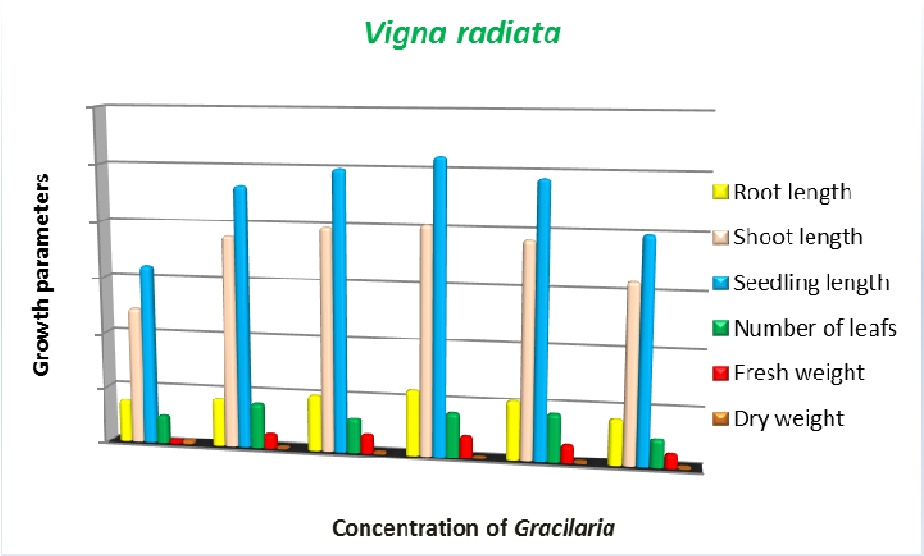

Figure 5.1.2: Effect on SLF from *Hypnea musciformis* on *Vigna radiata*

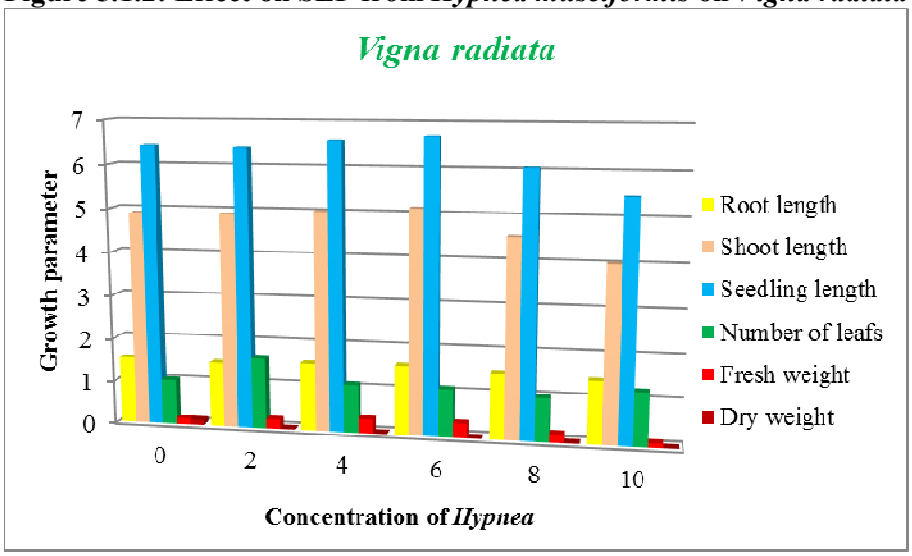

Table 5.5: Effect of Seaweed Liquid Fertilizer of *Gracilaria corticata* and *Hypnea musciformis* on shoot and root length of *Medicago sativa* L

Algae used	Concentration	No. of seeds germinated(%)	Seed vigour index	Root length	Shoot length	Seedling length	Number of leafs	Fresh weight	Dry weight
Gracilaria corticata	Control	80	674	1.75	6.676	8.426	0.4	0.597	0.0147
	2	100	677	1.667	5.11	6.777	0.53	0.589	0.0142
	4	93.3	745	2.012	5.98	7.992	0.667	0.609	0.0162
	6	100	1040	2.94	7.10	10.4	1.73	0.787	0.0176
	8	100	897	2.64	6.33	8.97	1.7	0.723	0.0169
	10	100	727	2.05	5.22	7.27	0.667	0.608	0.0158
Hypnea musciformis	Control	80	674	1.75	6.676	8.426	0.4	0.597	0.0147
	2	100	830	2.65	5.657	8.307	0.667	0.359	0.0123
	4	100	690	1.98	4.923	6.903	0.4	0.302	0.0120
	6	96	608	1.81	4.534	6.344	0.4	0.287	0.0128
	8	100	605	1.73	4.321	6.051	0.38	0.231	0.0119
	10	70	331	1.02	3.727	4.747	0.38	0.198	0.0118

Figure 5.1.3: Effect on SLF from *Gracilaria corticata* on *Medicago sativa*

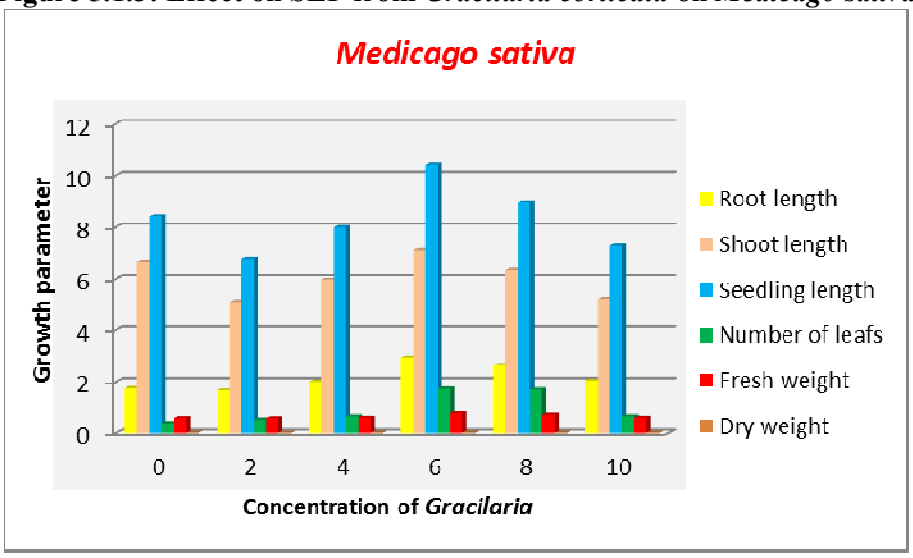

Figure 5.1.3: Effect on SLF from *Hypnea musciformis* on *Medicago sativa*

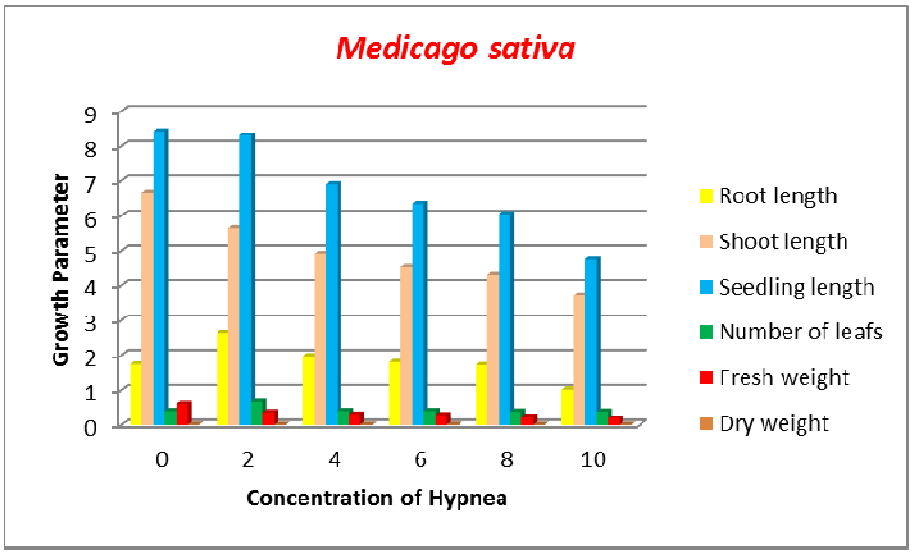

Figure 5.2: Phytochemical analysis of *Gracilaria corticata*

Figure 5.3: Phytochemical analysis of *Hypnea musciformis*

Table 5.6: Phytochemical analysis of *Gracilaria corticata* and *Hypnea musciformis*

S.No.	Test	*Gracilaria corticata*	*Hypnea musciformis*
1.	Alkaloid	++	+
2.	Flavonoid	+	-
3.	Phenol	+	++
4.	Tannin	+	+
5.	Steroid	-	+
6.	Phytosteroid	+	-
7.	Carbohydrates	++	+
8.	Protein	+	+
9.	Glycoside	++	+
10.	Coumarin	+	+

+ - present, ++ - moderately present, -- absent

Figure 5.4: *Gracilaria* jelly

Figure 5.5: *Ulva* jam

Sodium alginate

Beads

Artifical seed

Nutrient Agar

Figure 5.6: Artificial seeds of pomegranate encapsulated with sodium alginate

Phytochemical analysis of *Glacilaria corticata* and *Hypnea musciformis* confirms the presence of following components like alkaloid, phenol, carbohydrates, protein, glycoside and coumarin (Table 5.6, Figure 5.2 and 5.3).

The distribution of seaweeds on Indian coasts and due to their nutritive value a few recipes like *Ulva* jam and *Gracilaria* jelly are prepared since seaweeds are cheap sources for minerals and trace elements (Figure. 5.4 and 5.5).

Commercially, seed propagation is only used for cultivars that are difficult to propagate by cutting, but encapsulated seeds of pomegranate by alginate prepared from *Padina tetrastromatica* enhances seed germination (Figure 5.6).

Soil sample analysis of Hare Island reveals that the pH of the soil is 8.40 which indicates alkaline in the soil. Moisture content of the soil is 27.4%. Soil porosity of the soil is 29.6%. Bulk density of the soil is 1.9 and organic matter of the soil is 0.64.

DISCUSSION

6. DISCUSSION

The present study revealed the availability of 26 species of green, brown sand red seaweeds from the three selected locations of Gulf of Mannar. Jagadeesan *et al.* (2013) indicated that the direction of the ocean current around the Indian subcontinent during the South West monsoon is from West to the East, which brings Arabian Sea waters into the Gulf of Mannar. At Hare Island, 13 species of brown seaweeds were recorded with the dominance of *Padina tetrastromatica. Sargassum tennerimum* exhibited the highest biomass during January in this location, probably due to the fruiting period (Kesava Rao, 1994). This location is characterized with rocky corals, sandy habitats, semi exposed shallow water body and partly surrounded by land with moderate wave action. Water motion has been found to be one of the most important variables influencing seaweeds because it regulates turbidity, light penetration and nutrient availability (Kang *et al.,* 2011).

In the developing world, to use of Seaweed Liquid Fertilizer may be the solution of environmental pollution by heavy dose of chemical fertilizer. The effect of Seaweed Liquid Fertilizer on plant growth, yield and the ability sustained environmental conditions. The treatment with different Seaweed Liquid Fertilizer increased the seed germination. However, different concentration of Seaweed Liquid Fertilizer plays an important role to impact desired effects. The higher concentration of Seaweed Liquid Fertilizer affects on respiratory activity was higher and percentage germination was less. (Rinku, 2018).

At present, the use chemical fertilizers in great quantities to compensate the deficiency of nutrient in soil. It is observed that the abundant use of chemical fertilizers affects soil and plants in due course. Recent researchers proved that seaweed fertilizers are preferred not only due to their nitrogen, phosphorus and potash content but also because of the presence of trace elements and metabolites, similar to plant growth regulators. In India, as a step towards the expansion of native sources of natural manures, the seaweed fertilizers

application will be useful now for achieving higher production. Recently, seaweed extracts as liquid fertilizers (SLF) has come in the market for the simple reason that they contain many growth promoting hormones like auxin, gibberlin, trace elements, vitamins, amino acids and micronutrients (Sheela and Punitha, 2013). As the concentration of the SLF increased the growth parameters shows reduced with increased concentration.

The application of seaweed fertilizer for different crop was of great importance to substitute the commercial chemical fertilizers and to reduce the cost of production. Liquid fertilizers derived from seaweeds are found to be superior to chemical fertilizers due to high level of organic matter, micro and macro elements, vitamins and fatty acids and also rich in growth regulators (Gandhiyappan K, Perumal, 2001). The growth promoting effect of extract of seaweeds on seed germination, Vegetative growth and biochemical characteristics in agriculture crops has been reported (Kumar and Babu).

Seaweed extract have been marketed for several years as fertilizer additives and beneficial result from their use have been reported. The carbohydrates and other organic matter present in seaweeds alter the nature of soil and improve its moisture holding capacity (Lingakumar *et al.,* 2002). Thivy (1960) reported that the seaweeds improve the fertility of soil in cultured fields as their content helps in conditioning the soil, facilitating aeration, moisture retention and adsorption of nutrient elements.

In the present study, the seeds treated with 4% and 6% concentration of *Gracilaria* and 4% in Hypnea showed better results in growth parameters as compare to other concentration of Seaweed Liquid Fertilizer treatment. Seaweed extract different concentrations were evaluated to be more effective in different plant such as *Solanum lycopersicum* (Hernandez-Herrera *et al.,* 2014), *Vigna radiata* (Bai *et al.,* 2013; Parthiban *et al.,* 2013), *Mangifera indica* (Ahmed *et al.,* 2013), *Vigna mungo* (Kalaivanan *et al.,* 2012), *Lycopersicum esculentum* (Zodape *et al.,* 2011). Sekar *et al.* (1995) reported the fresh weight

of green gram seedlings tested with all the six seaweed extracts treatment was gradually decreased with increasing concentration of sea weed extract from *Ulva lactuca* promoted the growth of *Vigna unguiculata.* Kamaladhasan and Subramanian, (2009) also reported similar effect in red gram and Lignakumar *et al*. (2002) and Thevanathan *et al.* (2005) reported linear growth of both shoots and roots in *Vigna unguiculata* and *Phaseolus munga.* Similar typical growth promotion was observed in this study at lower concentration of the *Sargassum plagiophyllum* extract. Seaweed ingredients include macro- and microelement nutrients, amino acids, vitamins, cytokinins, auxins, and abscisic acid that affect cellular metabolism in treated plants, leading to enhanced growth and crop yield (Crouch and van Staden 1993; Stirk *et al.,* 2004; Wightman and Thimann, 1980).

The jam and jelly prepared from *Ulva* and *Gracilaria* are rich in nutritional content. The seaweeds in India also are used to certain extent as food, and in coastal areas of Tamil Nadu n particular type of seaweed *(Gracilaria edulis)* is being used since decades for making gruel (Krishnamurthy *et al.,* 1981).

Despite its usefulness, pomegranate remains mostly a neglected and underutilized species (da Silva *et al.,* 2013). Pomegranates are commonly propagated vegetatively in the spring by hardwood cuttings and in the summer by softwood cuttings. Work at our laboratory indicated that pomegranate seeds had low and irregular germination and stand establishment. Propagation and seedling availability are difficult because the hard seed coat inhibits water entry and gaseous exchange.

The present study reveals that it SLF binds soil particles together and improves the soil properties and mineral acquisition. SLF application is ecofriendly and can bring harmony to nature. SLF could be excellently used as one of the organic manures to improve the productivity of crop plants. Also algae has multidimensional purpose.

SUMMARY AND CONCLUSION

7. SUMMARY AND CONCLUSION

The present study is an important step towards the utilization of the extracts of seaweeds. *Gracilaria corticata* and *Hypnea musciformis* to improve the growth of *Vigna radiata* and *Medicago sativa*. The presence of nitrogen, magnesium, potassium and some trace elements in seaweeds make an excellent choice as organic fertilizers. The Seaweed Liquid Fertilizer prepared from the red alga *Gracilaria corticata* and *Hypnea musciformis*, applied to crop plant showed better results in all aspects of growth and pigments. It is probably due to the presence of growth promoting hormones and nutrients in more quantities in the red algae.

Seaweeds can be regarded as an under-exploited source of health benefit molecules for food processing and nutraceuticals industry. It is an excellent source of some essential macronutrient, minerals, vitamins, fatty acids and due to their high polysaccharides content which could also imply a high level of soluble and insoluble dietary fibre. Due to the high nutritional value, food grade seaweeds have a great potential and to provide healthy food.

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A STUDY ON THE MORPHOLOGICAL, PHYTOCHEMICAL AND FTIR ANALYSIS OF SELECTED SPECIES OF PORTULACACEAE

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By

SANTHIYA RAICHEL M

Reg.No.19APBO13

DEPARTMENT OF BOTANY

ST. MARY'S COLLEGE (Autonomous)

THOOTHUKUDI -628001

2020-2021

CONTENTS

CERTIFICATE

This is to certified that this dissertation entitled **A STUDY ON THE MORPHOLOGICAL, PHYTOCHEMICAL AND FTIR ANALYSIS OF SELECTED SPECIES OF PORTULACACEAE** Submitted by SANTHIYA RAICHEL.M Reg.No.19APBO13 to St.Mary's College (Autonomous) THOOTHUKUDI - 628001in partial fulfillment for the award of the degree of **'Master of Science in Botany'** is done by her under my supervision. It is further certified that this dissertation or any pare of this has not been submitted elsewhere for any other degree.

GUIDE 15.4.2024

M. Glory
HEAD OF THE DEPARTMENT

HOD of Botany
St. Mary's College (Autonomous)
Thoothukudi- 628 001.

EXAMINAR

Lucia Rose
PRINCIPAL
St. Mary's College (Autonomous)
Thoothukudi - 628 001.

DECLARATION

I do here by declare that this dissertation entitled **A STUDY ON THE MORPHOLOGICAL, PHYTOCHEMICAL AND FTIR ANALYSIS OF SELECTED SPECIES OF PORTULACACEAE.** Submitted by me in partial fulfillment for the award of the degree of **'Master of Science in Botany',** in the result of my original and independent work carried out under the guidance of **Dr. Mrs. S. Beulah Jerlin M.Sc, M.Phil., Ph.D.** Assistant Professor. Department of Botany, St.Mary's College (Autonomous) THOOTHUKUDI and it has not been submitted elsewhere for the award of any other degree.

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SANTHIYA RAICHEL.M

INTRODUCTION

INTRODUCTION

The medicinal plants continue to offer valuable therapeutic value for both modern and traditional medicine in resolving several health problems (Kshirsagar et al, 2010). The dependence of man on plant resources is as old as the various human civilizations. People throughout the world use medicinal plants for the treatment of various human as well as animal diseases. Since time immemorial, People have been using various medicinal plants for curing varieties of ailments (Bhattachariya *et al*., 2015).

Major sources of traditional medicines are plants with large variety of bioactive constituents, which are effective against different diseases. The significant biological activities of the plants are due to these bioactive constituents. Rich sources of antibacterial and antifungal agents are medicinal plants used in many countries as sources for potent and beneficial drugs. (Mahesh, 2008).

Plant derived drugs even today remain important resource especially in developing countries, to combat serious diseases. Approximately 62-80 of the world's population still relies on traditional medicines for the treatment of common illness (WHO, 2002, Zhang, 2004). Extracts of many plants are highly efficient against parasitic as well as microbial infections. In fact, plants produce a diverse range of bioactive molecules making them a rich source of different types of medicines. Higher plants, as sources of medicinal compounds, have continued to play a dominant role in the maintenance of human health since ancient times (Farombi, 2003).

Ornamental plants can provide multiple profits with regards to environment beauty, economy concern and human lifestyles. Ornamental plants attract pollinators which can feed them through nectar present in their flowers that has high nutritive value for humans too. Despite the increasing interest of ornamental plants, some of them are also cultivated for their medicinal

use as they have many bioactive compounds like phenolic compounds, carotenoids, antioxidants, essential oils and other secondary metabolites(Goleniowski M *et al*., 2013 and Kaushik P *et al*., 2015) Ornamental plants like *Ocimum* sp., *Nicotiana* sp., *Ixora*, *Aloe vera*, *Agave*, etc. and ornamental flowers like roses, nasturtium, hibiscus, marigold, *Calendula*, etc. are commonly grown in homes which also have many medicinal applications. Along with this, remedies from plants can be much cheaper and protect against free radicals without any side-effects than medicines formed by pharmaceutical companies.

Plant morphology or phytomorphology is the study of the physical form and external structure of plants, whereas plant anatomy is the study of the internal plant structure, mostly at the cellular/microscopic level (Armando Carrillo-López and Elhadi M. Yahia. 2019)

Phytochemicals are naturally occurring in the medicinal plants, leaves, vegetables and roots that have defense mechanism and protect from various diseases. Phytochemicals are primary and secondary compounds. Chlorophyll, proteins and common sugars are included in primary constituents and secondary compounds have terpenoid, alkaloids and phenolic compounds (DudukuKrishnaiah.*et al*., 2007). According to some generous estimates, almost 80% of the present day medicines are directly or indirectly obtained from plants (Vidhya and Umavandhana, 2016).

An antioxidant can be broadly defined as any substance that delays or inhibits oxidative damage to a target molecule (Yamagishi S and Matsui T. 2011). The main characteristic of an antioxidant are its ability to trap free radicals. Antioxidant compounds like phenolic acids, polyphenols and flavonoids scavenge free radicals such as peroxide, hydroperoxide or lipid peroxyl and thus inhibit the oxidative mechanisms that lead to degenerative diseases (WuYY*et al*., 2011).

FTIR used for the identification of functional groups of the compounds present in the sample (Payal Mittal1 *et al*., 2020). FTIR allows infrared spectrum simultaneously providing speed and accuracy in measurements of whole range of biological specimens (Griffiths *et al.,* 1986), and has been used as a requisite method to identify medicines in pharmacopoeia of many countries (Liu *et al.,* 2006).

Plant based medicines are safe and effective for the treatment of many ailments. Phytochemical screening is helpful to detect the various important compounds which could be used as the base of modern drugs for curing various diseases keeping this in mind the investigator select the two plants from Portulacaceae the purslane family of flowering plants, in the order Caryphyllales, with about 15 genera and 500 species of herbs or small shrubs, native primarily to the Pacific coast of North America and southern South America.

Portulaca grandiflora, Hook.is a common garden plant in Japan. The entire plant is depurative. It is used in the treatment of hepatitis, cirrhosis of the liver with ascites, swelling and pain in the pharynx. The fresh juice of the leaves and stems is applied externally as a lotion to snake and insect bites, burns, scalds and eczema.

Portulaca umbraticola, also known as the wingpod purslane, is a perennial succulent in the genus of flowering plant *Portulaca*. Although this species can be easily mistaken for *P. oleracea*, its foliage tends to be much smaller and wider than that of other species. Roots are edible. In fact, it is a better nutrient source than spinach. It can be eaten fresh or cooked and has no bitter taste at all. Since it has a mucilaginous quality it is great for soups and stews. Seeds may be eaten either raw or ground and made into bread. In addition recent studies suggest that Omega-3s may have positive effects on the brain and may aid in such conditions as depression, bipolar disorder, Alzheimer's disease, autism, schizophrenia, attention deficit disorder,

hyperactivity and migraines. Though very beneficial, there are few good dietary sources other than seafood for Omega-3s.

SCOPE AND OBJECTIVES

SCOPE AND OBJECTIES

Plants have been used for the treatment of several disorders since long before history. Ornamental plants are plants which are mainly grown for their aesthetic value, however, there are many ornamental plants that are being used as folk medicine in the treatment of various diseases. It is, therefore, imperative that in-depth knowledge is acquired on the medicinal properties of these ornamental plants (Larbie and Abboah-Offei, 2014). In current era, herbal medicines are used more often as human believe in natural therapies is increasing day by day. Knowledge of the chemical constituents of plant is helpful in the discovery of therapeutic agent. However it is essential to work on locally available resources to bring out their pharmaceutical values, for utilization as source of nutrient supplements, minerals, vitamins, enzymes and antimicrobials in medicine. Hence the present investigation was undertaken with the following objectives.

- To identify the morphological characters
- To perform qualitative analysis of phytochemical in various leaf extracts of selected species of Portulacaceae.
- To estimate quantitatively the percentage of elements and the various secondary metabolites present in selected species of Portulacaceae.
- To perform the FT-IR studies of leaf and stem powder of selected species of Portulacaceae.

REVIEW OF LITERATURE

REVIEW OF LITERATUR

Phytochemicals are knows as secondary plant metabolites and have biological properties such as antioxidant activity antimicrobial effect, modulation of detoxification enzymes, stimulation of immune system, decrease of platelet aggregation and modulation of hormone metabolism and anticancer property. There are more than thousand known and many unknown phytochemicals. It is well known that plants produce these to protect themselves, but recent researches demonstrate that many phytochemicals can also protect human against diseases (Rao. 2003).

These chemicals are often referred to as "secondary metabolites" of which there are several classes including alkaloids, flavonoids, Coumarins, glycosides, polysaccharides, phenols, tannins, terpenes and terpenoids (Okwu,2004)

Falodun *et al*., (2006) reported the occurrence of flavonoids, saponins, diterpenes and phorbol estersin in the aqueous and methanol extracts of *Euphorbia heterophylla.*

In addition to these substances, plants contain other chemical compounds. These can act as agents to prevent inconsiderable side effects of the main active substances or to assist in the assimilation of the main substances. Plants have an almost limitless ability to synthesize aromatic substances, mainly secondary metabolites of which 12,000 have been isolated, a number estimated to be less than 10% of the total (Mallikharjuna *et al.,* 2007).

Krishna *et al.,* (2009) conducted preliminary Phytochemicals, total phenolics and flavonoids content analysis in the methanol extract of *Justicia gendarussa*. The aerial parts of *Hypericum perforatum* were experimented to acquire knowledge about their composition of bioactive compounds (Gioti *et al.,* 2009).

Ravirajisingh *et al*., (2009) took methanol extract from *Clerodendron glandulosum* to study its qualitative and quantitative phytochemical constituents.

Jitin Ahuja et al., 2011 studied the phytochemicals present in various extract of aerial parts of *A. parviflora* and to determine the total phenolic and flavonoid content in ethanolic extract. Total phenol and flavonoid content was determined by folin-ciocalteu assay and aluminum chloride colorimetric assay respectively. Ethanolic extract showed the presence of alkaloids, sterols/ triterpenoids, flavonoids, tannins and coumarins. The phenolic and flavonoid content of ethanolic extract using gallic acid and rutin as standards was found out to be 1.09 ± 0.007mmgGAE/g and 1.163 ± 0.0208 mgRE/g respectively. The study showed significant amount of gallic acid and rutin equivalents were present in extract which may be responsible for valuable pharmacological property of extract. As phenolics and flavonoids are responsible for antioxidant activity of plant, present data implies that *A. parviflora* is a perfect candidate for in-vitro antioxidant activity and isolation of phytochemicals.

Johnson *et al.*, (2012) reported the phytochemical constituents of methanol flower extracts of *Helictresisora, Spathodea campunulata, Antigonon leptopus and Thunbergia grandiflora.* Kiruba *et al.,* (*2012*) studied the phytochemical analysis of various solvents extracts of the flower of *Rhododendron arboretum* spp. *nilagiricum.*

M. N. Abbas *et* al., 2012 studied the qualitative and quantitative phytochemical analysis of fifteen weed seed extracts. Alkaloids, saponins, glycosides, terpenoids, anthraquinine, steroids, flavonoids and tannins were detected from the weed seeds. Tannins and alkaloids were in high concentration. Tannins ranged from 7.97 to 24.17%, alkaloids 0.88 to 4.00%, saponins 0.54 to 1.29% and flavonoids 3.91 to 15.55%. Wheat weeds care medicianlly important but their phytochemical potential needs to be further investigated.

Atiq Mehsud *et al*., 2013 studied the morphology and anatomy of seven most common weed species infesting agricultural and non-agricultural lands of rainfed area of Bannu region were investigated during 2012. The study included *Datura metel L., Euphorbia hirta L., Fagonia cretica L., Heliotropium europaeum L., Parthenium hysterophorus L., Solanum surattense Burm f. and Withania somnifera (L.) Dunal.* Due to some special morphological and anatomical features, the capacity of rapid absorption of water along with minerals from the soil may be facilitated to compensate the rapid water loss, and thus can also be regarded as common xerophytes. Their morphological, anatomical and histological characteristics are suitable for their successful growth in rain-fed condition of the region.

Ajiboye B.O *et* al., 2013 studied to found out the presence of phytochemicals in the aqueous extracts of *Senecio biafrae* leaves of both qualitative and quantitative screening methods. In qualitative analysis, the phytochemical compounds such as alkaloids, saponin, tannin, phlobatannin, phenol, anthraquinoes, flavonoids, glycosides, steroids, terpenes, cardenolides, flavonoid and chalocones were determined in the sample aqueous extracts by using standard methods. Also, quantitative analysis of the important secondary metabolites such as alkaloids, phenolic compounds, flavonoids, saponins and tannins were tested in the sample extracts. Results concluded that the presence of these active compounds may be responsible for the medicinal purposes of the plan.

Gilberto Ocampo *et al*., 2013 studied not only the species with different C 4 biochemistry (NADP-ME and NAD-ME types) and C 3 -C 4 intermediacy, but also displays different leaf anatomical configurations. Photosynthetic pathways were assessed based on leaf anatomy and carbon isotope ratios. Information on the NADP- ME and NAD-ME C 4 variants was obtained

Here we addressed the evolutionary history of leaf anatomy and photosynthetic pathways in Portulacaceae.

Monika Gupta *et* al., 2013 studied the qualitative analysis of *Emblica officinalis, Acacia catechu, Acacia concina* and *Hibiscus rosa-sinensis* showed that tannins, saponins, flavonoids, terpenoids and alkaloids are present in all the plants except phlobatannins that is only present in *Acacia catechu*. The pet ether and chloroform extract of *Emblica officinalis* does not show potential for oil and fat components whereas all the extract of *Emblica officinalis* showed positive test for carbohydrates. The identification of colouring chemical constituents of natural products together with their therapeutic properties are discussed.

Shinde P. R *et* al., 2014 studied some parameters such as morphological, microscopical, physicochemical evaluation, florescence analysis; preliminary phytochemical analysis, thin layer chromatographic study and antimicrobial potential of alcoholic extract of *P. grandiflora* were carried out. Macroscopically the leaves are fleshy leaves, watery, needle shape. Flowers are racemes form; fruits are ovoid with small black colored seed. Chemomicroscopy revealed the presence of Rubiaceous stomata in leaf; Rosette calcium oxalate crystals and protoplast in mesophyll of leaf, cortex and pith of stem and root; pink colored cuticle of stem, collateral vascular bundle with lignified xylem, abundant of starch grains and mucilaginous cells in all aerial parts. Physicochemical evaluation used to determined standards showed a results with total ash, acid insoluble ash, water soluble ash, sulphated ash, ethanol soluble extractive, water soluble extractive, moisture content, swelling index and total crude fiber content in powder of stem. Preliminary phytochemical analysis revealed the presence of alkaloid, steroids, Triterpenoids, flavonoid, tannins, and carbohydrates. The total flavonoid content in alcoholic extract was found .The alcoholic extract of herbs showed significant inhibition of microorganisms.

Silvia Netala *et al*., 2014 compared the structural features and physicochemical properties of three species of *Portulaca.* Different Parts of *Portulaca* were examined for macroscopical, microscopical characters. Physicochemical, phytochemical and fluorescence were also analysed. The plants are succulent, prostrate herbs. Usually roots at the nodes of the stem. Leaves are opposite with paracytic stomata and characteristic Kranz tissue found in C-4 plants. Abundant calcium oxalate crystals are present in all vegetative parts of the plant. Quantitative determinations like stomatal number, stomatal index and vein islet number were performed on leaf tissue. Qualitative phytochemical screening revealed the presence of alkaloids, carbohydrates, saponins, steroids and triterpenoids.

InSun Kim, 2014 studied the vegetative and reproductive morphology and anatomy of two Hawaiian endemic *Portulaca* species were examined. Specifically, *P. molokiniensis* and *P. sclerocarpa* were compared to closely related species in the genus. The comparisons were both qualitative and quantitative, using characteristics of leaves, stems, roots, and fruits. Tissue organizations of vegetative and reproductive parts of the plants were assessed using microtechnique procedures, statistical analysis, and scanning electron microscopy. The most notable features of these two species were (1) the size and frequency of stomata in *P. molokiniensis*, and (2) the large number of sclerenchymatous cell layers in the thickest fruit walls of *P. sclerocarpa*. These findings may imply that stomata development in *P. molokiniensis* and thick fruit wall development in *P. sclerocarpa* are evolved features of survival.

Okafor I. A. and Ezejindu D. N. 2014 investigated its phytonutrients. The aerial parts of *Portulaca oleracea* were harvested, air dried and powdered for this study. Chemical tests were carried out on the aqueous extract and the powdered specimen to determine the phytoconstituents. The presence of Alkaloid, saponin, tannin, flavonoid, cardiac glycoside,

terpenoid, steroid, phobatannin, protein and starch were accessed qualitatively while flavonoid, tannin alkaloid and saponin were determined quantitatively and it was found not to contain steroid and phobatanin but containing 32% of saponin as its highest constituent with 26% alkaloid.

Phytochemical screening on bark extract of *Albizia lebbeck* revealed the presence of tannins, phenols, steroids, triterpinoids, Saponins, anthroquinones and phlobotannins (Kumar *et al.*, 2014).

Paula de Oliveira Amorim *et al*., 2014 studied the isolation and structural determination of four new compounds: one acrylamide and three new phaeophytins together with twelve known compounds, including four phaeophytins. The structures of the compounds were established on the basis of 1D and 2D NMR, IR, HRESI-MS spectra, including GC–MS, and HPLC–UV analysis, as well as comparisons with the literature data. The CD spectra data analysis were used to define the absolute configuration of phaeophytins.

Sohail Ahmad *et* al., 2014 studied the crude and numerous fractions of leaves, stem, and roots of the plant were investigated for phytochemical analysis and DPPH radical scavenging activity. Phytochemical analysis of crude and fractions of the plant revealed the presence of alkaloids, saponins, tannins, steroids, terpenoids, flavonoids, glycosides, and phenols. The antioxidant activity of various extracts was resolute against DPPH radical with the avail of UV at 517 nm. The stock solution (1000mg/mL) and then several dilutions of the crude and fractions were prepared. Ascorbic acid was used as a standard. The plant leaves (52.59 ± 0.84 to 90.74 ± 1.00), stem (50.19 ± 0.92 to 89.42 ± 1.10), and roots extracts (49.19 ± 0.52 to 90.01 ± 1.02) divulged magnificent antioxidant activities. For the ascertainment of the fatty acid constituents a gas chromatograph hyphenated to mass spectrometer was used. The essential fatty acids for

growth maintenance such as linoleic acid (65.70%), eicosadienoic acid (15.12%), oleic acid (8.72%), and palmitic acid (8.14%) were found in high percentage.

Chaitanya MVNL *et al*., 2015 investigated the microscopical, Phytochemical and *In-vitro* antioxidant studies of hydro alcohol, Aqueous, Ethyl acetate, Chloroform, Pet - ether and total saponin fractions of aerial parts of *Cestrum aurantiacum* and *Solanum mauritianum*. In this study, they founded that the selected plants posses' good amount of phenolics, alkaloids, flavanoids and saponins, among all the fractions the total saponin fractions showed better antioxidant activity in compare to other fractions ,The fractions also showed a good *In-vitro* cytotoxic activity on MCF-7 cell lines and moderate against HCT-116 cell lines in comparison to standard Salvicine.

Yan-Xi Zhou *et al.,*2015 isolated the compounds from *Portulaca oleracea*, such as flavonoids, alkaloids, polysaccharides, fatty acids, terpenoids, sterols, proteins vitamins and minerals. *Portulaca oleracea* possesses a wide spectrum of pharmacological properties such as neuroprotective, antimicrobial, antidiabetic, antioxidant, anti-inflammatory, antiulcerogenic, and anticancer activities. However, few molecular mechanisms of action are known.

Vidhya and Uma vandhana, 2016 reported the phytochemical analysis and GC-MS analysis of *Cleome viscosa* leaf parts and the presence of various metabolites including alkaloids, flavanoids, glycosides, tannis, saponins, steroids, terpenoides and phenolic compounds was analysed. The GC-MS results were noticed in the methanolic extracts of *Cleome viscosa*. The compounds were identified by comparing their retention time and peak area with that literature and by interpretation of mass spectra. The plant *Cleome viscosa* was used in various industries and its application includes wound healing, antioxidant, anti-inflammatory, antimicrobial and cancer preventive.

Sethupandian Geetha *et al*., 2016 carried out the qualitative phytochemical screening of *C. religiosa* leaves and stem were studied. Five solvents such as aqueous, methanol, ethyl acetate, chloroform and acetone were used to obtain extracts from powdered plant parts. The extracts were subjected to qualitative phytochemical screening using standard procedures. Results showed that the nine phytochemicals such as alkaloids, flavonoids, phenols, tannis, steroids, terpenoids, coumarins, quinines and saponins were found. However, catachin was absent in leaves and stem extracts. The diversity of photochemical present suggests that *C. religiosa* leaves and stem could serve as a source of useful drugs.

Sushmita Negi, 2018, studied the quantitative phytochemical analysis of *Portulaca oleracea* was carried for four main parameters. In addition to this total protein estimation was also carried. The plant samples were collected from garden area which was unpolluted site, and roadside area facing air pollution generated from traffic exhaust. Plant samples from polluted site also faced nutrient stress, water stress and stress of extreme temperature. Phytochemical analysis was carried separately for leaf and stem samples. Their percent values were found to be relatively high in samples collected from polluted site than the samples collected from unpolluted site. It shows that *Portulaca oleracea* has potential to grow in wasteland under nutrient and water stress conditions on one hand and at the same time the phytoconstituents values remain relatively high under stress.

Trupti P Durgawale *et* al., 2018 analysed the two plant species *Portulaca oleracea* and *Portulaca quadrifida*. Pharmacological investigations have revealed the importance of these plants as sources of antioxidants, essential fatty acids, and even antimicrobial agents.

Hemalatha K and Abirami P. 2018 studied the physicochemical and preliminary phytochemical analysis of *Talinum portulacifolium* leaf. Five different solvents like Petroleum

ether, Chloroform, Methanol, Ethanol, Aqueous were used to obtain the extract. These extracts were subjected for physicochemical and qualitative phytochemical analysis by using the standard procedures. Physico chemical parameters like moisture content, total ash, water soluble ash and sulphated ash values were calculated. Alkaloids, flavonoids, glycosides and phenols are present in the all the extracts. These bioactive compounds obtained from the phytochemical analysis may be the responsible for the pharmaceutical activity.

Maryam Sharif Shoushtari *et al*., 2018 investigated the phytochemical profiles for two models of aqueous (Aq) and methanolic (Me) pollen extracts of *F. excelsior* from three pollination periods from hermaphrodite flowers (H) of polygamous and male flowers of pure male (M) in order to identify their constituent compounds. There was a significant difference ($P < 0.001$) between the means of TPC for M and TFC for the H. Comparison of H and M antioxidant activities showed that DPPH (IC50) to be (2.977 ± 0.117 μM) during the second pollination period of M and (4.877 ± 0.021 μM) for first period of H. The majority of the compounds identified were linalool (35.42%) from the monoterpenoides in H and Delta-cadinene (43.22%) belonging to the sesquiterpenes in M. They concluded that there is a significant difference between the H and M compounds in pollen at different periods.

Deepak Kumar et al., 2018 studied anatomical and morphological features of different vegetative organs and reproductive organs were studied along with medicinal importance of the plant. *Portulaca oleracea*. In the morphological study it was observed that the plant have sessile leaves which were ovate, smooth, succulent, arranged in opposite manner, stem was aerial, weak and cylindrical, root consists of a long thick taproot as well as many fibrous lateral roots, flowers were single in leaf axils, fruit consists of round to egg-shaped capsules with glossy brown and black seeds. In anatomical studies, cross sections of the leaf, stem and root were examined.

Purslane has better nutritional quality than the major cultivated vegetables with higher beta-carotene, ascorbic acid, and alpha-linolenic acid along with high nutritive and antioxidant properties.

Nassima Boutaoui *et al*., 2018 evaluated the metabolite recovery from different extraction methods applied to *Thymus algeriensis* aerial parts. The experimental results show that microwave-assisted aqueous extraction for 15 min at 100 _C gave the most phenolics-enriched extract, reducing extraction time without degradation effects on bioactives. Sixteen compounds were identified in this extract, 11 phenolic compounds and five flavonoids, all known for their biological activities. Color analysis and determination of chlorophylls and carotenoids implemented the knowledge of the chemical profile of this plant.

H. R. H. Al-Newani, 2019 investigated the *Portulaca oleracea* is a succulent plant in Portulacaceae family distributed around different regions of Iraq as collected widely in the gardens of Baghdad governorate. The results of this study shown that a systematic significant of morphological and anatomical data. purslane with branched shoot stems. Stems and leaves are glabrous and leaves are alternate, the petiole is absent. Inflorescences were viewed as clustered in the form of small one carrying many male and female flowers as the inflorescences take the form of long stemmed. Anatomical techniques revealed two patterns of stomatal complex, paracytic which is the most common followed by tetracytic is limited distributed type and it is recorded for the first time in this species. Druses crystals have been found distributed in the stem with angular collenchyma alternating with xylem parenchyma cells with large intercellular space. As well as, root anatomy has been done and the results showed casperian strips cells clearly in section with xylem and phloem region

Rohita Singla and Saroj Kumar Pradhan, 2019, analysed the phenols, flavonoids content and antioxidant activity of common weeds growing around agriculture fieldsof Punjab plains. Maximum content of phenolics were reported in *Ageratum conyzoides* (flower, 9.51±0.00 mg CA/g DW), *Launaea procumbens* (stem, 7.94±0.01 mg CA/g DW), *Ranunculus muricatus* (flower, 7.15±0.07 mg CA/g DW) and *Sonchus asper* (flower, 8.12±0.34 mg CA/g DW). The flavonoid content was measured high in case of *Silybum marianum* (stem, 4.83±0.00 mg Q/g DW), *Ranunculus muricatus* (leaves, 2.96±0.01 mg Q/g DW), *Solanum nigrum* (leaves, 2.45±0.03 mg Q/g DW) and *Ageratum conyzoides* (leaves, 2.15±0.01 mg Q/g DW). All the species of weeds having high phenol and flavonoid content, also have strong antioxidant potential in terms of DPPH radical scavenging activity and total antioxidant capacity.

Ahmed, M.*et* al.,2019 examined the total phenolic, flavonoids content and antioxidant activities of *Citrullus colocynthis* L. and *Cannabis sativa* L. Phytoconstituents except terpenoids from *C. sativa* and *C. colocynthis* leaves were reported while, in contrast, steroids, tannins and phenols were absent in *C. colocynthis* roots. The methanol derived maximum phenolic contents from *C. sativa* and *C. colocynthis* leaves were 36.42 and 37.69 mg gallic acid equivalent GAE/g respectively. However, total flavonoids registered from *C. sativa* leaves and *C. colocynthis* leaves and roots were 59.03, 50.58 and 43.32 mg quercetin equivalent QE/g respectively. Interestingly, *C. colocynthis* leaves produced the highest flavonoids 119.63 mg QE/g using ethyl acetate extract. DPPH inhibition (%) was high in acetone 55.57, hexane 45.98 and distilled water 35.5% from *C. sativa*, *C. colocynthis* leaves and roots respectively. Our findings suggest that studied plants contain phytochemicals, reasonable quantity of phenol and flavonoids content confer to the potential antioxidant activity responsible for insecticidal properties as safer alternatives of synthetic pesticides.

Pranabesh Ghosh *et* al., 2019 studied five common medicinal herbaceous weeds of West Bengal, and India namely; *Heliotropium indicum*, *Tridax procumbens*, *Cleome rutidosperma*, *Commelina benghalensis,* and *Euphorbia hirta* have been chosen from five different families describe their phytochemical, and anti-oxidant properties. This investigations have concluded that *Euphorbia hirta* possesses a significant amount of phytochemicals, and it exhibits the highest anti-oxidant activities in comparison with the other four medicinal weeds. In *Euphorbia hirta* leaves acetone extract highest amount of phytochemicals were detected by qualitative assays.

R.R.Saswade . 2019 studied the qualitatively preliminary analysis of some different weed species. Discovery of active compounds and their role in curing diseases from this plant leads its importance. The presence of these secondary bioactive phytochemicals signifies the importance of these medicinal plants as an efficient source of therapeutic agent.

Muhammad Aslam Tahir and Muhammad. S. A. Abbasi, 2020 studied the two members of Portulaca Family namely, *Portulaca pilosa* and *Portulaca quadrifida Linn*. Dried twigs of *Portulaca pilosa* and *Portulaca quadrifida* ground to fine powder and then analyzed using FTIR technique. Functional groups of phytochemicals were identified through FTIR spectral lines. Appropriate correlations of absorption peaks to medicinal compounds have been discussed. As a result, both herbs are found to be rich source of bioactive compounds like alkaloids, flavonoids, fatty acids, tannins, triterpenoids, amino acids and saponins.

MATERIALS AND METHODS

MATERIALS AND METHODS:

(a) *Portulaca grandiflora* Hook.

Common Name : Sun plant, Rose moss, Moss-rose purslane

Class : Dicotyledons

Subclass : Caryophyllidae

Order : Caryophyllales

Family : Portulacaceae

(b) *Portulaca umbraticola* Hook.

Common Name : Crownpod Purslane, Wingpod Purslane

Class : Dicotyledons

Subclass : Caryophyllidae

Order : Caryophyllales

Family : Portulacaceae

Selected materials are a succulent flowering plant in the family Portulacaceae, native to Argentina, southern Brazil, and Uruguay and often cultivated in gardens. Both taxa are small, but fast-growing plant.

METHODS

Collection and processing of the plant materials

Portulaca grandiflora and *Portulaca umbraticola* were collected from home garden Thoothukudi. The Leaf and stem of *Portulaca grandiflora* and *Portulaca umbraticola* were cut into pieces, sun dried to reduce the moisture level. After the completion of drying, the plant material was pulverized to get coarser powder material, which was stored in air tight plastic container. About 10 g of each fine powdered sample was weighed and separately soaked in 100

ml of Methanol and distilled water. These were allowed to stand for 24 hours at room temperature. All the extracts were filtered through Whatman No. 41 filter paper and the filtrate were used for further analysis.

Preliminary Phytochemical Screening: **Qualitative analysis:**

Tannins (Braymer's Test)

To 1 ml of the leaf extract was mixed with 2 ml of water. To these 2 drops of 5% ferric chloride solution was added. Appearance of dirty green precipitate indicated the presence of tannins (Harbone, 1973)

Saponins (Foam Test)

1 ml of leaf extracts was mixed with 5 ml of distilled water and shaken and observed for the formation of froth, which is stable for 15 min for a positive result (Harbone, 1973)

Flavonoids

To 1 ml of the leaf extract was mixed with 1 ml of sulphuric acid. Appearance of orange colour indicates the presence of flavonoids (Kokate, 2000)

Coumarins

To 2 ml of the leaf extract was mixed with 3 ml of 10% sodium hydroxide. Appearance of yellow colouration indicated the presence of coumarins (Yadav *et al*., 2014)

Terpenoids

To 2 ml of the leaf extract was mixed with 2 ml of acetic acid. To these 2 drops of conc. sulphuric acid. Deep red colour development showed the presence of terpenoids (Yadav *et al*., 2014)

Quinones

To 1 ml of the leaf extract was mixed with 5 ml of HCL. Appearance of yellow colour precipitate indicates the presence of quinones (Kokate, 2000)

Test for Alkaloids

Wagner's test

A fraction of extract was treated with Wagner"s reagent (1.27 g of iodine and 2 g of potassium iodide in 100 mL water) and observed for the formation of reddish brown colour precipitate. There was a formation of reddish brown colour confirming the presence of alkaloid.

Steroids : (Salkowski test)

To 1 ml of the leaf extract, with 2 ml of water. To these 2 drops of 5% ferric chloride solution was added followed by conc. sulphuric acid. Formation of reddish brown ring at the junction showed the presence of steroids (Yadav *et al*., 2014).

Test for Carbohydrate

Fehling's test

5 ml of Fehling"s solution was added to 0.5 mg of extract and boiled in a water bath. The formation of yellow or red precipitate indicates the presence of reducing sugars. (Harbone, 1973)

Test for Glycosides

0.5 mg of extract was dissolved in 1 ml of water and then aqueous NaoH solution was added. Formation of yellow colour indicates the presence of glycosides.

Test for Protein

Ninhydrin test:

0.5 mg of extract was taken and 2 drops of freshly prepared 0.2% ninhydrin reagent was added and heated. The appearance of pink or purple colour indicates the presence of , peptides or amino acids. (Harbone, 1973)

Test for phenol

To 1 ml of the extract, 2 ml of distilled water was added and followed by few drops of 10% aqueous ferric chloride. Appearance of blue or green colour indicates the presence of phenols. (Harbone, 1973)

PHYTOCHEMICAL QUANTITATIVE ESTIMATION

Total Soluble Protein (Lowry *et.al*.,1951)

Requirements

- Alkaline copper reagent
- Solution A- 20% Sodium carbonate in 0.1 N sodium hydroxide
- Solution B- 1% Sodium potassium tartarate
- Solution C- 0.5% copper sulphate

To prepare 100 ml alkaline copper reagent, 98 ml of solution A, 1ml of B and 1 ml of C were mixed together freshly. Folin-ciacalteau reagent (commercial reagent was diluted in the ratio of 1:1 with distilled water at the time of use).

Procedure

100 mg of sample was homogenized in 10 ml of distilled water and filtered through a muslin cloth and centrifuged at 3000 rpm for 10 minutes. To the supernatant 10% trichloro acetic acid (TCA) was added in 1:1 ratio and left in an ice bath for 30 minutes to precipitate protein. Then centrifuged at 3000 rpm for 5 minutes and discarded the supernatant. The precipitate was dissolved in 0.1N sodium hydroxide and diluted to a known volume.

To 0.5 ml of protein extract, 5 ml of alkaline copper reagent was added. After thorough mixing 0.5 ml of folinciocalteau reagent was added and allowed to stand for 30 minutes; the blue colour appeared and absorbance was measured at 650 nm using UV visible spectrophotometer (Model No: UV 2371). The analysis was performed in triplicates and the amount of protein was calculated and expressed as mg/g DW. Bovine serum albumin (BSA) was used as standard.

Estimation of Carbohydrate

The total carbohydrate content was determined according to Dubosis *et al*.,(1956). An aliquot of 1ml sample (1mg/ml) was mixed with 5ml of 5% phenols and conc. Sulfuric acid. Tubes were vortexed and incubated for 15 minutes at boiling water bath. After cooling to room temperature and the absorbance was measured against blank at 490nm using UV-VIS spectrophotometer.

Total flavonoid (Zhinshen *et al.,* 1999)

Reagent

- 5% Sodium nitrate
- 10% Aluminum chloride
- 1N Sodium hydroxide
- Quercetin standard

Procedure

100 mg of sample was homogenized with 10 ml of distilled water and filtered through muslin cloth. 0.5 ml of extract was added with 2.5 ml distilled water and mixed . After 6 minutes, 0.15 ml NaNO was added of and again after 6 minutes, 0.3 ml of 10% AlCl3 was added. After 5 minutes 1 ml of 1M NaNH and 0.5% ml of water were added . Following thorough mixing of the solution, absorbance against blank was determined at 510 nm. Quercetin was used as standard and the results were expressed as mg quercetin equivalents (QE)/g dry weight

Vitamin-C (Ascorbic acid) – Baker and Frank, (1968)

Reagent

- 5% of TCA
- Indophenol reagent (20 mg of dichlorophenol indophenol was dissolved in 10 ml of warm distilled water).
- 20 mg of dichlorophenol indophenols ws dissolved in 10ml of warm distilled water
- DT reagent (2 g of 2, 4 dinitrophenyl hydrazine and 1 g of thiourea were dissolved in 100 ml of 9 N sulphuric acid).
- 85% sulphuric acd
- L – ascorbic acid – standard

100 mg of each sample was homogenized with 10 ml of 5% trichloro acetic acid (TCA). The homogenate was centrifuged at 3000 rpm. To 2 ml of protein free supernatant, 1 drop of indophenol reagent and 0.5 ml of DT reagent were added and incubated at 10°C for 1 hour. Then cooled in ice bath and 2.5 ml of 85% sulphuric acid was added. After intermittent shaking for 30 minutes (until red colour appeared), 30 absorbance was measured at 540 nm. L-ascorbic acid was used as standard and the results were expressed as mg/g DW.

Vitamin E (Tocopherol) (Rosenberg, 1992)

The sample (2.5 g) was homogenized in 50 ml of 0.1 N sulphuric acids and allowed to stand overnight. The content in the flask was shaken vigorously and filtered through Whatman No.1 filter paper. Aliquots of the filtrate was used for estimation. Into stoppered centrifuge tubes, 3 ml of extract and 3 ml of water were pipetted out separately. To both the tubes, 3 ml of ethanol and 3 ml of xylene were added, mixed well and centrifuged. Xylene (2.0 ml) layer was transferred into another stoppered tube. To each tube, 2.0 ml of dipyridyl reagent was added and mixed well. The mixture (3 ml) was pipetted out into a cuvette and the extinction was read at 460 nm. Ferric chloride solution (0.66 ml) was added to all the tubes and mixed well. The red colour developed was read exactly after 15 minutes at 520 nm.

ANTIOXIDANT ACTIVITY

Crude samples extracts were prepared by pouring 100ml of disttiled water in a conical flask containing 10g of each samples separately in the ratio of 10:1 (V/W). after 24 hours, the mixture was filtrated through whatman no:1 filter paper and the filtrate was evaporated to dryness. Crude (aqueous) extracts of all samples (1mg/ml) were used for the determination of free radical scavenging activity.

Free radical scavenging assay (Hatano et al., 1998).

Free radical scavenging assay was measured by 2-2 Diphenyl, 1-picryl hydrazine (DPPH) method proposed by with slight modifications. 1ml of aliqut of test sample was added to 3ml of 0.004% DPPH solution prepared in methanol. The mixture was vortexed for 1min and kept at room temperature for 30 minutes in darkness the absorbance was read at 517 nm. Allow absorbance of the reaction mixture indicated a high free radical scavenging activity. Ascorbic acid was used as standard.

DPPH scavenging activity(%)

A control –A test / A control * 100 Where, A control is the absorbance of the DPPH solution without test solution. A test is the absorbance of DPPH with the test solution. Aqueous extract was used as blank.

FT-IR analysis A little powder of plant specimen was mixed with KBr salt, using a mortar and pestle, and compressed into a thin pellet. Infra -red spectra were recorded as KBr pellets on a Thermo Scientific NicotiS5ID1 transmission, between 4000-400 cm-1.

RESULT AND DISCUSSION

RESULT AND DISCUSSION

Plants are very rich and useful source of primary and secondary metabolites like proteins, lipids, carbohydrates, alkaloids, flavonoids, terpenoids, tannins, etc. These metabolites are useful for the human being for the treatment of various illnesses. Determination of phytochemical profile of plants is an indication of the class of compounds present in the plant. Various pharmacological activities are expressed by medicinal plants based on the type and common of secondary metabolites (Jegede *et al.,* 2011)

The morphological characters of the studied taxa are presented in (Table1). Both taxa were ornamental perennial herb. In *P.grandiflora* stem are whitish herbaceous, prostrate, cylindrical thick and succulent with scarcious or hairy stipular appendage. Leaves are needle like, simple alternate one leaf per node along the stem, small group at the end of the branched. In *P.umbraticola* stem reddish herbaceous prostrate, cylindrical, thick, full and luscious. It is completely hairless and succulent. Leaves are simple alternate along the stem flat, ovate and succulent. They are sus sessile. The lamina is obovate, thick and succulent 3-4 cm long The margin is entire smooth and both sides are glabrous.

In *P.grandiflora* the flowers are solitary, axillary, bisexual, small groups at the end of branches, but flowers only flourish one after another and comes in various colours. Flowers bracteates, hermaphrodite, actinomorphic, showy, complete and hypogynous. Life span of flower is one day they are opening during day time. Subconnate at the base, sepals 2, green in colour persistant, united at the base. Petals many, obovate with entire margin. Stamens 8-9 in one whorl, adnate to petals inserted on calyx, filaments basally connate, about 2.5-6 mm long, anthers 2-4

locular, dorsifixed. Ovary semi inferior, unilocular, ovules 4-many on free central placentation, style 5 fid, about 8-13 mm long, stigmas 5, linear, about 2.5-3 mm long.

In *P.umbriticola* the flowers axillary, solitary or small groups at the end of branches, but flowers only flourish one after another. The flowers are sessile, yellow 2-3 cm wide. Life span of flower is one day they are opening during day time. Flowers bracteates, hermaphrodite, actinomorphic, showy, complete and hypogynous. The calyx consists of 2 sepals broad-based welded to the ovary and upper free. The corolla has 5 petals bilobed to trilobed at the top 6-12 stamens, filament 4mm long. The ovary is surrounded by a style divided in to 4-6 stigmas linear ciliate. The quantitative macromorphological characters were measured and summarized inTable2. Morphological characters were taxonomically significant for taxa identification and delimitation in Portulacaceae. This result agreed with that of Anhar et. al.2018.

QUALITATIVE PHYTOCHEMICAL SCREENING

The term 'phytochemical' is reserved for those plant chemicals that have a beneficial effect on human-health but are not essential from the point of view of nutrition. A medicinal herb is considered to be a chemical factory as it contains a multitude of chemical compounds like alkaloids, glycosides, Saponins, resins, oleoresins, sesquiterpene lactones and oils (essential and fixed). Antifungal activity of medicinal plants is mainly due to the presence of Phytochemicals like alkaloids, glycosides, phenols, tannins and flavonoid (Sarojini *et al*., 2011). Moreover, phytochemical screening of the drug is significant for proper identification, which further exerts importance on therapeutic activity of the medicinal plant.

The current study was attempted to find out the presence of preliminary Phytochemicals aqueous solution of leaf and stem of selected medicinal plants such as *Portulaca grandiflora* and *Portulaca umbraticola.* Presence or absence of certain important compounds in an extract is

determined by colour reactions of the compounds with specific chemicals which act as dyes. This procedure is a simple preliminary pre-requisite before going for detailed phytochemical investigation. The results were presented in **Table 3.** Flavonoids have been reported to possess many useful properties, including anti-inflammatory, oestrogenic, enzyme inhibition, antimicrobial, anti-allergic, antioxidant, vascular and cytotoxic antitumour activities (Havsteen, 1990). Alkaloids exhibit anti-hyperglycemic and anti-inflammatory properties, alongwith anaesthetic and analgesic properties. Terpenoids are attributed for analgesic and anti-inflammatory activities (Santhi *et al.,* 2011). Coumarins are potential antioxidants (Tseng, 1999).

The result of preliminary phytochemical screening indicated that tannins, saponin, phenol and steroids are totally absent in both the species. Alkaloids were present in all the samples. Leaf sample of *P. grandiflora* contain flavonoids, glycosides, terpenoids and Quinonesones in both aqueous and Methanol extracts. Leaf sample of *P.umbraticola* contain glycosides in aqueous extract anthraquinone is present in stem extract.

Quantitative Analysis

Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties (Kumar *et al*.2009). Many herbaceous and medicinal plant contain important phytochemicals and vitamins such as alkaloids, flavonoids, tannins, cyanogenic glycosides, phenolic compounds, saponins, lignins, vitamin-C, vitamin-E and carotenoids, which are utilized both by humans and animals as important components of diets (Hussain *et al*.,2011).

Protein level is maximum in all the samples. Carbohydrates are very less. The antioxidant activity such as flavonoids, vitamin C and vitamin E were analysed in leaf and stem of selected species of Portulacaceae. The results were presented in Table 4.

Vitamin C is a vital component in human diet. Vitamin C is a non-enzymatic, water soluble antioxidant Vitamin C functions in enzyme activation, oxidative stress reduction and immune function. It protects against respiratory tract infection and reduces risk for cardiovascular disease and some cancer (Ueta *et al*., 2003).

Vitamin E is a fat-soluble nutrient found in many foods (Jacob, 1995). In the body, it acts as an antioxidant, helping to protect cells from the damage caused by free radicals. Free radicals are compounds formed when our bodies convert the food we eat into energy.

The results of anti – oxidant DPPH scavenging activity shows more percentage in stem than leaf of P.umbraticola. In *Portulaca pilosa* leaf shows maximum than stem.The result is present in Table 5.

The FTIR spectrum was used to identify the functional group of the active components based on the peak value in the region of infrared radiation. The results of FTIR peak values and functional groups were represented in Table 6-9. The FTIR spectrum profile was illustrated in the Fig.1-4. FTIR spectrum confirmed the presence of alcohols, phenols, alkanes, alkynes, alkyl halides, aldehydes, carboxylic acids, aromatics, nitro compounds and amines in different extracts.

Table1: Morphological characters of selected species of Portulacaceae

Characters		*P.grandiflora*	*P.umbraticola*
Stem	Strength	Prostrate	Prostrate
	Colour	Whitish	Reddish
	Texture	Smooth	Smooth
Leaf	Arrangement	Alternate	Alternate
	Composition	Simple	Simple
	Shape	Needle	Obovate
	Margin	Entire	Entire
	Base	Round	Round
Flower	Position	Axillary	Axillary
	Calyx colour	Green	Green
	Calyx Type	persistant, united at the base	Broad based welded to the ovary
	Corolla colour	Various colour	Yellow
	Corolla Shape	obovate with entire margin	obovate with bi or tri lobed
	Stamen number	8-9	6-12
	Stigma	5	4-6

Table2: Quantitative macromorphological measurements of studied taxa

Plant Parts	Size	
	P.grandiflora	*P.umbraticola*
Shoot length	13.47±4.64209 Cm	15.06±4.510285
Shoot width	1.4±0.362093 Cm	0.91±0.191195
Nodal space	0.15±0.152388 Cm	0.9±0.309121
Leaf length	4.15±0.62388 Cm	3.15±0.52388 Cm
Leaf width	0.96±0.084327 Cm	1.65±0.310813
Leaf area	0.611±0.276223 Cm	1.215±0.340857
Calyx length	0.711±0.276223 Cm	1.611±0.476223 Cm
Corolla length	2.611±0.376223 Cm	3.611±0.176223 Cm

Table 3 Priliminary phytochemical screening of selected plant species of Portulacaceae

TEST	*Portulaca grandiflora*				*Portulaca umbraticola*			
	Leaf		Stem		Leaf		Stem	
	Aqueous	Methanol	Aqueous	Methanol	Aqueous	Methanol	Aqueous	Methanol
Tannins	–	–	–	–	–	–	–	–
Saponins	–	–	–	–	–	–	–	+
Flavanoids	+	+	–	+	–	–	–	+
Alkaloids	+	+	+	+	+	+	+	+
Glycosides	+	+	–	–	+	+	–	–
Phenol	–	–	–	–	–	–	–	–
Steroids	–	–	–	–	–	–	–	–
Carbohydrates	–	+	–	+	–	–	–	+
Protein	–	–	+	–	+	–	–	–
Coumarins	–	–	–	–	–	+	–	–
Terpenoids	+	+	–	–	+	–	–	–
Betacyanin	+	–	+	–	+	–	–	+
Quinones	+	+	–	–	–	–	–	–
Anthraquinone	–	+	–	+	–	+	+	+

Table 4 Quantitative analysis of phytochemical from selected species of portulacaceae

Qualitative analysis	*P.grandiflora* Leaf/100g	*P.grandiflora* Stem/100g	*P.umbraticola* leaf/100g	*P.umbraticola* Stem/100g
Protein	43.27	63.38	37.29	57.40
Carbohydrate	0.0043	0.0116	0.0043	0.007
Flavanoid	0.0022	0.0022	0.0038	0.0051
Vitamin -C	0.033	0.033	0.024	0.048
Vitamin -E	0.308	0.147	0.364	0.317

Table 5 Antioxidant of free radical scavenging DPPH activity of selected species of Portulacaceae

Portulaca grandiflora		*Portulaca umbraticola*	
Leaf	Stem	Leaf	Stem
66.2%	73.3%	16.3	63.4

Table 6: Functional group of *P. grandiflora* leaf

Peak value	Functional group
517.85	C-Br
617.18	C-Cl
689.5	C-Br
780.15	C-Cl
1082.96	C-O-C stretch
1163.96	C-OH stretch
1317.29	NO_2 stretch
1338.51	NO_2 stretch
1379.01	CH_3 bend
1511.12	C=O amide
1641.27	C=C alkene
1679.88	C=C alkene
1747.39	C=O ester
2311.53	C≡N stretch
2885.31	-C-H aldehydic
3502.49	N-H stretch
3734.9	water OH Stretch

Table 7: Functional group of *P. grandiflora* Stem

Peak value	Functional group
517.85	C-Br
592.11	C-Br
653.82	C-Cl
689.5	C-Cl
1085.85	C-O-C stretch
1163	C-OH stretch
1338.51	NO_2 stretch
1393.47	NO_2 stretch
1510.16	C=O amide
1548.73	C=O amide
1687.6	C=O amide
1712.67	C=O amide
1747.39	C=O ester
2360.71	C≡N stretch
2885.31	- C-H stretch
3565.17	alcohol OH stretch

Table 8: Functional group of *P. umbraticola* leaf

Peak value	Functional group
456.13	C-I
517.85	C-Br
548.71	C-Br
592.11	C-Br
653.82	C-Cl
689.5	C-Br
966.27	C-F
1082.96	C-O-C stretch
1120.56	C-O-C stretch
1163.96	C-O-C stretch
1316.33	NO_2 stretch
1338.51	NO_2 stretch
1392.51	NO_2 stretch
1431.08	C=C aromatic
1454.23	CH_3 bond
1510.16	C=O amide
1604.66	C=C alkene
1680.85	C=O amide
1747.39	C=O ester

Table 9: Functional group of *P. umbraticola* stem

Peak value	Functional group
517.85	C-Br
592.11	C-Br
640.32	C-Cl
690.47	C-Cl
780.15	C-Cl
1081.99	C-F
1163	C-F
1316.33	NO_2 stretch
1338.51	NO_2 stretch
1394.44	NO_2 stretch
1510.16	NO_2 stretch
1604.66	C=C alkene
1686.63	C=O amide
1747.39	C=O ester
2311.53	C≡N stretch
2885.31	-C-H aldehydic
2976.92	carboxylic acid OH stretch
3545.88	alcohol OH stretch

SUMMARY AND CONCLUSION

SUMMARY AND CONCLUSION

There is a growing body of evidence indicating the benefits of medicinal plants for their use against pathogenic microorganisms. Plant-based remedies used in human and animal medicine are an essential part of the primary health care system in many countries. Extensive screening programs of plants used mainly in traditional medicine have resulted in the discovery of thousands of Phytochemicals with inhibitory effects on different types of diseases. The leaf and stem of *P.grandiflora* and *P umbraticola* were collected from home garden, Thoothukudi, Tamilnadu for the current study. Morphological, Preliminary Phytochemicals like alkaloids, phenol, flavones, steroids, tannins, Coumarins, and quinines are quantitatively screened.

Morphologicaly they are similar we can differenciate this plant only by the leaf and flower. Leaves are needle in *P.grandiflora* and obovate in *P. umbraticola*. Petals are numerous in *P.grantiflora* petals are 5 in *P. umbraticola*.

The result of preliminary phytochemical screening indicated that leaf and stem of both species. Both plants were free from steroids. Moreover, quantitative estimation of phytochemical also exhibited that leaf and stem of both species. The plants rich source of secondary metabolites with interesting biological activities in general these secondary metabolities are an important source with a variety of structural arrangements and properties. They have rich source of protein and have high antioxidant scavenging activity. Therapeutic value also rich. Therefore further efficacy and safety studies are encouraged on this potential herb with the hope of replacing some less effective ones in clinical practice especially for antidiabetic and anticancer.

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EXTRACTON OF FABRIC DYES FROM BROWN SEAWEEDS AND THEIR PROPERTIES

A dissertation submitted to

St. Mary's College (Autonomous), Thoothukudi.

Affiliated to

MANONMANIAM SUNDARANAR UNIVERSITY, Tirunelveli.

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Master of Science in Botany.

By

SOWNDHARYA.N

Reg.No.19APBO14

DEPARTMENT OF BOTANY

ST. MARY'S COLLEGE (Autonomous)

THOOTHUKUDI -628001

2020-2021

CERTIFICATE

This is to certified that this dissertation entitled **'EXTRACTON OF FABRIC DYES FROM BROWN SEAWEEDS AND THEIR PROPERTIES'** submitted by SOWNDHARYA.N, Reg.No.19APBO14 to St. Mary's College (Autonomous) Thoothukudi - 628001 in partial fulfillment for the award of the degree of **'Master of Science in Botany'** is done by her under my supervision. It is further certified that this dissertation or any pare of this has not been submitted elsewhere for any other degree.

C.P 15/4/2021
GUIDE

M. Glory
HEAD OF THE DEPARTMENT

HOD of Botany
St. Mary's College (Autonomous)
Thoothukudi- 628 001.

S.D
EXAMINAR

Lucia Rose
PRINCIPAL

St. Mary's College (Autonomous)
Thoothukudi - 628 001.

DECLARATION

I do here by declare that this dissertation entitled **"EXTRACTON OF FABRIC DYES FROM BROWN SEAWEEDS AND THEIR PROPERTIES"** submitted by me in partial fulfillment for the award of the degree of **'Master of Science in Botany',** in the result of my original and independent work carried out under the guidance of **Dr. Mrs. G. Flora M.Sc., M.Phil., Ph.D.** Assistant Professor. Department of Botany, St. Mary's College (Autonomous) Thoothukudi- 628001 and it has not been submitted elsewhere for the award of any other degree.

Station: Thoothukudi

Date: 15.04.2021

N. SOWNDHARYA

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CONTENTS

\

Introduction

Introduction

Dyes are widely used in a various industries such as food, textile, cosmetics, paper, paint etc. Dyes are a colorful organic compound that absorbs light in visible area and can firmly bind to substrates (fibers, food etc.) by chemical and physical bonding between the dye group and the group present on the substratum.

Dyes, especially natural dyes, have been of unspoken importance in our lives for thousands of years, providing not only aesthetic satisfaction but also other useful applications. The global demands for natural dyes of great interest now a days because the general awareness of the therapeutic properties of natural dyes. Natural dyes are eco-friendly colorants that derived from biological sources (such as plants, animals) and minerals.

A variety of natural dyes are obtained from different plant parts examples: roots, barks, leaves, fruit, seed, flower etc. that produce wide ranges of colour such as red, orange, yellow, green, The oldest and most widely used dye is indigo blue (*Indigofera tinctorum*), which has been used in India for the last four thousand years. (Arora, *et. al.,* 2017)

Rather than indigo, there are so many plants and algae are have ability to wild range of color dyes due to the presents of categorized colorant compounds correspondingly photosynthetic pigments (chlorophylls, carotenes, xanthophylls, phycobilins(Phycocyanin and phycoerythrin), and fucoxanthin) and secondary metabolites (phenolic compounds) like anthro quinones, naphtho quinones, anthocyanin, usnic acid, tannins and vitamins (B compounds, C). (Osório, *et. al*., 2020).

Moreover, the sources used to produce natural dyes especially plants will not produce pollutant unlike the sources used in synthetic dyeing process. The colors of natural dyes consist of pigments and bioactive components that can be enhance the environment and do not have any harmful effect on the other environmental factor. (Moldovan.et. al., 2016)

At the beginning of the twentieth century, the cost of production of synthetic dyes was reduced and those reasons caused the reduction of natural dyes usages. So, there is a drastic decline of the usage of natural dyes was happened then.

In this era, almost all colors which used everywhere in everything are chemically manufactured synthetic dyes. Because of their profitable nature such as more color production, low cost, brighter texture, more color fastness and easily mixed with product. Although these dyes have many beneficial uses, they also can cause side effects in our health and environment. Chemicals which is used in the production of synthetic dyes (Hg, Sn, Cr, Co, $NaCl_2$, Benzene) are toxic and carcinogenic factors that leads to form lethal mutation in any organisms in environment especially humans who exposed to dye in factories and consumed the product which contain these artificial dyes . Wastewater from textile dye industries are released into drinking water resources, which can lead to contamination of surface water, ground water as well as land also. Because of these shortcomings, researchers are considering to develop safer and less polluting dyes. (Khandare, *et. al.,* 2015)

Resent years, researchers show more interest towards, the discovery of various and better natural dyes for replacing the current usage of synthetic dyes which have some lack

of desirable fastness properties on textiles and lack of scientific information on the chemistry of dyeing and standardized dyeing methods. (Çalıs*, et. al.,* 2009)

This study will give the some possibilities for using seaweed as a source of natural dye and which are the conditions or processes will improve the effectiveness of different solvents and mordants on the natural dyes extracted from the three selective brown seaweeds.

Scope and Objectives

Scope and objectives

Now a days, due to the excelling advantages of natural dyes, it's becoming an enticing option over synthetic dyes. They are biodegradable, non-toxic, eco-friendly, and easy extraction by boiling the plant materials. By keep these in mind, the present work is concentrated on extraction of natural dyes from seaweeds.

The major objectives were as follows:

1. Collection of seaweeds from the local region (Hare island-Thoothukudi).
2. Extraction of dyes with different solvents (acetone, ethanol, methanol and water)
3. Application of dyes to the untreated cotton threads which treated with different mordant- uncoated cotton threads
4. To study the effects of different types of mordants on the dyeing process.
5. Observation of fastness properties of natural dyes.

Review of literature

Natural dyes

Recently, overall world took initiative for the using natural dyes which have eco-friendly and biodegradables material to avoid environment pollution and health issues. Geetha *et. al.,* (2013) did research work on the extraction of dyes from plants like *Allium cepa (Skin part), Beta vulgaris(root), Bougainvillea glabra (flower), Brassica oleracea (fruit),* and *Ceasalpinia pulcherima (flower)* then they analyzed the biochemicals in the extracts and displayed the dyeing capacity of dye extracts by using different mordants such as vinegar, alum and cream of tartar. Finally, they concluded that developing the natural dyes will give rural employments, medically useful products (chemo theraphic dyes) and textile products.

The use of natural colorants offers promise in developing antimicrobial textiles for aesthetic, hygienic, and medical applications owing to the presence of potent highly active agents such as tannins, flavonoids, quinines carotenoids, and alkaloids in their extracts. This article presents a concise account of the state-of-the art sustainable technology derived from natural colorants and will be useful to the textile and polymer chemists engaged in development of health care bioactive textiles. (Kadolph, *et. al.,* (2004)).

Disadvantages of synthetic dyes

Textile manufacturing used a wide range of chemicals and most of them are harmful to the environment, to the people working in textile processing and potentially to consumers (Al-Ghouti *et. al., (*2003)). Different levels of toxicity are produced at different

textile processing stages. However, there are limited data available on the biological effects of the treated textiles with these chemicals.

Samanta *et. al.,* (2009) and Ali *et. al.,*(2007) concluded their studies that the textile dyes form a major group of textile chemicals and comprise of over 8,000 different compounds with almost 40,000 commercial names. There are approximately 10,000 different dyes and pigments produced globally in which the production capacity per year is over 700,000 tons. Statistically, during the dyeing process, up to 15% of the dyes and pigments produced are lost in the effluent and released into the environment.

Seaweed resources

Seaweed is one of the first commercially useful marine resources belonging to the first group of non-flowering plants (Thalophyte) which grow submerged in intertidal, shallow sometimes subsurface water up to a depth of 100 m, as well as submerged in saline estuaries. These sea algae grow abundantly on the coasts of Tamil Nadu and Gujarat on the mainland and in the islands of Lakshadweep and Andaman Nicobar. In late 20^{th} century, there are about 700 species of seaweed from different parts of the Indian coast. Of these, about 60 species are of commercial importance and can be used in the production of agar, algin 'and carrageenan, and as raw materials for food, fertilizer and pharmaceuticals. 6,000 tons of agar yielding seaweed, 16,000 tons of algin, 8,000 tons of carrageenan, and the remaining 70,000 tons of edible and green seaweed are the total support products of all seaweeds in Indian waters.(Devaraj, 1999.)

Seaweeds are promising plants of the millennium because it is a renewable and sustainable source which can be harvested at 6 to 8 weeks after the cultivation. (Ahemad *et. al.,* 2006)

However, now not most effective do seaweeds play an essential function within the ecological machine (algae are the idea of all food chains inside the sea offering as much as half of the earth's oxygen), however a few have additionally been used for hundreds of years as food, as manure or as a supply of chemical compounds. Alginates, a form of brown seaweed, have been accrued in Scotland for the reason that 18th century for the treatment of blisters and lacerations. From the 1960's, seaweeds have been farmed and nowadays, alginates locate vast applications in both the fabric, food and the pharmaceutical industries. (Burrows. *et. al.,* (1983))

Prasanna *et al.* (2007) stated that algae has a wide variety of natural pigments like chlorophyll, carotenoids and phycobiliproteins, which exhibit colors ranging from green, yellow, brown and red. Algae pigments have great commercial value as natural colorants in nutraceutical, cosmetics and pharmaceutical industry as well as their health benefits.

Uses of seaweeds dyes

Kasimala *et al.,* (2015) discussed about the consumption of seaweeds in Asian countries. Marine algae have been utilized in Japan as raw materials in the manufacture of many seaweed food products, such as jam, cheese, wine, tea, soup and noodles and in the western countries, mainly as a source of polysaccharides for food and pharmaceutical applications. Seaweeds are an important source for food, fodder fertilizer and medicine from the ancient times. Because of their high protein content, Protein Concentrates of

seaweeds have become more important for the food industry, especially in developed countries. Their recent utilization as an animal feed is on the increase due to their nutritive importance. Generally most of the seaweeds contains high ash (indicates appreciable amount of diverse minerals), high fibers, low protein and moderate amount of fatty acids. The Rhodophyta members contain high protein content (32%), whereas Chlorophyta members contain highest carbohydrate content (35%). The most common edible seaweeds in Asian countries are *Porphyra* spp., *Padina* spp., *Undaria* spp., and *Laminaria* spp. Sea weeds are good additive to improve the nutritive quality of various foods.

Some researches explained about the nutraceutical, pharmaceutical and cosmeceutical value of the seaweeds. Some of their diverse properties are anti-cancer, antiviral, antifungal, anti-diabetic, antihypertensive, immuno-modulatory, cytotoxic antibiotic, anticoagulant, anti-inflammatory, anti-parasitic, antioxidant, UV-protective and neuroprotective. It has also been confirmed that several species of seaweed have powerful antioxidant compounds such as phloro tannins, carotenoids and sterols, making seaweed a source of compounds with possible neuro protective effects, useful in the treatment of neurodegenerative diseases such as Parkinson's and Alzheimer's. Sulfated polysaccharides from seaweed have shown important potential pharmacological uses, such as their anti-ulcer effects, by preventing adhesion of the infection caused by the bacteria *Helicobacter pylori* (Stengel *et. al.,* (2011) and Leandro *et. al.,* (2020)).

Natural dyes have been used since ancient times. After the invention of chemically synthesized dyes, the use of natural dyes has rapidly decreased. Natural dyes and colorants have been used for many years. For example, they were used to color fur and leather, as well as natural fibers such as wool, cotton and silk. In addition to this, they

served to color cosmetics and produce inks, watercolors and artist paints. As the world's environmentally aware ecology and pollution control increase, natural dyes become more attractive. Currently, the government is forcing the dye industry to reduce toxic waste and stop production of potentially hazardous dyes and pigments. (Glover, (1995)).

Extraction of seaweed dyes

Moldovan, *et. al.,* (2017) engaged with identify the alternative sources of dyes, with characters like biodegradable, non- carcinogenetic and sustainable colorant matter from algal biomass. They were selected red algae *Gracilaria spp.* for extract the red pigment phycoerythrin was made by 3 steps: freezing, thawing and extraction by magnetic stirrer.

Another research (Kadir *et. at*., (2014)) also found on the extraction of natural dye from green seaweeds of *Caulerpa letillifera* as a textile colorant. This colorant was extracted using boiling water and ammonia fermentation methods. For dyeing process, three types of mordants (alum, acetic acid and Iron) were added with dye baths that which produced different shades from greenish yellow to reddish brown. This study, resulting that *Caulerpa letillifera* can be exploited as a natural dye source which can give unique and interesting shades on textile field.

Researchers Asmida., *et. al.,* (2014) was studied on the extraction of natural dyes from red, green and brown strains of *Kappaphycus alvarezii* for textile colorant. The green, brown and red strains of *Kappaphycus alvarezii* seaweeds were extracted using boiling water and microwave assisted extraction methods. The extracted colorant was measured with UV–Vis spectrophotometer to analyze the absorbance value at the maximum wavelength or peak absorbance (λmax) as well as to determine the compounds present

which contribute to the shades obtained. The results ranged from good to excellent rating except for light fastness which gave poor rating.

Seaweeds (macro algae) have many commercial applications like foodstuffs, animal feed, cosmetics, pollution abatement, and therapeutics. Currently, algae find a promising future as an alternate fuel. But the cost of algal biodiesel offset its chances of commercialization. Renita. *et. al.,* (2015) was worked about extraction of dye from marine macro algae and work was concluded that the dye extracted from the marine seaweed *Sargassum myriocystum* has potential application as a food colorant. Since it has photosynthetic pigments like chlorophyll and carotenoid it also finds application in dye sensitized solar cells. Currently, algae are researched upon as a potential biofuel resource. But the cost offsets the economical production of biofuel. Value added materials like dyes and pigments extracted from the seaweeds can make the cost of biofuel economical.

Wiraningtyan, *et.al.,* (2020) were studied the use of dyes and alginates from seaweed *Sargassum* sp. in coloring Bima woven cloth Seaweed *Sargassum* spp. is a species of seaweed that can produce alginates. In the industrial world, alginate is used as a thickener in the fabric printing process. Besides producing alginate, seaweed *Sargassum* spp. also contains dyes or pigments that can be used as natural dyes. Several types of pigments contained in brown seaweed include fucoxanthin, chlorophyll, carotene and other pigments, but fucoxanthin is very dominant which gives dark brown to yellow brown in the dyeing results. Seaweed type *Sargassum* sp. gives a golden brown color and fastness test results show good value 5.

Calogero, *et. al.,* (2014) concentrated their investigation on the brown macroalga *Undaria pinnatifida* (Harvey) Suringar. to extract the photosynthetic pigments and to

prepare colored solution using appropriate solvent; to fabricate a titania-based dye-sensitized solar cell (DSSC); to test the photovoltaic performance of the chlorophyll based devices measuring current voltage curves and the incident photocurrent efficiency (IPCE). Algal species were collected in Venice Lagoon where they are highly invasive species, forming huge biomass disturbing human activities, and presently removed as a waste.

Edible pigments can be extracted from seaweeds which can be used as natural food coloring agent due to substitute hazards in artificial (synthetic) food coloring. Chlorophyll, carotenoid, and phycobiliproteins are major photosynthetic pigments presence in micro algae. Extraction of high quality natural food coloring and efficient impact of these coloring on chemical, microbial and sensory quality of gel dessert were evaluated. The main objectives of the present study Chlorophyll and carotenoids were extracted from *Ulva lactuca* and *Sargussum wightti* using acetone, methanol and water as solvents while and phycobiliprotein was obtained from *Gracilaria verrucosa* grinding with ice cold potassium phosphate buffer. The extracted natural food colors were found to be in higher rangers of nutrition indicating that these dies can be used as food supplement. (Jayasinghe, *et. al.,* (2016)).

Light absorption of dyes by using UV- Vis spectrophotometer

Experimentally, light absorbance is measured using a UV-Visible (UV-Vis) spectrophotometer. This instrument utilizes a light source that is transformed by a monochromator into specific wavelengths of light that will pass through a sample and into a detector at the other end. Different colored dyes vary in the wavelength of light that they

absorb. Most dyes are conjugated compounds with alternating double and single bonds and typically absorb light in the visible region (De Caro. *et. al.,* (2015)).

FTIR- spectral analysis

Fourier transform infrared (FTIR) spectroscopy has a large application range, from the analysis of small molecules or molecular complexes to the analysis of samples. (Levin, *et. al.,* (2005))

FTIR is a rapid, nondestructive, time saving method that can detect a range of functional groups and is sensitive to changes in molecular structure. FTIR provide information on the basis of chemical composition and physical state of the whole sample. Fourier Transform-Infrared spectroscopy (FTIR) has been used as a rapid, accurate and nondestructive technique for measuring many wheat quality parameters. (Cocchi *et. al.,* (2004)).

Mordants and tests

Mordants are substances that have affinity for both textile fibres and dyes, thus they act as a link between the fibre and the dyestuff. The mordanting process was reported to improve colour strength and colour fastness properties due to stable dye complex formed on the fabrics (Kumaresan *et al.* 2011).

Different types of mordants or their combinations can be applied on textile materials to obtain varying color shades, to increase the dye uptake and to improve the color fastness behavior of natural dyes. (Samanta, *et. al.,* (2009))

Souissi, *et. al.,* (2018) investigated the improvement of colour fastness with biological mordants compared with metallic mordants. The fastness properties of dyed samples were also evaluated. Obtained results indicated interesting sweat, washing and rubbing fastness in the range of 4-5. Three metallic mordants (*Alum*, *ZnSO4* and *CuSO4*) and three biological mordants (*gall nuts*, *chlorophyll (a)* and *green almond shell*) were used to improve the degree of absorption as well as the color fastness of cotton fabrics dyed with aqueous extract from date palm pits (*Phoenix dactylifera)*. The changes in shade for wash fastness and crock fastness tests were evaluated by gray scales. For light fastness, the dyed samples and the blue scales were exposed, using Sun test CPS Plus apparel (Atlas Material Testing Technology) to a xenon lamp light with a light intensity of 765 W/m2 for 72 h.

Yaqub *et. al.,* (2018) were used the acid mordants (citric acid, tartaric acid, acetic acid and tannic acid) for differentiate the shades which shown after dyeing the acidic and basic *Beta vulgaris* peel dyes on the silk fabrics. The results were shown that light shadiness and better fastness results in dyed silks with acidic dye solution.

Kampeerapappun *et. al.,* (2010) determined the influence of dyeing methods with mordants, i.e. pre-mordanting, meta-mordanting, and post-mordanting on the knitted cotton fabrics that with chitosan. Each mordant gave different shades of color and mordant techniques affected to color intensity and fastness of fabric. The effect of mordants on color intensity (K/S) of chitosan-treated and untreated fabric was examined by spectrophotometer. These results were explained that post-mordanting can form more stable dye-mordant complex than other two methods.

Common metal mordants used are Alum, Copper sulphate, ferrous sulphate, Potassium dichromate, Stannous Chloride and Stannic Chloride. Based on the final color

produced by natural dyes, these metal mordants are further divided into two types: Brightening Mordants and Dulling Mordants. Alum, potassium dichromate and tin (Tin chloride) fall into the category of whitening mordants, while copper sulfate and iron sulfate are dull mordants. (Prabhu *et. al*., (2012))

The low affinity between the dye and the fibre is mainly due to the low dyeing ability, narrow shade range and low color fastness of natural dyes on textile material. Researchers to deal with these issues and paid a way to improve the color properties of dyed textiles by using metal salts (mordants) enhance molecular interactions and various functional properties between fiber and dye. Dye-Mordant-Fiber interaction is also responsible changing the hue of certain dyes, darkening and brightening the final color of the dyed fibers. Mordanting can be achieved by pre-mordanting, simultaneously mordanting and post-mordanting methods (Rather et al. 2017).

Metal ions used as mordants in natural dyeing discharged in the effluents have negative impact on the environment and public health. Potassium aluminum sulphate (Alum) and ferrous sulphate (Iron) can be considered ecologically safe as they are naturally present in the environment in large amounts and stannous chloride (Tin) is not restricted by many eco-labels so can be used at very low concentrations (Dalby 1993; Rather *et al.,* 2017)

Ali *et al.,* (2009) work was to extract more colouring component with keeping the environment friendly extraction procedure (extraction in aqueous alkaline (Sodium hydroxide) medium) excluding the extensive application of organic solvents. This medium was chosen to view the acidic chemical nature of lawsone. This will help to make natural dye as co-partner of synthetic reactive dyes. Furthermore, the dyeing and mordanting

characteristics of colouring matter on cotton have also been studied. Finally, the comparative studies have also been conducted between the dyeings with optimized Henna alkaline extracts and synthetic dyes and evaluated the optimum conditions as well.

Presently as the climate changes, the requirement for functional clothing arises. Besides providing colour, natural dyes have inherent functional properties such as resistance for bacteria, fungus and moth, UV protection, etc. Fabric as a second skin covers the major part of the body and hence can be used as a preventive measure from near environment. At present, the researches on utilization of natural dyes in functional finishing of textiles have increased because of the efficiency of natural dyes which provides protection against various harmful agents as well as provides greater comfort. Dyed fabric remains fresh and odour-free in use.

CIE L*a*b* analysis

The difference of mordant types showed different in color of fabric expressed as CIE L*a*b*. Three values of CIE L*a*b* are L* (white-black), a* (red-green), and b* (yellow-blue). The increased values of L*, a*, and b* define whiter, more reddish, and more yellowish, respectively. The wash fatness and light fastness of the dyed fabrics are identified by the comparing with grey scale to obtain the color change compared with fabric before testing. Grey scale has scale from 1 to 5 that scale 1 indicates the most color difference and scale 5 means no color difference. (Kampeerapappun *et. al.,* (2010)).

Materials and methods

Materials preparation

Seaweeds collection

The three brown seaweeds were collected from Hare Island of Gulf of Mannar, Thoothukudi District (Jan- Mar 2021). The seaweeds were identified with a help of Phycology of Indica. Voucher deposited and preserved in St. Mary's College, department of botany herbarium, Thoothukudi, Tamilnadu, India.

Species description

1. ***Padina tetrastromatica*** Hauck

Characteristics

Thalli flabelliform, usually divided into several small lobes, regularly and distinctly concentrically zonate due to the regular row of fructiferous organs; easily recognized due to dark double lines of sporangia; enclosing a line of colorless hairs in between; blades composed of two layers of cells, in the young apical involute portion, about 3 0 - 40 µ thick, in the middle three cell layered partitions about 80 - 90 µ thick, lower down replaced by the typical four cell layer, about 130 - 150 micron or more near the basal portion.

2. ***Sargassum tenerrimum*** J.Agardh

Characteristics

Plants pyramidal in form, delicate and with a disc shaped holdfast, yellowish-brown in color, reach a height of 30-40cm, axis glabrous and rounded, ultimate branchlets modified into arranged, being larger and broader in the lower portion becoming smaller

and narrower towards the apex marginal leaves somewhat dentate, midrib more or less prominent, vesicles stalked and spherical, receptacles richly branched and spinose.

3. ***Turbinaria ornata*** (Turner) J. Agardh

Characteristics

Thalli erect upto 13 cm tall. Dark brown in color attached to substrate with branched holdfast; erect axis is sub cylindrical supporting alternatively and polystichously arranged stalked turbinate leaves; leaves are 13 - 20 mm long with substrate stalk slightly tapering below and becoming expanded and triangular above; distal marginal blade may be vesiculate or evesiculate 10 -15 mm broad, concave at the centre which may be surrounded fully or partially by an inner crown of teeth, the outer margin is lined by sharp prominent teeth; paniculate receptacular clusters are racemose attached to the stalk at 1/4 distance from the margin

	***Padina tetrastromatica* Hauck**	***Sargassum tenerrimum J.* Agardh**	***Turbinaria ornata* (Turner) J. Agardh**
Order	Dictyotales	Fucales	Fucales
Family	Dictyotaceae	Sargassaeae	Sargassaceae
Genus	*Padina*	*Sargassum*	*Turbinaria*
species	P. *tetrastromatica*	S. *tenerrimum*	T. *ornata*

Selection of fibres

As a substrate for dyeing process, 100% cotton untreated threads with three different diameters (1.0mm, 2.10mm and 2.20 mm) were used for the experiment.

Selection of mordants

For differentiate the effect of acidic, alkali and metallic mordants in the dyeing, two percent (2%) of acetic acids, sodium bicarbonate and ferrous sulphate were used as mordants for each different dyeing process.

Brown seaweeds dye extraction (Mona *et. al.,* (2019), Luque de Castro. *et. al.,* (2010)).

Dye was obtain by the soxhlet extraction methods in the radio 1:20. Fresh seaweeds were dried and made into powder. For extraction, 10g of dry powdered samples were taken and loaded separately into the thimble. They were operated for 5 cycles with 200ml of 4 different solvents viz., acetone, ethanol, methanol and water with their respective boiling temperature. After these process, the extracted solvents were collected in separate reagent bottles.

Estimation of concentration of dye in the extract

One gram of dye extracts were took in weighed beakers separately and placed into the boiling water bath till the extract became dry. After boiling, dry weight of extracts were noted and concentration of dye was calculated.

Estimation of light absorption capacity

This estimation were contacted for identify the absorption of colour lights in selective wavelengths by each dye extracts of brown seaweeds The dye solution was

subjected to UV spectroscopy for absorption measurement at wavelength of 400-663 nm. The peaks present in the diagram were analyzed for which compound was absorbed that particular light wavelength.

Fourier transform infrared (FTIR) spectrometric analysis

FT IR spectrometric analysis of three seaweed samples done by Instrumentation Centre, Ayya Nadar Janaki Ammal College, Sivakasi. The peaks were analyzed by the reference table for FT IR spectrum. In this analysis, the identification of dye enhance compound was found.

Preliminary phytochemical analysis (Geetha, *et. al.,* (2013))

1. **Test for glycosides (Keller test)**

   Test solution was treated with few drops glacial acetic acid and ferric chloride solution and mixed. Concentrated sulphuric acid was added, and observed for the formation of two layers. **Lower reddish brown laye**r and **upper acetic layer which turns bluish green** would indicate a positive test for **glycosides.**

2. **Test for alkaloids: (Wagner's test)**

   About 2ml of extract was taken and few drops of Wagner's reagent was added. The formation of **a reddish brown precipitate** and that indicated the presence of **alkaloids**.

3. **Test for flavonoids:** (**Shinoda test**)

About 2ml of extract was added to pinch of magnesium turnings and 1-2 drops of concentrated hydrochloric acid was added. Formation of **pink color** indicated the presence of **flavonoids.**

4. **Test for tannins (Ferric chloride test)**

About 2ml of extract was taken and 0.5ml of 5% ferric chloride was added. The development of **dark bluish black** color indicated the presence of tannins.

5. **Test for phenols: (Sodium hydroxide test)**

About 2ml of extract was dissolved in 0.5 ml of 20% Sulphuric acid solution. Few drops of aqueous sodium hydroxide solution added. It turns **blue** which indicated the presence of **phenols.**

6. **Test for saponins**

1 ml of extract was dissolved with 1 ml of distilled water and appearance of **foam** indicated that presence of **saponins.**

7. **Test for quinine**

1ml of extract is mixed with few drops of concentrated hydrochloric acid and appearance of **green** indicated the presence of **quinine.**

8. **Test for steroids**

2ml of extract was mixed well with drops of chloroform. Then, a drop of acetic acid was added and the mixture was heated for few minutes after which few drops of sulphuric acid was added. Appearance of **brown color** indicated the presence of **steroids**.

Quantitative analysis

Estimation of carbohydrate (Dubois, *et. al.,* 1956)

Grind 1 gm. of sample with 20 ml distilled water using a mortar and pestle. Filter through a cheese cloth, centrifuge the filtrate for 10 minutes around 6000rpm, make up the filtrate to 100 ml with water and discard pellet; Take 1 ml of the extract in duplicates and add 1 ml of phenol (5%) and 5 ml Conc. H_2SO_4. Mix well and keep in boiling water bath for 10 minutes (until color develops). Cool to room temperature and measure the OD at 490 nm using the blank. Extrapolate the OD in the standard graph of the glucose. From this calculate the amount of glucose present in 100 ml, which will be equal to the amount of sugar present in 1 gm. of plant material.

Estimation of protein (Bradford, 1976)

Weigh 1gm of sample and grind it with 5ml of phosphate buffer (pH 7.5). Filter through a cheese cloth. Centrifuge at 3000 rpm for 20 mins. and collect the supernatant that contains protein. Make up the final volume to 10 ml using phosphate buffer. Take 1 ml from the extract add 2ml of distilled water and 1.5 ml of Bradford reagent to each tube. Mix thoroughly and incubate for 5 mins. Measure absorbance at 595 nm.

Estimation of lipids (Loehr *et al.,* 1964)

1g of sample was taken in a test tube and 5 ml of chloroform methanol mixture (2:1 by vol.) was added. After 6 hours the content was centrifuged at 3000 rpm for 10 minutes. The supernatant was carefully collected and to this 10ml of 0.01 N of KCl was added to remove the non- lipid content. Of the 2 separate layers formed, the upper layer with non-lipid content was removed and the lower layer was transferred carefully to a previously

weighed container and the solvent was evaporated. After complete evaporation, the container was again weighed accurately. The difference between the two weights gave the amount of lipid present in the sample.

Estimation of crude fibre (Dougall. 1956)

Take 2g of the sample and transferred into the conical flask to mix with 200ml of 0.128 M sulfuric acid solution. The flask was placed on the hot plate and boiled for 30 mins. After 30 mins the solution was filtered. The filtrate was washed with hot water for remove the acid residue completely. Then, 200ml of 0.313 M NaOH solution was poured into the conical flask by washing the filtrate and placed on hot plate for another 30 mins. After boiling process, the solution was filtered and the filtrate was washed by hot water to remove NaOH residues. Collect the fiber by clean and dried crucible till no filtrate is left. The crucible was dried in the hot air oven at 230°C for 2 hours. After drying, take out the crucible and cool in desiccator and take weight. Then, place the crucible into the muffle furnace incinerated the sample 550°C and time for 2hrs and cool the content in desiccator for 20 mins. Take weight of the crucible containing ash. Then, the percentage of crude fibre present in sample was calculated.

Mordanting and dyeing process (Arora *et. al.,* 2017)

Mordanting the fibres were done selectively before dyeing process (Pre mordanting process). The pre-soaked fibres were dipped in required mordanting solution (2%) such as acetic acid, ferrous sulphate and sodium bicarbonate for 20 – 30 min at 80˚C. Dyeing was done with natural dye at 85˚C for 40 - 60 min. after dyeing, the fibres were cold washed and dried under shade.

Colour Fastness analysis of dyed threads

Wash fastness of dyed threads

Wash fastness of dyed threads was tested by 3 different types of treatments namely,

i. Cold water treatment

In this test, the dyed threads were placed in cold water for five days and colour change was recorded for each day.

ii. Hot water treatment

In this test the dyed threads were placed 5 times in hot water bath for30 mins. and the colour changes were recorded for each time.

iii. Soap water treatment

In this test the dyed threads were placed in the soap water (5g/lit) for 5 days and the colour changes were recorded for each day.

Colour change was recorded by 0-5 scales that means,

5- Excellent
4- Good
3- Moderate
2- Poor
1-Very Poor
0-No Efficient Results

CIE- L* a* b* analysis

This assessment was conducted for measuring the colour of the dyed fibres. In this method L* defined as the lightness of the colour, *a** is the axis that extends from red (positive) to green (negative), *b** is the axis that extends from yellow (positive) to blue (negative). By these values, the dyed fibres dominant colours and shades will be detect easily. In this study, L* a* b* values were calculated with a help of android applications (color detector, color analysis and color grab).

Results and discussion

The dye solution was procured from the seaweed by solid-liquid extraction (Soxhlet) method using acetone, methanol, ethanol and water as solvent system.

Dye colour

Natural dyes were extracted from seaweeds with different solvents like acetone, ethanol, methanol and water. Due to their different solutes presence, dyes were shown different colour. Dyes extracted by water was brown in colour other dyes gave green, greenish yellow and yellowish green shaded colours. (Table 1)

Dye concentration in seaweeds extracts

This analysis estimated the percentage of dyes dissolved in the seaweed extracts. According to our results, except acetone extracts other extracts extracted by ethanol, methanol and water have more dyes in them. (Table 2)

Light absorption capacity of seaweeds dye extracts

The dye solution was subjected to UV spectroscopy for absorption measurement at wavelength of 400-663 nm**.** UV- Vis spectrometry analysis explains that which colour region, absorbed maximum by seaweeds dye extracts. Light absorption capacity of dyes were mentioned in the Figures 1-3. It shows the absorption values. The obtained absorption spectrum was very sharp for all the extracted dyes at different wavelengths, varying their intensity levels. The results showed that water extracts of three brown seaweeds were absorbed the blue (480nm) and orange (626nm) light highly. Ethanol extracts of *P. tetrastromatica* and *T. ornata* and the water extract of *S. tenerrimum* were absorbed the

light from orange region at 626 nm. Ethanol extracts of three brown seaweeds were absorbed the red light at 645nm and 663nm. The absorbance peaks indicated the presence of flavonoids and chlorophyll. In comparison, γ carotene (497 nm), and phycocyanin (590 nm, and 614 nm) are active in the water solvent. The phycoerythrins (537 nm, 540 nm, 549nm, 578 nm, and 588 nm), phycocyanins (618 nm), β carotene (478 nm), and Chlorophyll b (627 nm) are active in the ethanol solvent.

FT-IR Spectrometry analysis

FTIR – spectral peaks were the indications for the presence of the compound functional groups in the specific absorption wavelength (cm-1). The composition of natural dyes has been affirmed using FTIR spectroscopy, has been shown in Figure 4- 6. A brief analysis of the FTIR spectra of three sample has been displayed in Table 3-5.

Since various peaks obtained in FTIR studies of individual samples are approximately very similar, the strong peak is in spectrum Figure 4 and 5 is seen at 1510.16. P. *tetrastromatica* , S. *tenerrimum* and T. *ornata* in Figure 4 and 6, all dyes with ethanol solvent shows sharp peaks due to the aromatic ν C–H and aliphatic ν C–H stretching frequencies, respectively.

In the wavenumber range of 1000–1500 cm-1 , considerable isotropic properties (Figure 4(-6) have been seen in P. tetrastromatica , S. tenerrimum and T ornata with low bending groups such as –CH2, and –CH3. Further investigations revealed that all the samples within the same region demonstrated the isotropic behavior with higher bending groups –CH2, and –CH3. Our results are in good agreement with earlier reports.)

Preliminary phytochemical analysis

It was performed to analyze the presence of bioactive compounds (alkaloids, flavonoids, glycosides, phenols, quinine, saponins, steroids and tannins) in seaweeds dye extracts. In overall seaweeds extracts dye extracted by ethanol and methanol were recorded more positive results. Dyes were extracted by water had poor results in qualitative analysis. Among the four different extracts, ethanol extract of *T. ornata* showed the presence of maximum number (8) of compounds**.** Next to that, Methanol extracts of *S. tenerrimum* showed seven compounds. (Table 6)

Quantitative analysis of primary metabolites

The results of these tests were shown in figure 7 and 8. When compared to other two seaweeds *S. tenerrimum* is have more quantity of primary metabolites like carbohydrates (149±1mg), protein (63.5 ± 1.26) and lipids (5.03 ± 0.26). Due complex constructions of thalli, *T. ornata* has more quantity of crude fibres (9.42%) than other two seaweeds.

Dyeing process

Dyed fibres

The plates 1-4 shows the dyed uncoated cotton fibres (treated with and without mordants) with twelve different dyes extracted from three brown seaweeds. The shades of dyed fibres by usage of different mordant can be compared. In plate 2, the acetone-*T. ornata* dye extract was not fixed on the cotton fibres with and without application of mordants.

L*a*b* values

L*a*b* values for dyed cotton fibres with twelve different dye extracts from three brown seaweeds were figured in figure 9-12. The L* values indicate perceived lightness or darkness. Value of 0 indicates black and 100 indicates white. The values of a* indicate red (+a) and green (-a), while b* indicates yellow (+b) and blue (-b). The darkest shades obtained from the fibres treated with ferrous sulfate as a mordant. Cotton fibres with different diameters were obtained a same colours.

On the other hand, the lightest shades obtained from fibres treated with acetic acid and fibres which did not treated with water and these two samples produced almost similar shades in terms of lightness.

Except aqueous seaweed dye extracts, other extracts have negative a* value that indicates the dyed fibres have a green shaded colour. The b* value of all extracts are positive that conclude that the yellow colour is present in the dyed fibres.

Colour Fastness Properties

Washing fastness properties

The washing fastness properties of dyed cotton fibres recorded with a treatment of cold water, hot water and soap water are given in Tables 7, 8 and 9.

In cold water wash treatment, the dyed samples of aqueous dye extracts (all 3 seaweeds) have excellent fastness properties and rated between 4 and 5. The poor fastness properties rate was obtained by cotton fibres were dyed acetone (0) and methanol dye extracts (2-3).

In hot water wash treatment, the fibres were dyed with ethanol dye extracts have good to excellent fastness properties and rated between 4 and 5. The poor fastness properties rate was obtained by cotton fibres were dyed acetone (0) and methanol dye extracts (0).

In soap water wash treatment, all dyed cotton fibres shown the poor fastness properties and rated between 2 and 3. The fibres were dyed with aqueous dye extracts have better fastness properties and rated between 2 and 3. The poor fastness properties rate was obtained by cotton fibres were dyed acetone (0) and methanol dye extracts (0).

There is no differentiation in fastness properties by mordant used and diameters of cotton fibres.

Durability colour fastness of dyed fibres

Stored dyed fibres were shown the colour difference in storage period. Fibres which dyed with aqueous extracts (5) and ethanol extracts (4-5) have an excellent dyeing properties. Other two dye extracts didn't have durability fastness (0). It may be caused by the evaporation property of solvent used for dye extraction. (Table 10)

Summary and conclusion

Summery and conclusion

In this work, the three brown seaweeds (*Padina tetrastromatica, Sargassum tenerrimum* and *Turbinaria ornata*) were used as a sources for obtain the natural dyes for fabrics. There are different types solvents (acetone, ethanol, methanol and water) used for dye extraction and different types of mordants (acetic acid, ferrous sulphate and sodium bicarbonate) for obtain different shades and excellent fastness properties in the uncoated cotton fibres with different diameters (1mm, 2.10mm and 2.20mm.).

After extraction of dyes by soxhlet extraction method, uncoated cotton fibres which underwent pre- mordanting process (2% mordant solution) were dyed. The dyed fabrics shown different shades of colours based on the mordants used and solvent which used for dye extraction.

Colour fastness assessments were provided the significant features of dyed cotton fibres will have excellent dyeing properties or not. From the results the aqueous and ethanol dye extracts have an excellent fastness properties than acetone and methanol dye extracts. Especially, the species *Sargassum tenerrimum* dyes (aqueous and ethanol dyes) have more excellent properties than other two seaweeds. Fastness properties were not depended on the mordants which used on the cotton fibres.

To know the phytochemicals which gave colour on fabrics by seaweeds, different experiments were done like preliminary phytochemical analysis (Qualitative analysis), Quantitative analysis of primary phytochemicals and FTIR analysis. It is confirmed that the secondary metabolites like flavonoids and tannins are the reason for colouring the fabrics. Light absorption capacity of seaweeds dye extracts by UV Spectroscopy also

studied. This study confirms the presence of flavonoids, chlorophyll, γ carotene, phycocyanin, phycoerythrins, phyco cyanins, β carotene and Chlorophyll b. Hence all these pigments may responsible for colouring the fabrics.

Brown seaweeds have been used as biological source of the specialized polysaccharides align that have numerous commercial values like the thickener of paint, cosmetics, food, medicines etc., There are some seaweeds processing industries present in India for the extraction of alginate form brown seaweeds. If we commercialized these dyes, alginate processing industries can extract the dyes before the processing the extraction of alginates cultivation of brown seaweeds will increases the income of the seashore living peoples.

It is time to take action to document these natural dyeing techniques for future applications. Otherwise, we will lose important information about the use of natural resources around us. In conclusion, there is an urgent need for proper collection, documentation, evaluation and characterization of dye-producing plants and algae and their dyes, as well as research to overcome the limitations of natural dyes.

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Table 1 Colours of dyes extracted from brown seaweeds

S. No	Brown algae	Solvent used	Colors
1.	*Padina tetrastromatica*	Acetone	Green
2.		Ethanol	Green
3.		Methanol	Green
4.		Water	Brown
5.	*Sargassum tenerrimum*	Acetone	Greenish yellow
6.		Ethanol	Yellowish green
7.		Methanol	Green
8.		Water	Brown
9.	*Turbinaria ornata*	Acetone	Yellowish green
10.		Ethanol	Greenish yellow
11.		Methanol	Yellowish green
12.		Water	Brown

Table 2. Percentage of dye extracted from brown algae

S. No	Brown algae	Solvent used	Concentration of dye (%)
1.	*Padina tetrastromatica*	Acetone	86.02
2.		Ethanol	98.77
3.		Methanol	99.1
4.		Water	98.26
5.	*Sargassum tenerrimum*	Acetone	88.42
6.		Ethanol	98.33
7.		Methanol	99.9
8.		Water	98.31
9.	*Turbinaria ornata*	Acetone	98.56
10.		Ethanol	99.72
11.		Methanol	97.67
12.		Water	98.8

Table 3 **FTIR spectral peak values and functional groups obtained from *Padina tetrastromatica* sample**

S. No	Peaks	Appearance	Group	Compound class
1.	517.85	Strong	C-I, C-Br	Halo compound
2.	620.07	Strong	C-I, C-Br	Halo compound
3.	654.79	Strong	C-I, C-Cl	Halo compound
4.	781.12	Strong	C- H, C-Cl	1, 2, 3- trisubstituted
5.	990.38	Strong	C=C	Alkene
6.	1116.71	Strong	C-O, C-F	Secondary alcohol, aliphatic ether, fluoro compound.
7.	1175.53	Strong	C-O, C-F	Ester, fluoro compound.
8.	1269.07	Strong	C-O, C-F	Alkyl aryl ether, fluoro compound.
9.	1316.33	Strong, medium	S=O, C-N, O-H, C-F	Sulfone, aromatic amine, phenol, tannic acid, fluoro compound.
10.	1338.51	Strong , medium	S=O, C-N, C-F, O-H	Sulfone, sulfonamide, Sulfonate, aromatic amine, fluoro compound, alcohol.
11.	1383.83	Medium, strong	O-H, C-F, S=O,C-H	Phenol, tannic acid, tannic acid, alcohol, fluoro compound, sulfonyl chloride, sulfate, alkene, aldehyde
12.	1434.94	Medium, weak	O-H, C=C	Carboxylic acid, aromatic
13.	1510.16	Strong	N-O , C=C, C=O,	Nitro compound, aromatic, amide
14.	1584.41	Medium, strong	C=C, N-H, C-H, C=O, NO2	Cyclic alkene, amine, aromatic compound, anhydride, amide
15.	1681.81	Medium, strong, weak	C=N, C=O, C-H	Imine/ oxime, conjugated ketone, aromatic compound, anhydride, amine, amide
16.	1713.64	Strong, weak	C=O,C-H	Aliphatic ketone, carboxylic acid, anhydride, aromatic compound
17.	1748.35	Weak, strong	C=O	Anhydride, aromatic compound, ester, δ-lactone
18.	1796.57	Strong, weak	C=O, C-H	Acid halide, conjugated acid halide, anhydride, aromatic compound
19.	2883.38	Medium, Strong broad,	C-H, O-H, N-H	Alkene, Carboxylic acid, amine salt
20.	2976.92	Strong, weak	O-H, N-H	Carboxylic acid, alcohol, amine salt, primary amine
21.	3524.67	Medium, Strong	N-H, O-H	Secondary amine, alcohol

Table 4 **FTIR spectral peak values and functional groups obtained from *Sargassum tenerrimum* sample**

S. No	Peaks	Appearance	Group	Compound class
1.	517.85	Strong	C-Br, C-I	Halo compound
2.	548.71	Strong	C-Br, C-I	Halo compound
3.	649	Strong	C-Br	Halo compound
4.	688.54	Strong	C=C, C-Cl, C-Br	Alkene , halo compound
5.	816.8	Strong	C-Cl, C-H	Halo compound , 1, 4 di-subtituted or 1,2,3,4- tetra substituted
6.	992.31	Strong	C=C	Alkene
7.	1082.96	Strong	C-F, C-N, C-O	Fluoro compound, amine, primary amine
8.	1122.49	Strong	C-F, C-N, C-O	Fluoro compound, amine, alphatic ether, secondary alcohol
9.	1268.11	Strong	C-F, C-N, C-O	Fluoro compound, aromatic amine, aromatic ester, alkyl aryl ether
10.	1316.33	Strong, medium	S=O, C-N, O-H, C-F	Sulfone, aromatic amine, phenol, tannic acid, tannic acidtannic acidfluoro compound.
11.	1338.51	Strong , medium	S=O , C-N, C-F, O-H	Sulfone, sulfonamide, Sulfonate, aromatic amine, fluoro compound, alcohol.
12.	1384.79	Strong, medium	S=O, O-H, C-F, C-H	Sulfate, sulfonyl chloride, phenol, tannic acid, tannic acidfluoro compound, aldehyde, alkane
13.	1418.55	Medium	O-H	Alcohol, carboxylic acid
14.	1511.12	Strong	N-O	Nitro compound
15.	1604.66	Weak, strong	C=O, C=C	Amide, alkene
16.	1680.85	Strong, medium	C=O, C=N	Conjugated acid, conjugated ketone, imine/ oxime
17.	1748.35	Weak, strong	C=O	Anhydride, aromatic compound, ester, δ-lactone
18.	2887.24	Medium	C-H	Alkyne
19.	2975.96	Strong, medium, weak.	N-H, C-H, O-H	Amine salt, alkyne, alcohol, carboxylic acid
20.	3588.32	Strong	O-H	Alcohol

Table 5 FTIR spectral peak values and functional groups obtained from *Turbinaria ornata* sample

S. No	Peaks (cm^{-1})	Appearance	Group	Compound class
1.	517.85	Strong	C-Br, C-I	Halo compound
2.	592.11	Strong	C-I, C-Br	Halo compound
3.	649	Strong (n, o)	C-Br	Halo compound
4.	678.9	Strong	C=C, C-Cl, C-Br	Alkene , halo compound
5.	874.66	Strong	C-H	1,2, 4-tri substituted
6.	1001.95	Strong	C=C	Alkene
7.	1033.77	Strong, medium	C-F, C-N, C-O	Fluoro compound, amine, primary amine
8.	1056.92	Strong	C-F, C-N, C-O, S=O	Fluoro compound, amine, alphatic ether, primary alcohol, sulfoxide
9.	1164.92	Strong	S=O, C-O	Sulfone, tertiary alcohol, ester
10.	1267.14	Strong	C-F, C-N, C-O	Fluoro compound, aromatic amine, aromatic ester, alkyl aryl ether
11.	1315.36	Strong, medium	C-F, S=O, O-H	Fluoro compound, sulfone, phenol
12.	1338.51	Strong, medium	S=O , C-N, C-F, O-H	Sulfone, sulfonamide, Sulfonate, aromatic amine, fluoro compound, alcohol.
13.	1384.79	Strong, medium (n, o)	S=O, O-H, C-F, C-H	Sulfate, sulfonyl chloride, phenol, tannic acid, tannic acid, tannic acidfluoro compound, aldehyde, alkane
14.	1433.98	Medium	O-H	Alcohol, carboxylic acid
15.	1510.16	Strong	N-O	Nitro compound
16.	1603.7	Weak, strong	C=O, C=C	Amide, alkene
17.	1680.85	Strong, medium, weak	C=O, C=N, C-H	Conjugated acid, conjugated ketone, imine/ oxime, aromatic compound
18.	1712.67	Weak	C-H	Aromatic compound
19.	1748.35	Weak, strong	C-H ,C=O	Anhydride, aromatic compound ester, δ-lactone
20.	1781.14	Strong, weak	C=O, C-H	Vinyl/ phenyl ester, conjugated acid halide, aromatic compound
21.	2885.31	Medium	C-H	Alkyne
22.	2976.92	Strong, weak	O-H, N-H	Carboxylic acid, alcohol, amine salt, primary amine

Table 6. Preliminary phytochemical analysis of different extracts of seaweeds

Seaweeds	Solvent used	Alkaloids	Flavonoids	Glycosides	Phenols	Quinine	Saponins	Steroids	Tannins
Padina tetrastromatica	Acetone	+	-	-	+	+	+	-	+
	Ethanol	+	+	+	+	+	+	-	+
	Methanol	+	+	+	+	+	+	-	+
	Water	+	-	+	-	-	-	+	+
Sargassum tenerrimum	Acetone	-	-	+	+	+	+	-	+
	Ethanol	+	-	-	+	+	+		+
	Methanol	-	-	+	-	+	+	-	-
	Water	-	-	-	-	-	-	-	+
Turbinaria ornata	Acetone	-	-	+	+	+	+	-	-
	Ethanol	-	+	+	+	+	+	+	+
	Methanol	+	+	+	+	+	+	-	+
	Water	+	-	-	+	+	-	+	+

Table 7. Cold water wash treatment used for colour fastness assessment.

COLD WATER WASH					
Species name	Mordant name	AQ-E	AC-E	ET-E	ME-E
Padina tetrastromatica	NONE	5	0	4-5	2
Padina tetrastromatica	CH_3COOH	5	0	4-5	0
Padina tetrastromatica	$FeSO_4$	5	0	5	0
Padina tetrastromatica	$NaHCO_3$	5	0	4-5	0
Sargassum tenerrimum	NONE	5	0	4-5	1
Sargassum tenerrimum	CH_3COOH	5	0	4-5	2
Sargassum tenerrimum	$FeSO_4$	5	0	0	0
Sargassum tenerrimum	$NaHCO_3$	5	0	3-4	0
Turbinaria ornata	NONE	4-5	0	5	0
Turbinaria ornata	CH_3COOH	5	0	4-5	0
Turbinaria ornata	$FeSO_4$	4-5	0	3-4	3-4
Turbinaria ornata	$NaHCO_3$	4-5	0	0	4-5

Scales: 5- Excellent, 4- Good, 3- Moderate, 2- Poor, 1-Very Poor, 0-No Efficient Results.

Note: AQ-E- aqueous dye extract, AC-E- acetone dye extract, ET-E- ethanol dye extract, ME-E- methanol dye extract.

Table 8. Hot water wash treatment used for colour fastness assessment.

HOT WATER WASH					
Species name	Mordant name	AQ-E	AC-E	ET-E	ME-E
Padina tetrastromatica	NONE	4-5	0	4	0
Padina tetrastromatica	CH_3COOH	4-5	0	4	0
Padina tetrastromatica	$FeSO_4$	5	1	4-5	1
Padina tetrastromatica	$NaHCO_3$	2-3	0	4	0
Sargassum tenerrimum	NONE	4-5	0	4-5	0
Sargassum tenerrimum	CH_3COOH	3-4	0	4-5	0
Sargassum tenerrimum	$FeSO_4$	5	0	2-3	0
Sargassum tenerrimum	$NaHCO_3$	2-3	0	3-4	0
Turbinaria ornata	NONE	1-2	0	4-5	0
Turbinaria ornata	CH_3COOH	1-2	0	2-3	0
Turbinaria ornata	$FeSO_4$	4-5	0	4-5	3
Turbinaria ornata	$NaHCO_3$	0	0	4-5	0

Scales: 5- Excellent, 4- Good, 3- Moderate, 2- Poor, 1-Very Poor, 0-No Efficient Results.

Note: AQ-E- aqueous dye extract, AC-E- acetone dye extract, ET-E- ethanol dye extract, ME-E- methanol dye extract.

Table 9. Soap water wash treatment used for colour fastness assessment.

SOAP WATER WASH					
Species name	Mordant name	AQ-E	AC-E	ET-E	ME-E
Padina tetrastromatica	NONE	2-3	0	1-2	0
Padina tetrastromatica	CH_3COOH	2-3	0	2-3	0
Padina tetrastromatica	$FeSO_4$	3-4	0	1	0
Padina tetrastromatica	$NaHCO_3$	3-4	0	0	0
Sargassum tenerrimum	NONE	4-5	0	2-3	0
Sargassum tenerrimum	CH_3COOH	2-3	0	2-3	0
Sargassum tenerrimum	$FeSO_4$	2-3	0	0	0
Sargassum tenerrimum	$NaHCO_3$	3-4	0	0	0
Turbinaria ornata	NONE	4-5	0	0	2
Turbinaria ornata	CH_3COOH	1-2	0	2	1
Turbinaria ornata	$FeSO_4$	2-3	0	0	0
Turbinaria ornata	$NaHCO_3$	2-3	0	0	0

Scales: 5- Excellent, 4- Good, 3- Moderate, 2- Poor, 1-Very Poor, 0-No Efficient Results.

Note: AQ-E- aqueous dye extract, AC-E- acetone dye extract, ET-E- ethanol dye extract, ME-E- methanol dye extract.

Table 10 Durability colour fastness of dyed fibres which were stored

Durability of dye on cotton fibres					
Species name	Mordant name	AQ-E	AC-E	ET-E	ME-E
Padina tetrastromatica	NONE	5	0	4-5	0
Padina tetrastromatica	CH_3COOH	5	0	5	0
Padina tetrastromatica	$FeSO_4$	5	0	4-5	0
Padina tetrastromatica	$NaHCO_3$	5	0	5	0
Sargassum tenerrimum	NONE	5	0	5	0
Sargassum tenerrimum	CH_3COOH	5	0	4-5	0
Sargassum tenerrimum	$FeSO_4$	5	0	5	0
Sargassum tenerrimum	$NaHCO_3$	5	0	4-5	0
Turbinaria ornata	NONE	5	0	5	0
Turbinaria ornata	CH_3COOH	5	0	5	0
Turbinaria ornata	$FeSO_4$	5	0	4-5	0
Turbinaria ornata	$NaHCO_3$	5	0	5	0

Scales: 5- Excellent, 4- Good, 3- Moderate, 2- Poor, 1-Very Poor, 0-No Efficient Results.

Note: AQ-E- aqueous dye extract, AC-E- acetone dye extract, ET-E- ethanol dye extract, ME-E- methanol dye extract.

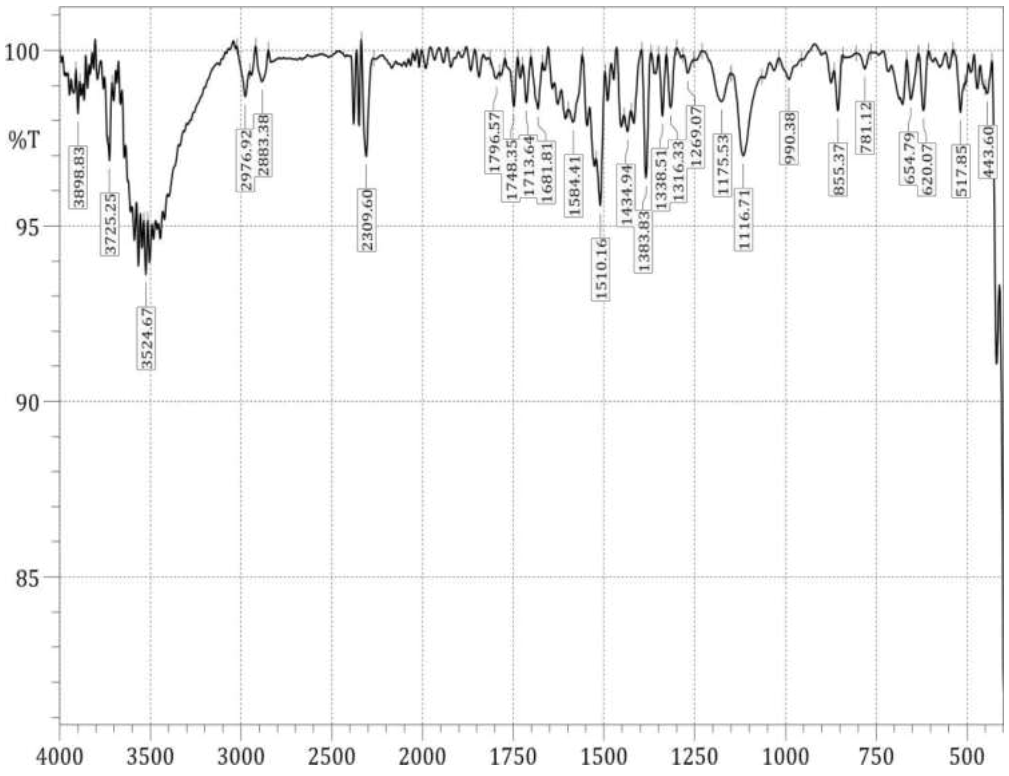


Figure 4 FTIR spectral peak values (cm^{-1}) and functional groups obtained from *Padina tetrastromatica* sample

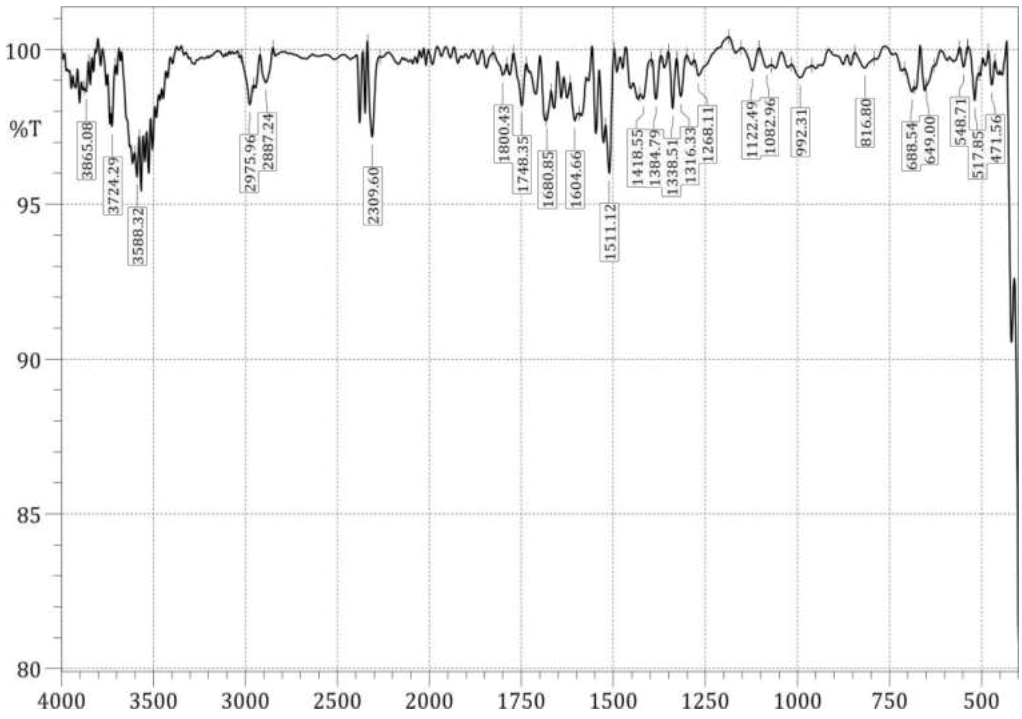


Figure 5 FTIR spectral peak values (cm^{-1}) and functional groups obtained from *Sargassum tenerrimum* sample

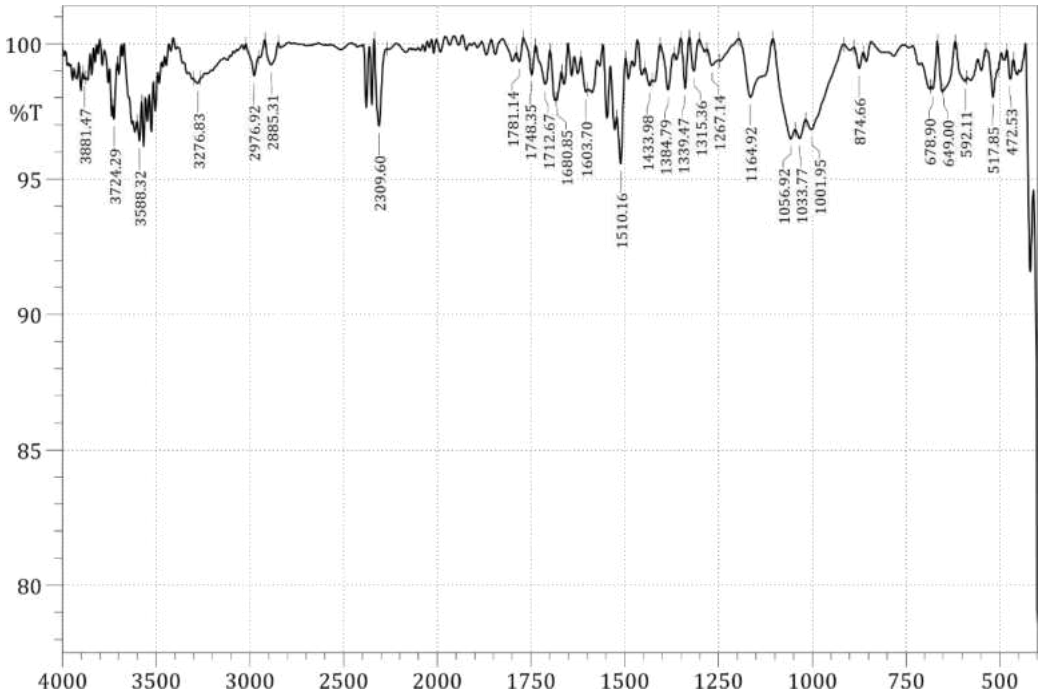


Figure 6 FTIR spectral peak values (cm^{-1}) and functional groups obtained from *Turbinaria ornata* sample

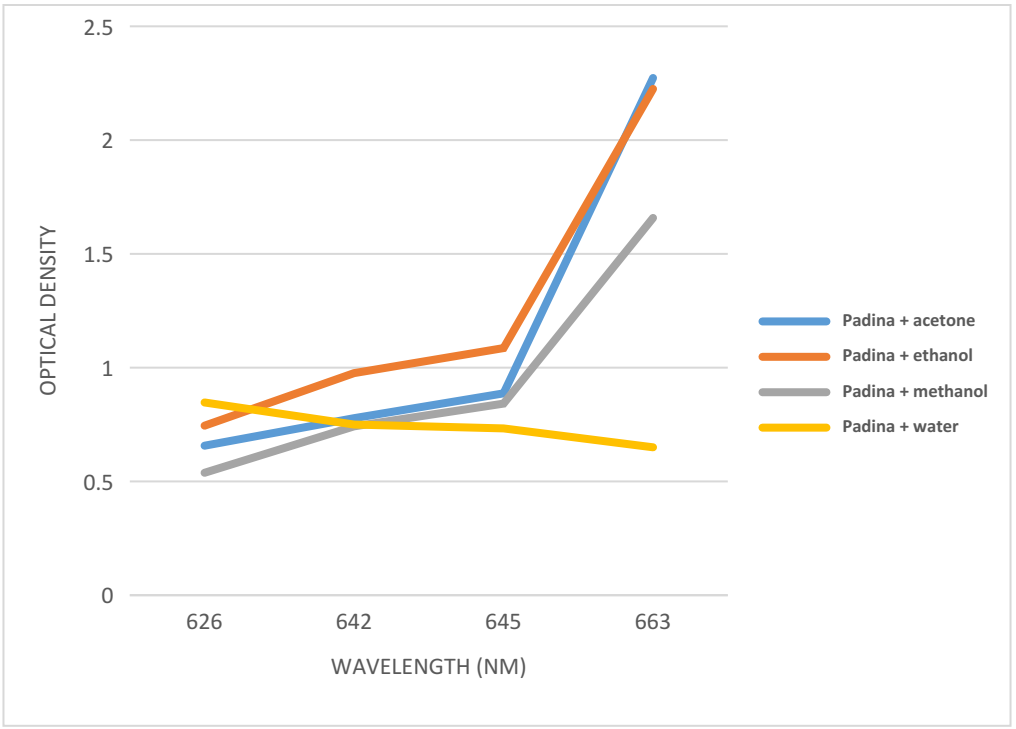
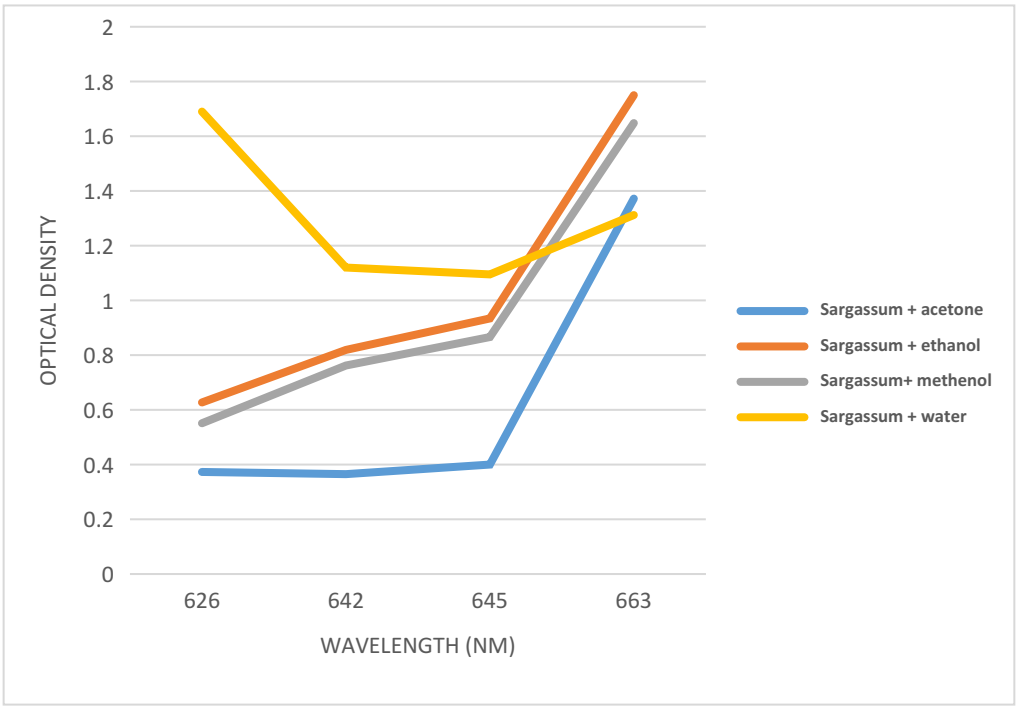


Figure 1 Estimation of light absorption capacity of *Padina tetrastromatica* dye extracts



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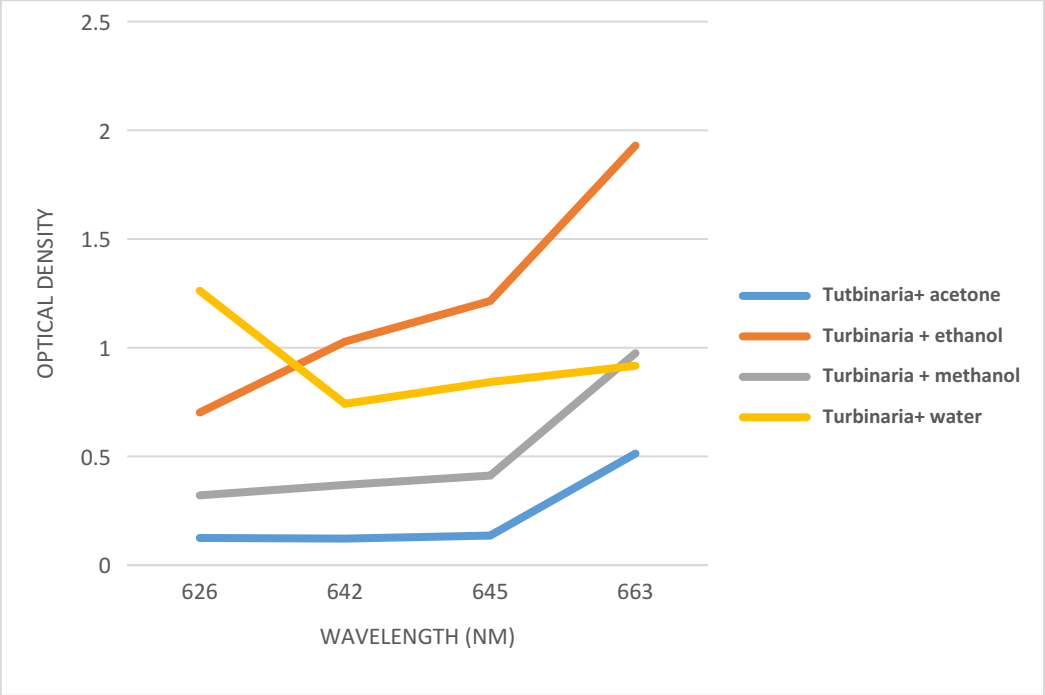


Figure 3 Estimation of light absorption capacity of *Turbinaria ornata* dye extracts

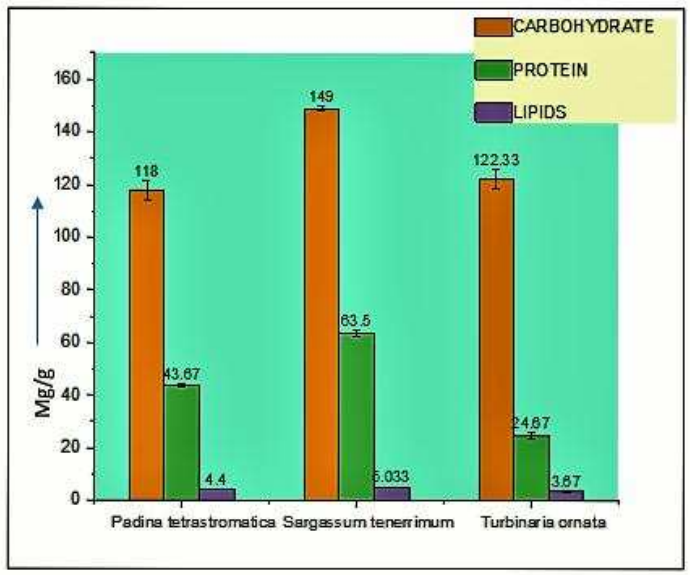


FIGURE 7. Estimation of primary metabolites in seaweeds collected from Hare Island.

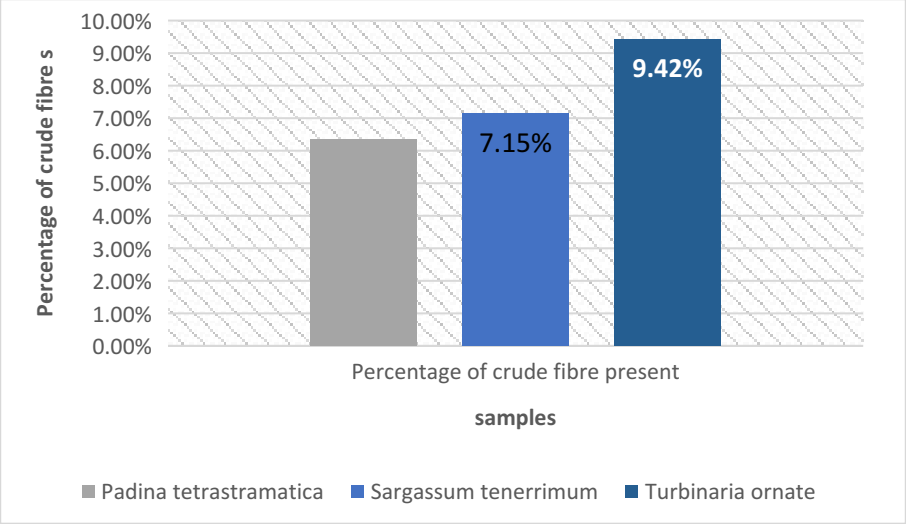


FIGURE 8 Diagrammatic representation of amount of crude fibre present in three brown seaweeds (in %)

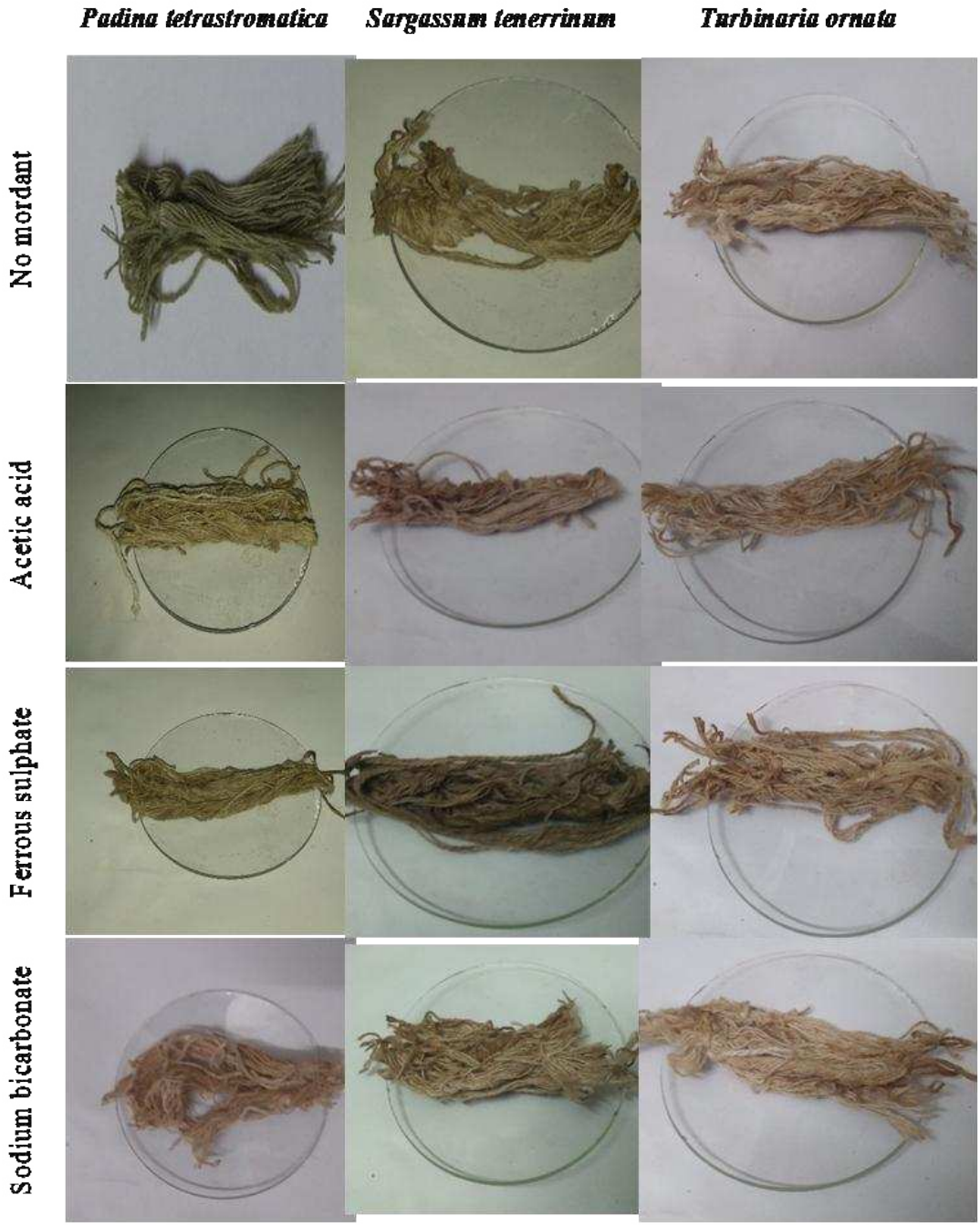


Plate 1. Colours intensity showed in uncoated cotton threads after the treated by aqueous extracts of brown seaweeds

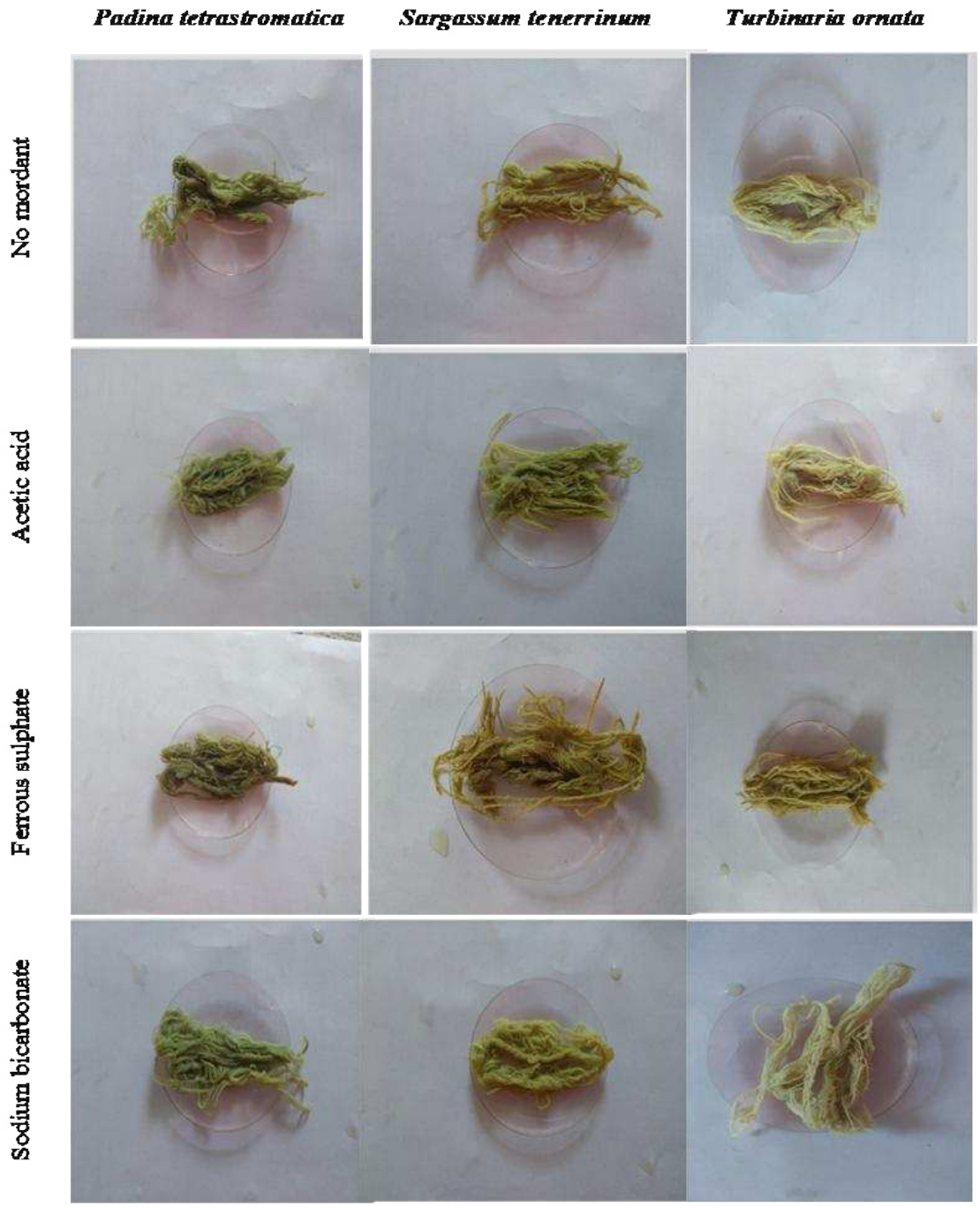


Plate 2. Colours intensity showed in uncoated cotton threads after the treated by acetone extracts of brown seaweeds

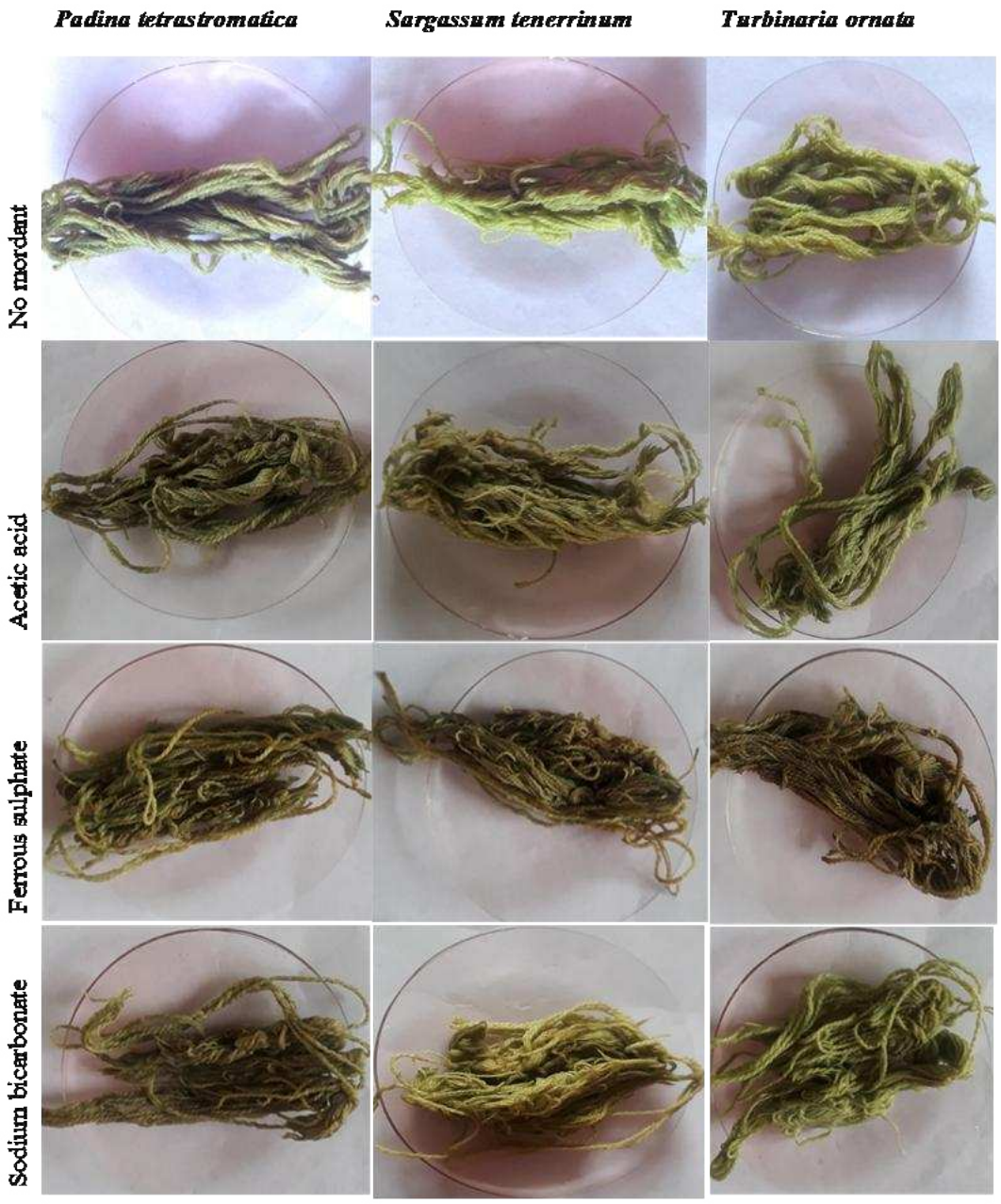


Plate 3. Colours intensity showed in uncoated cotton threads after the treated by ethanol extracts of brown seaweeds

Plate 4. Colours intensity showed in uncoated cotton threads after the treated by methanol extracts of brown seaweeds

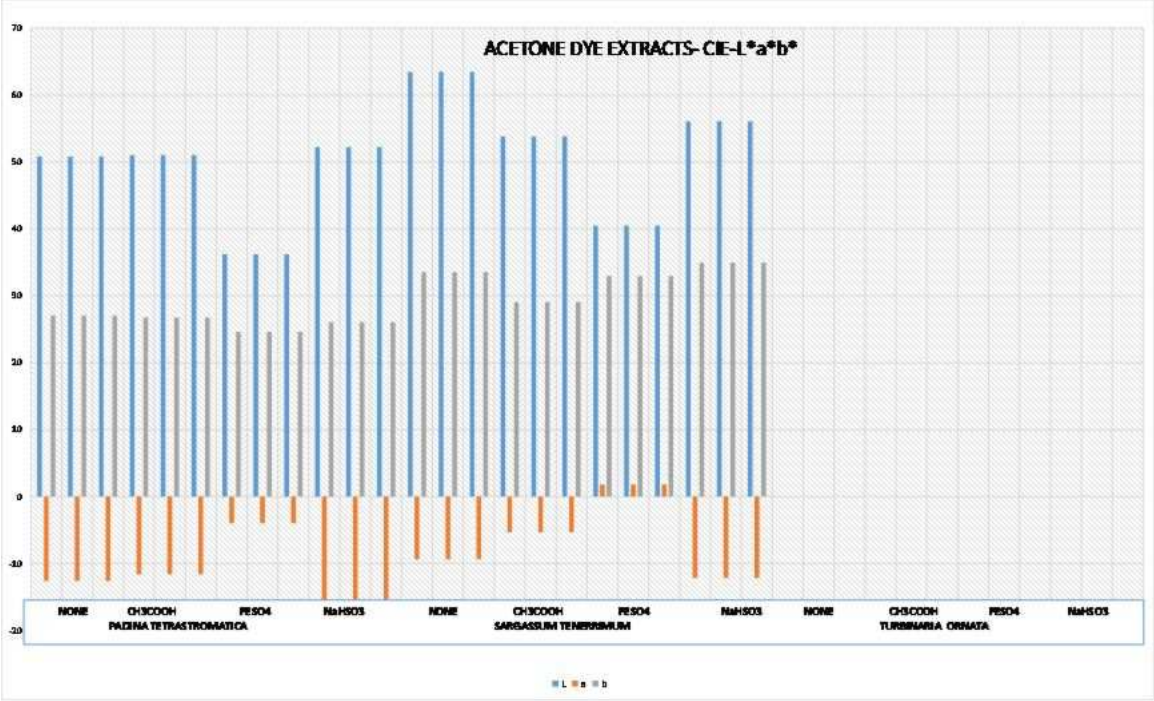
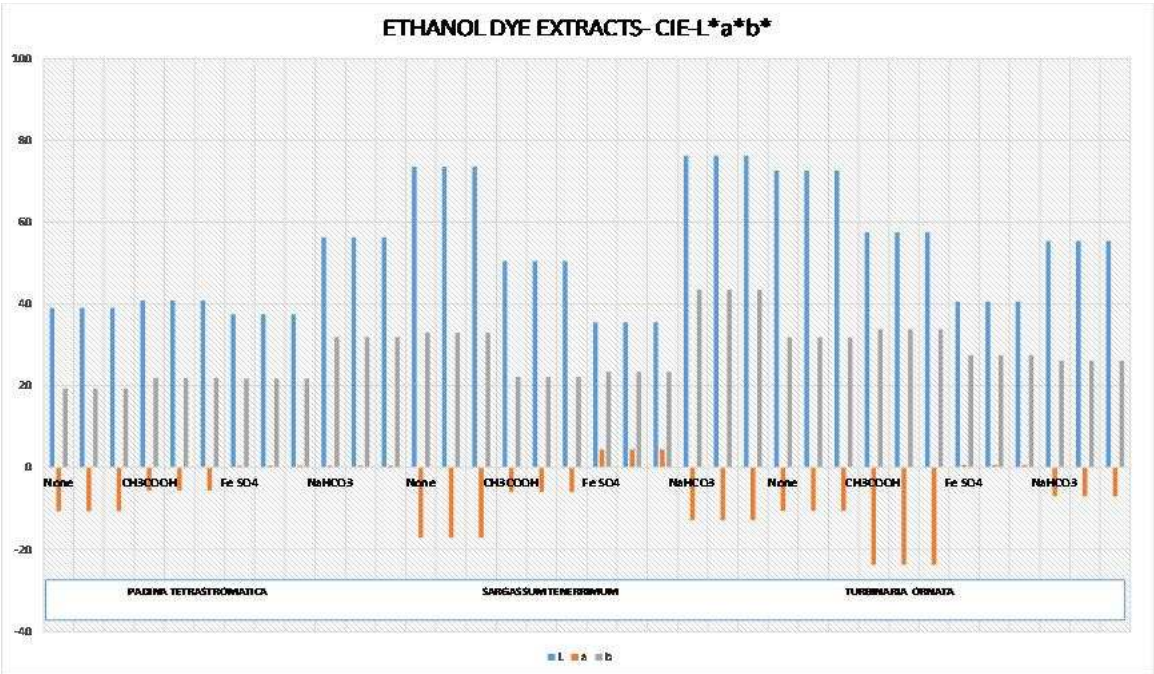


*Figure 9 L*a*b* values for dyed cotton fibres with seaweeds dye extracts extracted by acetone*



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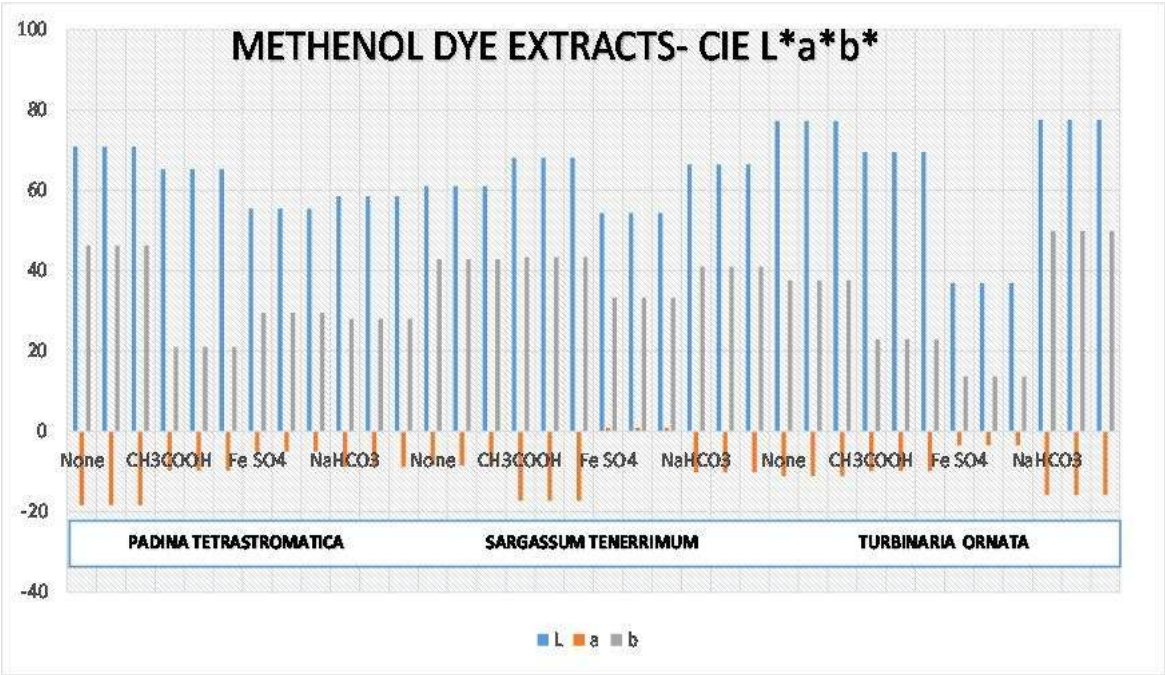

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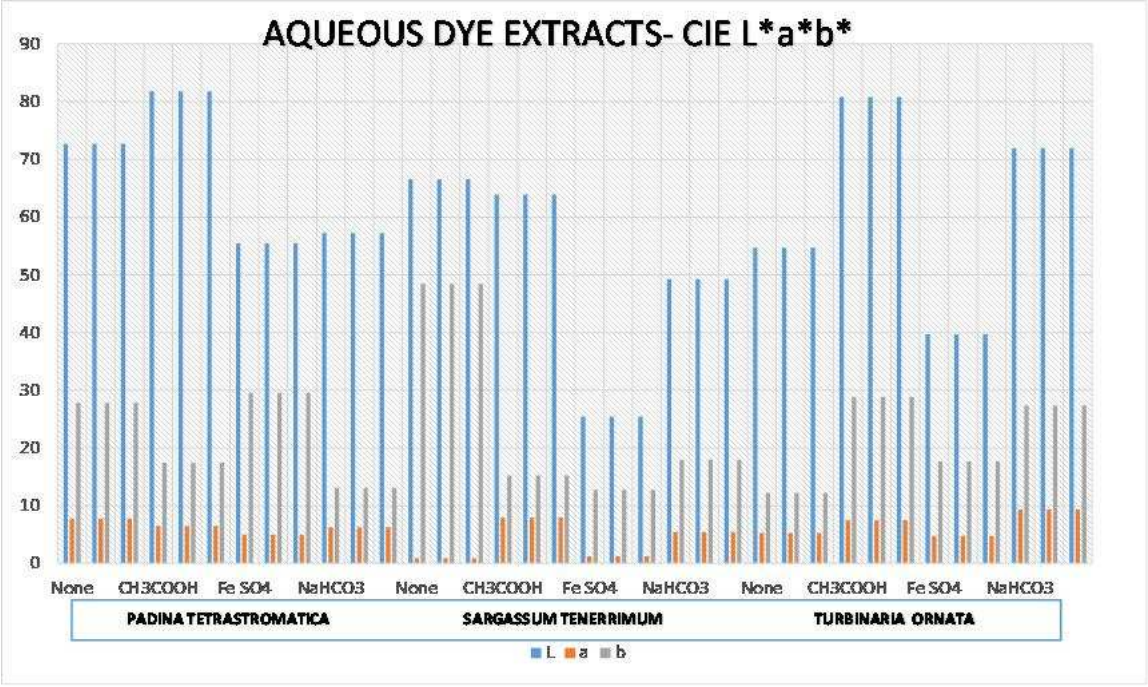

Figure 12 L*a*b* values for dyed cotton fibres with seaweeds dye extracts extracted by water

CHARACTERISATION OF ENDOPHYTIC BACTERIA IN *SYRINGODIUM ISOETIFOLIUM*

A short term project work submitted to

ST. MARY'S COLLEGE (AUTONOMOUS)

Affiliated to

MANONMANIAM SUNDARANAR UNIVERSITY

in partial fulfilment of the requirement for the degree of

Master of Science in Botany

By

D. SULAMITHIYAL

19APBO15

DEPARTMENT OF BOTANY

ST. MARY'S COLLEGE (Autonomous)

THOOTHUKUDI-628001

APRIL, 2020 – 2021

CERTIFICATE

It is certified that this short term project work entitled **CHARACTERISATION OF ENDOPHYTIC BACTERIA IN *SYRINGODIUM ISOETIFOLIUM*** submitted by **D. SULAMITHIYAL** in partial fulfilment of M.Sc. degree in Botany to **ST. MARY'S COLLEGE (Autonomous)** affiliated to **MANONMANIAM SUNDARANAR UNIVERSITY**, Thirunelveli is based on the results of studies carried out by her under my guidance and supervision. It is further certified that this short term project work or any part it has not been submitted elsewhere for any other degree.

GUIDE

HEAD OF THE DEPARTMENT
HOD of Botany
St. Mary's College (Autonomous)
Thoothukudi- 628 001.

EXAMINER 16/04/2021

PRINCIPAL
St. Mary's College (Autonomous)
Thoothukudi - 628 001.

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INTRODUCTION

INTRODUCTION

Plant makes associations with beneficial microbes in the environment they survive to make their survival easy. These microbes can either be bacteria or fungi. Plant-beneficial bacteria provide numerous benefits to their host plants, helping them in tolerating various biotic and abiotic stresses. Bacteria can associate with their host either endophytically or ectophytically. Endophytic bacteria are the beneficial bacteria that thrive inside the plant body. The endophytic bacteria are associated with roots or rhizomes of terrestrial and aquatic plants. The population of endophytic bacteria are either gram-positive or gram-negative bacteria (Kobayashi and Palumbo, 2000) and help plant growth either by establish the availability of limited nutrients, responding to pathogens, stress and pollutants through bacteria-plant interactions (Dalton *et al.*, 2004, Chi *et al.*, 2005, Siciliano *et al.*,2001, Ikeda *et al* .,2010 and Li *et al* .,2011).

Seagrasses are primary producers, developing rich and highly productive meadows in shallow coastal region with several ecological and biogeochemical significances. Seagrasses balance water quality by purify unwanted matter in the water column (Short and Short 1984), by inhibiting marine pathogens (Jensen *et al.*,1998 and Marhaeni *et al.*,2011) and provide a link between sediment and water column nutrient cycles (Vissini *et al.*,2002 and Papadimitriou *et al.*,2005).This studies indicate the mutual interactions found between the seagrasses and associated microbial communities.

Bacteria are living endophytically within plant tissues (e.g., Hallmann and Berg, 2006). Endophytic bacteria are living inside plant tissues not harming the host plant (Schulz and Boyle, 2006), promote plant growth by for contribute nutrients and restrict plant pathogens (e.g., Hallmann and Berg, 2006; Ikeda *et al.*, 2010; Li *et al.*, 2011).

Endophytes are bacteria or fungi that present in healthy plant tissues without causing any distinct symptoms of disease (Wilson, 1995). They are omnipresent in all plants. There are interactions between plants and hosts which involves mutualism and antagonism (Carroll, 1988). In symbiosis, endophytes produce numerous compounds that promote growth of plants (Lee *et al* .,2004).

The composition of symbiotic bacterial population is abundant in terrestrial and freshwater plants (Ueda *et al.*, 1995). The presence and signification of symbiotic bacteria in marine plants are less explored.

Seagrasses are marine angiosperms that developed from freshwater angiosperm ancestors (den Hartog, 1970). The seagrass flora is constricted to approximately 50–60 species. (Hemminga and Duarte, 2000; Short *et al.*, 2007). They play essential roles in marine ecosystems. Although angiosperms are the most diverse terrestrial plant with over 250,000 species, there are only around 70 species of seagrasses. They are individually moved adapted to the marine ecosystem and formed between 70 and 100 million years ago (Les *et al.*, 1997; Wissler *et al.*, 2011). Seagrasses are keystone species in coastal regions. (Costanza *et al.*,1997). The seagrass beds are increases by climate change, pollution and habitat fragmentation. The restoration is expensive and has a low success rate (Orth *et al.*, 2006).

Seagrass are important global carbon sinks, induce coastal biodiversity and prevent coastal erosion (Hemminga and Duarte, 2000; Orth *et al.*, 2006). Bacterial population play a vital role in seagrass meadows (Hemminga and Duarte, 2000). The information about bacterial communities associated with seagrasses is limited, with most studies focusing on bacterial communities in seagrass sediments (Cifuentes *et al.*, 2000; Bagwell *et al.*, 2002; Garcia-Martinez *et al.*, 2009) or associated with plant surfaces (i.e., epiphytic

bacterial community) above (Weidner et al., 2000; Jensen *et al.*, 2007; Uku *et al.*, 2007; Crump and Koch, 2008) or belowground (Garcia-Martinez *et al.*, 2009). The endophytic bacteria in seagrass tissues have been reported using optical microscopy (Kuo, 1993). *Clostridium* glycolicum has been isolated from the *Halodule wrightii* (Küsel *et al.*, 1999), and a new species has been isolated (Ivanova *et al.*, 2004) from the roots of *Z. marina* (Nielsen *et al.*, 1999). These studies indicate that endophytic bacteria occur in seagrass tissues.

Symbiotic microorganisms can play an important role in regulate seagrass population dynamics. They can promote the uptake of elements like nitrogen, which can be limited in marine environments. The growth rates of marine angiosperms in marine sediments receiving high organic matter information. The decomposition of organic matter under depleted dissolved oxygen conditions can invade into seagrass tissues (Pedersen *et al.*, 2004), with negative outcome for seagrass meristematic activity (Garcias-Bonet *et al.*, 2008). Endophytic symbionts including bacteria and fungi live within plant tissues without causing negative effects. It became evident that endophytes are rich source of bioactive natural products, and many different agents have been isolated from these microorganisms (Berdy, 2005).

The several reports on composition and ecological roles of symbiotic bacteria population associated with terrestrial and fresh water plants. The presence and relevance of endophytic bacteria in marine plants is almost unexplored. The present study is aimed at exploring the endophytic bacteria in *Syringodium isoetifolium*. The roots, rhizomes, and leaves were analysed in search of endophytic bacteria. This is the first report characterizing the endophytic bacteria in *Syringodium* leaf.

SCOPE AND OBJECTIVE

SCOPE AND OBJECTIVE

The endophytic bacteria belong to a larger group of microorganisms that have their life-cycle partly or entirely inside the plant and are located in intra and inter-cellular spaces or in the vascular tissue. The association between endophytic bacteria and plants features important benefits such as significant increases in growth, plant biomass, length of roots, dry matter production, and grain yield. Studies show that there is a great diversity of endophytic bacteria colonizing plant structures that result in several benefits to the host plant. Endophytic bacteria are one of the alternative control methods to support sustainable agriculture. Endophytic bacteria have PGPR traits like phosphate solubilization, production of auxin (IAA), ammonia production, HCN production, siderophore production, nitrogen fixation and sulphate solubization. They can be used for phytoremediation. They are also tested for the production of extracellular enzymes like urease, amylase, lipase, cellulase and asparginase. They have potential to increase the soil fertility and control disease.

The present study aims at

- isolating the bacterial strains from leaf, rhizome and roots of the seagrass *Syringodium isoetifolium*,
- test the gram staining properties of the isolates
- characterise any one pure endophytic bacterial colony from the leaf

LITERATURE REVIEW

REVIEW OF LITERATURE

Beneficial plant–microbe interactions promote plant growth, health and development. Endophytic bacteria have been associated with almost all the plants. The International Union for Conservation of Nature and Natural Resources estimates that there are about 297,326 species of plants (Gymnosperms, Ferns, Mosses Monocotyledons and Dicotyledons,), but only a few of them have been studied for their endophyte microbiota (Strobel and Daisy, 2003). They colonize the internal tissues of their host plants and can form mutualistic, symbiotic, trophobiotic and commensalistic. Most endophytes appear to originate from the rhizosphere. But some may be transmitted next generation through the seed. Endophytic bacteria can promote plant growth and yield. Endophytes produce a range of natural products that could be utilized for potential use in agriculture, medicine, or industry. It has been shown that they have the potential to remove soil contaminants by enhancing phytoremediation. They play a role in soil fertility through nitrogen fixation and phosphate solubilization. They can act as biocontrol agents. There is increasing interest in developing the potential biotechnological applications of endophytes for improving phytoremediation and the sustainable production of nonfood crops for biomass and biofuel production.

The most abundant metabolite producing Gram-positive bacteria endophytes found within diverse environments are *Bacillus* and *Streptomyces* species (Reinhold-Hurek and Hurek, 2011; Frank *et al.*, 2017). Lipopeptides are among the most important classes of secondary metabolites produced by endophytic bacteria, which are formed by cyclic or short linear peptides linked to a lipid tail or lipophilic molecules. Lipopeptides may show

antimicrobial, cytotoxic and surfactant activities; they are synthesized by non-ribosomal peptide synthetases (NRPS), or polyketide synthase (PKS) and have great structural diversity based on a hydrophobic fatty acid acyl chain of 13 to 17 carbons, linked to a hydrophilic peptide of 7–25 aminoacids. Lipopeptides are important for both, their antibiotic activity and for inducing plant defense mechanisms (Stein, 2005; Raaijmakers *et al.*, 2010One bacterial strain may synthesize several polypeptide isoforms. Bacillus and Paenibacillus-related lipopeptides are the most studied ones (Villarreal-Delgado *et al.*, 2018), whereas several Bacillus amyloliquefaciens strains have been recognized as higher lipopeptides producers (Ongena and Jacques, 2008).

Among plant microbiota, endophytic bacteria can be found in most plant species and be recovered from roots, leaves, stems, and a few from flowers, fruits, and seeds. They have the potential to produce a variety of secondary metabolites with application in agriculture and pharmaceutical and industrial biotechnology. Bacterial endophytes live within cell walls and xylem vessels intercellular regions and they may colonize seeds, fruits, and flowers, among other tissues. It is known that endophytic bacteria are located in the apoplast, and plant roots are proposed to be the entry point. It is also suggested that they are transmitted using an alternative vertical strategy due to their presence in flowers and seeds. The potential explanation for their ubiquitous presence into plant tissues is the diversity of positive effects on plant growth and fitness they have shown, by stimulating the host phenylpropanoid pathway or by producing several linked-metabolites to the plants' metabolism. Many reports indicate that bacterial endophytes help to provide nutrients as plant growth-promoters, and induce tolerance/resistance against biotic and abiotic stress.

Secondary metabolites spinosyn A and D, are produced by the soil actinomycete *Saccharopolyspora spinosa*, highly effective against lepidopteran and dipteran pests,

among others, which commercial product named spinosad, has been commercialized for ~250 countries and adopted in integrated pest management programs worldwide. Furthermore, there are reports of endophytic *Saccharopolyspora* species, although their potential as bioinsecticide has not yet been elucidated (Qin *et al.*, 2011). Many endophytic actinomycete compounds are isolated and have found application not only as antimicrobial agents but also as cytotoxic agents against tumor cells. Some members of the Gram-positive bacteria group have been recently found as endophytes in different plant species (Eljounaidi *et al.*, 2016). Endophyte extracts have demonstrated to be a better choice versus chemotherapy agents due to their antitumor activity efficacy and lower side-effects, since they are less toxic to normal cells and more effective against several drug resistant microorganisms. As a consequence, the natural endophyte-derived metabolites have attracted peculiar attention with the purpose of being human cancer-chemo preventive compounds and anticancer chemotherapeutic drugs (Cardoso-Filho, 2018).

Singh *et al.*, (2017) studied that endophytic inoculation of the plants modulates the synthesis of bioactive compounds with high pharmaceutical properties besides promoting growth of the plants. Hydrolases, the extracellular enzymes, produced by endophytic bacteria, help the plants to establish systemic resistance against pathogens invasion. Phytohormones produced by endophytes play an essential role in plant development and drought resistance management. The high diversity of endophytes and their adaptation to various environmental stresses seem to be an untapped source of new secondary metabolites. Their review summarizes the role of endophytic bacteria in synthesis and modulation of bioactive compounds.

Gouda *et al.*, (2016) analyze that Endophytes are an endosymbiotic group of microorganisms that colonize in plants and microbes that can be readily isolated from any microbial or plant growth medium. They act as reservoirs of novel bioactive secondary metabolites, such as alkaloids, phenolic acids, quinones, steroids, saponins, tannins, and terpenoids that serve as a potential candidate for antimicrobial, anti-insect, anticancer and many more properties. The review aims to comprehend the contribution and uses of endophytes as an impending source of drugs against various forms of diseases and other possible medicinal use.

Afsal *et al.*, (2019) highlighted that endophytic bacteria are the plant beneficial bacteria that thrive inside plants and can improve plant growth under normal and challenging conditions. They can benefit host plants directly by improving plant nutrient uptake and by modulating growth and stress related phytohormones. Indirectly, endophytic bacteria can improve plant health by targeting pests and pathogens with antibiotics, hydrolytic enzymes, nutrient limitation, and by priming plant defenses. The review elaborates the factors affecting diversity of bacterial endophytes, their host specificity and mechanisms of plant growth promotion and also accentuates various methods used to study endophytic communities, wild plants as a source of novel endophytic bacteria, and innovative approaches that may improve plant-endophyte association.

Nair and Padmavathy (2014) investigated that Impact of Endophytic Microorganisms on Plants, Environment and Humans. Endophytes are microorganisms (bacteria or fungi or actinomycetes) that dwell within robust plant tissues by having a symbiotic association. They are ubiquitously associated with almost all plants. They produce a wide range of compounds useful for plants for their growth, protection to environmental conditions and sustainability, in favors of a good dwelling place within the hosts. They protect plants

from herbivory by producing certain compounds which will prevent animals from further grazing on the same plant and sometimes act as biocontrol agents. A large number of bioactive compounds produced by them not only are useful for plants but also are of economic importance to humans. They serve as antibiotics, drugs or medicines, or the compounds of high relevance in research or as compounds useful to food industry. They are also found to have some important role in nutrient cycling, biodegradation, and bioremediation. In the review, they have tried to comprehend different roles of endophytes in plants and their significance and impacts on man and environment.

Galaviz-Silva *et al.*, (2018) identified marine microorganisms from Mexican coasts that have antimicrobial activity against *Staphylococcus aureus* and *Vibrio parahaemolyticus*, which are known worldwide to be food-poisoning agents. Of the 42 tested strains, 15 inhibited these pathogens. *Bacillus* and *Virgibacillus* strains are identified by 16S rRNA gene sequencing. Biofilm production by all strains was moderate, but *B. acillus altitudinis* produced a stronger biofilm. This is the first study to isolate *Bacillus aerius*, *Bacillus oryzicola*, *Bacillus safensis*, *Bacillus boroniphilus*, *Bacillus altitudinis* and *Virgibacillus. senegalensis* from marine ecosystems in Mexico as well as the first study to report their inhibitory effects against both *Staphylococcus aureus* and *Vibrio parahaemolyticus.*

The Importance of Seagrass

Seagrasses are marine flowering plants fall under in monocotyledonae. They are adapted to the marine environment and complete their life cycle under water In contrast to other submerged marine plants (e.g. seaweeds), sea grasses flower, fruit and produce seeds. They also have true roots and internal system for the transport of gases and nutrient. They are classified in three separate families. Worldwide there are about 12 major divisions,

consisting of approximately 60 species of sea grass. The 72 species of sea grasses are commonly divided into four main groups: Zosteraceae, Hydrocharitaceae, Posidoniaceae and Cymodoceae. Most common names are applied to sea grass species, such as turtlegrass, eel grass, tape grass, spoon grass, and shoal grass. Sea grasses are often confused with sea weeds, but it is not sea weeds.

Torre-Castroa *et al.*, (2014) analyzes the importance of Seagrass for a small-scale fishery. Seagrasses provided highest fish catches and income at community level. This study investigated the Seagrass are key ecosystems supporting Small scale fishing and protection and management are urgently needed. Adoption of a seascape approach considering all ecosystems underpinning Small scale fishing and the social aspects of fishing and a shift in emphasis from pure conservation to sustainable resource management would be desirable.

Short and Coles (2001) highlighted the importance of seagrasses. Seagrasses provide food for Sea turtles, nearly 100 fish species, water fowl and for the marine mammals the Manatee and the dugong. Seagrasses also support complex food webs by virtue of their physical structure and primary production and are well known for their role as breeding grounds and nurseries for important crustacean, finfish and shellfish populations. Seagrasses are the basis of an important detrital food chain. The plants filter nutrients and contaminants from the water, stabilize sediments and act as dampness to wave action. Seagrasses rank with coral reefs and mangroves as some of the world's most productive coastal habitat and strong linkages among these habitats make the loss of seagrasses a contributing factor in the degradation of the world's ocean.

Seagrasses are submerged flowering plants found in shallow marine waters, such as bays and lagoons and along the continental shelf in the Gulf of Mexico. A vital part of the

marine ecosystem due to their productivity level, seagrasses provide food, habitat, and nursery areas for numerous vertebrate and invertebrate species. The vast biodiversity and sensitivity to changes in water quality inherent in seagrass communities makes seagrasses an important species to help determine the overall health of coastal ecosystems. Seagrasses perform numerous functions. They are stabilizing the sea bottom, Providing food and habitat for other marine organisms, maintaining water quality and supporting local economies.

Torre – Castro (2006) analyze the societal importance of seagrass by investigating the linkage between humans and seagrass system. Seagrass meadows provide Socio ecological resilience. The goods and services associated with seagrass ecosystem and also appreciated by locals are fishing and collection grounds as well as substrate for seaweed cultivation. Torre – Castro (2006) studied that seaweed farmers and fisherman considered seagrass ecologically and economically important. Seaweed farmers consider seagrass an important indicator for seaweed cultivation. However, the relation between farmers and seagrasses is complex. Seagrasses are used directly in the community, as fertilizers, medicines and indicators of current and seasonality. Seagrasses are a very important component of the local economy.

Hemminga and Duarte (2000) studied the seagrasses provide numerous goods and services linked to the structure and function of the whole ecosystem. The capacity of Seagrasses to provide habitat, refuge, foraging, nursery and spawning areas due to their morphological characteristics, nutrient recycling abilities, dual reproduction strategies and their great plasticity.

Gokulakrishnan *et al.*, (2016) highlighted that function of Seagrass ecosystem. Seagrass ecosystem is one of the most common and productive marine habitats which play an

important role in the overall health of coastal ecosystems. High standing crop produces large amounts of dissolved and particulate detritus which form the basis of important food chains both within the seagrass ecosystem and shore ward and offshore as the materials are washed away from the seagrass. The leaves and erect shoot surfaces are home for epibiotic organisms. This increase both primary and secondary productivity, as well as providing a large amount of food sources for fish and invertebrates. Because, the seagrass are rooted in their substrate and produce shoots with leaf bundles, they stabilize their habitat. The leaves form a baffle, which slows and retards current and wave activity, which promotes sedimentation of particles as well as inhibiting re-suspension of organic and inorganic materials. Seagrass creates an active environment for nutrient cycle. Overall, seagrass ecosystems enhance the ecological function of coastal zones by increasing the productivity and biomass of the region.

Fonseca (1986) analyze that seagrass meadows are the nursery grounds for many commercial fishes and crustacean species. The juveniles come into the seagrass meadows for protection against predators, to feed on the epiphytes growing on seagrass plants and to feed on the organic detrital rain that falls into the meadow from the water above. The juvenile tiger prawns (*Penaeus esculentus* and *P. semiscatus*) and endeavour prawns (*Metapenaeus ensis* and *M. endeavouri*) have seagrass meadows as their nursery grounds. Post-larvae of both tiger and endeavour prawns settle from the water column into the shallow inshore seagrass meadows and they move into the deeper meadows after getting the matured growth.

The juveniles of the Western rock lobster forage in seagrass meadows close to the reefs in which they shelter. This meadow is helpful to improve the diversity of most of the organisms which are economically important. Seagrass meadows reduce the speed and

change the pattern of currents. This process results in depositional environments. Few larger animals possess the ability to actually digest seagrass leaves (dugong, turtle, geese, brants and some herbivorous fish). Seagrass leaves often harbour a multitude of organisms such as algae and invertebrates, which serve as food for transient fish, as well as the permanent fauna within the seagrass meadow. Moreover, adult fish migrate from adjacent habitats, like coral reefs and mangrove areas, to the seagrass meadows at night to feed on the rich food sources within the seagrass meadows. Many small subsistence fishing practices are totally depended on seagrass meadows for their fishing grounds. The coastal populations in such areas receive most of their protein from fishing within seagrass meadows.

Koch *et al.*, (2001) carry out the leaf canopy and the network of rhizome and root fix and stabilize the sediment over which seagrass grow, and reduce the re-suspension of the sediment by currents and waves. This role is driven by reduced water motion due to canopy friction and by the structural frame that rhizomes and roots provide to the sediments. Sediments vegetated by seagrass are less likely to be mobilized by waves and currents, so that seagrass can reduce the erosion of the coastline. Detached seagrass leaves, which are lost either at the end of their life or earlier due to waves and storms, and their accumulation in the beaches, represent another way by which seagrass has a role in the protection of the shoreline.

Large accumulation of leaves, such as those of *Posidonia oceanica* in the dissipate wave energy and directly protect beach sediments from the impact of waves. Seagrass are important elements of coastal protection through the sediments being eroded. In the Mediterranean, the particles that constitute the sediment have in many cases a biological origin being fragments of the skeletons, shells of spines of marine animals or being the

calcareous remains of benthic algae. As seagrass harbor a large diversity of marine organisms, the meadows can be considered a net source of new sediment. Biogenic particles can be the main component of sediment in coastlines with no rivers or with low fluxes of particulate matter from land to the sea. In such areas sediment produced by seagrass meadows may contribute significantly to feed the beaches, further contributing to curb coastal erosion.

Fortes (1990) studied that Seagrass play a very important role as basic land builders and shore stabilizers, similar to that of sand dune and mangrove vegetation. Seagrass, although of limited direct economic profit, have been used for various purposes in different parts of the world. Coastal people use rhizomes of *Cymodocea sp.* (nicknamed as sea sugarcane) as food, for the preparation of salad. Seagrass are also used as raw materials in paper industry and in the production of fertilizer, fodder and feed. Most of the seagrass are used extensively as soil fertilizer for coconut and other plantations. A variety of medicines and chemicals are also prepared from them. Agar like substance and *zosterin* is extracted from *Zostera sp.*

Terrados *et al.*, (2004) analyze that traditionally, seagrass have many uses. Seagrass are used to prepare the baskets, extracted for soda salt and used as minor fuel, stuffing, insulating and packing material, fertilizers, etc. In addition to that, they have been used as a sewage filters, coastal stabilizers, paper manufacture, fodder and compost. Further, it can be utilized for roof covering and after removing the excess salts in the leaf, it can be used as house insulation.

Torre- Castro and Ronnback (2004) investigated that Seagrass used as a traditional medicine against skin diseases. Seagrass seeds of several species are used as a food source. Seagrass leaves from several species viz., *Z. marina, T. ciliatum, E. acoroides, P.*

oceanica, *P. iwatensis* and *P. torreyi* are gathered from the wrack line or cut above the surface of the sediment, dried and used for thatch, animal bedding, mattress and pillow stuffing and cordage. Bandeira and Gell (2003) are carry out the seeds (raw) and rhizomes (ground into flour) of *E. acoroides* are also consumed as food. *H. ovata* leaves are principal ingredient in a paste used to treat various skin ailments.

Connolly *et al.*, (1995) highlighted the coastal Chumash began to fashion cordage, thatch and footwear from the leaves of *Phyllospadix torreyi*. Coastal people used the thin and silicate strengthened leaves of this plant as raw material to weave fishing line and thatch shelters for thousands of years.

Gokulakrishnan *et al.*, (2016) studied that the abundance of seagrass is being destroyed due to the several activities particularly anthropogenic activity. Direct human impacts to seagrass which includes; fishing and aquaculture, introduced exotic species, boating and anchoring, and habitat alteration viz., dredging, reclamation and coastal construction, etc. Fishing methods such as dredging and trawling may significantly affect seagrass by direct removal. Damage to *Zostera marina* by scallop dredging reduces shoot density and plant biomass and digging for clams can also exert extensive damage.

Rasmussen (1977) studied that the exploitation of marine resources and the use of certain types of fishing gear like bottom trawls have detrimental effects on seagrass beds. Mussel harvest in the Dutch Wadden sea is believed to be a major factor in the loss of *Z. marina* and *Z. noltii*. Moreover, the use of dynamite poison contributes to the rapid destruction of the seagrass habitat. The impact of dynamite on the reproductive capacity of fish will surely lead to a decline in fish population in a certain habitat. Likewise, the residual effects of cyanide are irreversible or it may take several years to recover the seagrass ecosystem. In addition to that, the large scale loss of seagrass that occurred on both sides

of the North Atlantic Ocean in the early 1930s, a result of "eelgrass wasting disease" have many effects on the ecosystem . Associated with this loss are a collapse of scallop fisheries and dramatic reductions in waterfowl populations. In addition, it result extinction of a marine gastropod.

Endophytic bacteria in seagrass

Tarquino *et al.*, (2019) investigated that the presence of microbes in seagrass is important for the development of seagrasses from seed germination and protect against pathogens and saprophytes.

Ugarelli *et al.*, (2017) studied that the activities of various groups of heterotrophic bacteria in the sulfate-rich water-column and sediment. These bacteria stimulated to increased nutrient concentrations and these bacteria detoxify sulfide by oxidizing it to sulfur or sulfate.

Garcias-Bonet *et al.*,(2020) analyse the endophytic and total bacterial communities of leaves, rhizomes, and roots of six Red Sea seagrass species. They provide information towards the understanding of the role of microorganisms in seagrass ecosystem functioning framed under the seagrass holobiont concept.

Venkatachalam *et al.*, (2015) reported that the rhizome and leaf tissues of 10 seagrass species collected along the coast of Tamil Nadu state, southern India are sampled for the presence of fungal endophytes. The Colonization frequency of the endophytes was more for the rhizome than for the leaves. Species of *Aspergillus, Paecilomyces* and *Penicillium* occurred in high Colonization frequency and could be isolated from both the tissue types of seagrasses belonging to both the families.

Devarajan and Suryanarayanan (2002) were carried out the leaf blade, petiole and rhizome of the seagrass *Halophila ovalis* are examined for the presence of endophytic fungi. The seagrass protect endophytic fungi and their colony population in seagrass tissues are low.

Torta *et al.*,(2014) reported that presence of a fungal endophyte in the roots of *Posidonia oceanica*. Staining techniques on root fragments and sections, in combination with microscope observations, are used to visualize the fungal presence and determine the percentage of fungal colonization in this tissue. Various isolation techniques used to obtained fungal colonies of both sampling sites and identified these colonies as *Luhwoana* sp as DSE in roots of *Posidonia oceanica*.

Garcias-Bonet *et al.*, (2012) conducted a survey of the endophytic bacterial population of marine angiosperm in surface-sterilized tissues of roots, rhizomes, and leaves. Endophytic bacterial sequences are detected in the samples analyzed. The Operational Taxonomic Units found in roots different from of rhizomes and leaves. They provide information about presence of bacterial endophytes that differed from locations and tissue types.

Ettinger and Eisen (2020) despite that fungi having critical roles on land as decomposers, pathogens or beneficial endophytes and carry out fungi associated with the seagrass *Zostera marina*, also bacteria and oomycete isolates in the process. These isolates are taxonomically identified using a combination of molecular and phylogenetic methods. This study generates a culture collection of fungi, bacteria and oomycetes which gives the knowledge of the diversity of Z. marina associated microbes and functional and evolutionary roles of microbial eukaryotes associated with seagrasses.

Li *et al.*, (2011) investigated the structure of endophytic bacteria in seagrass roots and identified these endophytic bacteria are capable of fixing nitrogen and improve plant growth. They identified these endophytic bacteria used in the process of phytoremediation for the restoration of eutrophic systems.

Cho *et al.*, (2007) identified the roots of ginseng associated with endophytic bacteria. The isolated bacteria contain potential activity as biocontrol agents against phytopathogenic fungi. Kobayashi and Palumbo (2000) highlighted that endophytic bacteria give beneficial activities to agriculture. These endophytic bacteria enhance growth potential of host plants, disease control and suppression of pathogenic infection and contain as pest control property. They are effective in reducing insect boring and increase control.

Dalton *et al.*,(2004) carried out nitrogen fixing dune grasses. These grasses contain endophytic and diazotrophic bacteria. These bacteria helpful for nitrogen fixation process and contribute to the phenom enol success of these grasses on nutrient poor sand.

Marhaeni *et al.*,(2011) explored that possible role of bacterial symbionts of seagrass. They isolated antifouling activity against marine biofilm forming bacteria and these isolated bacteria capable of inhibiting the growth biofilm forming isolates. Further test reveals, crude extracts of the active bacterial symbionts supported the potential of these symbionts as the alternative source of environmentally friendly marine antifoulants.

Donnelly *et al.*, (1999) investigate that bacterial interactions in the rhizhophore of seagrass communities and electron microscopy identified the rhizome as the main site of colonization for a diverse range of morphological groups of bacteria. Sulphate reducing bacteria are identified as the key group of bacteria involved in Nitrogen fixation in the rhizosphere.

Berg *et al.*, (2009) carried out heterotrophic bacteria in association with cyanobacterial water blooms. Various growth media are used to isolate the strains of bacteria in cyanobacteria. Several strains also induce and increase the growth of cyanobacteria. These strains supply a large group of bacteria and controlling the harmful effects of cyanobacteria.

Pollard and Kogure (1993) introduced multidisciplinary investigation, to evaluate the role of epiphytic and epibenthic algae in a tropical seagrass, *Syringodium isoetifolium*. Hanington *et al.*, (2015) highlighted that seagrass ecosystems play a significant role in coastal biogeochemical processes, but are under threat from both natural and anthropogenic disturbances. In this study, they investigated the changes in benthic macroflora composition result in a shift in benthic metabolism and *Syringodium isoetifolium* loss is likely due to combined effects of low salinity and light. This study highlights the need for monitoring seagrass meadows to a dominant species or community level and demonstrates the importance each community can play in the broader ecosystem. Sathyanathan *et al.*, (2016) evaluated the antibacterial efficiency of the noodle grass *Syringodium isoetifolium*, which is commonly found in the Indian coastal waters. Also, this study characterizes the active compound and predicts the mode of action in silico. Ravikumar *et al.*, (2012) identify the antibacterial potential of seagrass (*Syringodium isoetifolium*) associate microbes against bacterial pathogens. The study indicates *Streptomyces* sp. (GU045544.1) from *Syringodium isoetifolium* could be used as potential antibacterial agent.

Mayavu *et al.*, (2009) reported the antimicrobial properties of seagrass species against biofilm forming bacteria. Seagrass species have a very potential groups are producing several secondary metabolites. The bioactive potential of two different seagrass species

viz., *Cymodocea serrulata* and *Syringodium isoetifolium* are selected and preliminary effort has been made against the marine biofilm forming bacteria's *Pseudomonas aeruginosa, Bacillus cereus, Proteus vulgaris, P. mirabilis, E. coli, Listeria monocytogenes, Salmonella enteritidis, Staphylococus aureus* and *Vibrio paraheamolyticus*, which also the human pathogens. Ethanol and methanol extracts of *Syringodium isoetifolium* was inhibited the biofilm forming bacteria such as *E. coli* (14 mm), *P. aeruginosa* (8 mm) and *Vibrio paraheamolyticus* (7 mm) and it showing Minimum activity against S. aureus (2 mm). The crude extract of ethanol and methanol of *Cymodoces serrulata* was inhibited the growth of all the 9 species of the biofilm forming microbes. The results of present study are concluded that seagrasses have potential bioactivity against marine biofilm forming microorganisms.

With the above background information, the isolation of endophytic bacteria in *Syringodium isoetifolium* and characterization of the endophytic bacterium associated with the leaf was carried out.

MATERIALS AND METHODS

MATERIALS AND METHODS

MATERIALS

Instruments: Centrifuge, Laminar Air flow chamber, water bath, PCR, GelDoc, Agarose Gel electrophoretic system, DNA sequencer

Consumables: Petridishes, bottles, parafilm, conical flask, borossil bottle, non-absorbent cotton, streaking loop, pipettes, microscopic slides, coverslips, eppendorff tubes, PCR tubes

Chemicals: Autoclaved sea water, LB-agar powder, carbendazim, ethanol, crystal violet, Gram's iodine, saffranin, TE buffer, isoamyl alcohol, sodium acetate, proteinase K, PCR buffer, Taq polymerase, primers, nucleotides, agarose powder, ethidiumbromide, DNA ladder, Agarose gel extraction kit

METHODS

Sample Collection

Syringodium isoetifolium samples were collected from the coastal region of Mottakopuram in Thoothukudi district. The samples are taken between January 2021 and March 2021. Bulk leaf tissue was collected using gloves and placed in plastic bags. Seawater was also collected in 1 L plastic bottles. The plants were transported to the laboratory in seawater from the same location and processed immediately.

Sterilization

Once the samples were brought to the lab, clean, undamaged, non-epiphyte containing, young, and visually good samples were isolated and washed in clean tap water. The

leaves, rhizomes, and roots of were separated. The samples were cut into around 5cm short pieces. They were passed through a series of surface sterilants such as 10% hydrogen peroxide for 3 min, 20% tween for 5min, 75% ethanol for 5 min, and finally washed gently with autoclaved sea water. All the surface sterilant solutions contained 20% Carbendazim fungicide

Media Preparation

Weighed 2.5 g of LB-agar broth powder (Himedia) and poured autoclaved sea water of 100ml together in a borosil bottle. The mixture was kept for autoclaving under around 121°C temperature and 30psi for 30 minutes. After autoclaving, ones the temperature became bearable the mixture was poured into previously autoclaved petridishes inside the Laminar Air Flow Chamber. When the petridishes became cooled enough all are sealed using parafilm and kept at 4°C for future use.

Isolation of Bacteria

The surface sterilized seagrass tissues of leaf, root or rhizome are grind using autoclaved mortar and pestle, inside the Laminar Air Flow Chamber. A pinch of fungicide was added while grinding. The extract was filtered using sterile cheese cloth. The supernatant was collected. By using sterile pipettes around 3ml of the supernatant was pipette to the LB-Agar plates. The solution was spread by shaking the petridish, keeping it on the floor. All the petridishes were kept under 37°C. After 16 hrs, the colonies started appearing.

Gramstaining

Samples from the bacterial colonies were taken using sterilized loop and placed on a clean microscopic slide. Prepared the smear of suspension on the clean slide, air dried and heat fixed the sample. Crystal Violet was poured and kept for about 30 seconds to 1

minute and rinsed with water. Flooded the gram's iodine for 1 minute and washed with water. Then, washed with 95% alcohol for about 10-20 seconds and rinsed again with water. Added safranin for about 1 minute and washed with water. The samples were observed under microscope and imaged.

DNA Isolation: DNA extraction from bacterial cells (phenol, chloroform method)

Took 1.5 ml of the liquid bacterial culture in LB, centrifuged it at 5000 rpm for 5 minutes and collected the pellet. 200 µl of TE buffer was added to the pellet, and mix well. To the tubes 20 µl of 10 % SDS and 10 µl 10 mg/ml of Proteinase K and 10 µl of 10 mg/ml lysozyme were added and mixed gently. The reaction mixture was kept in water bath at 55°C for 30 minutes. 250 µl of phenol, 240 µl of chloroform and 10 µl of isoamyl alcohol, were added and mixed well and centrifuged at 12,000 rpm for 10 minutes. Collected the aqueous phase in new eppendorf tubes and added 0.2 volume of 3 M sodium acetate and 2 volume of isoproponal and mixed well. Centrifuged the mixture at 10,000 rpm for 10 minutes at 4 °C, collected the pellet. To the pellet added 700 µl of 70 % ethanol, mixed well and centrifuged it for 5000 rpm for 5 minutes at 4 °C. Air dried the pellet and added 50 µl of TE buffer and stored at -20 °C used for PCR. DNA was visualized using 0.8% Agarose and documented.

PCR Amplification of 16S rRNA

The 16S rDNA sequences were amplified by polymerase chain reaction (PCR) using universal primers.

The buffer, taq polymerase, dNTPs, primers and DNA sample were thwed on ice bring to 4° C from -20° C. Mixed the chemicals in a PCR tube in the below order and quantity using micro pipette. For the polymerase chain reaction (PCR), a total of 50 µl of PCR reaction mixture was prepared having 5 µl of 1X Taq buffer, 5 µl of 200 µM of each

deoxynucleotide, 1.5 µl of 0.3 µM of each forward and reverse primer, 0.25 µl of 5U Taq DNA polymerase and 2 µl of genomic DNA extract. Amplification of DNA for 50 µL reaction was carried out under the following condition. The PCR conditions were set for 28 cycles with initial denaturation at 95°C for 5 min then final denaturation of 95°C for 1 min, annealing at 55°C for 1 min and final extension at 72°C for 2 min using Thermal cycler (Applied Bio system Thermal Cycler). PCR products were electrophoresed on 1.5% agarose gel and documented. The amplicon was cut out and extracted using Agarose Gel Extaction kit (Genei, Bangalore, India) by following the manufacture protocol.

DNA Sequencing and Similarity Search

The nucleotide sequences of the PCR product was determined by using the automated DNA sequencer with forward and reverse primers (Bio-serve Bio Technologies Pvt. Limited Hyderabad, India). Sequence comparison with the databases was performed using Basic Alignment Search Tool (BLAST) through the National Centre for Biotechnological Information (NCBI).

Phylogenetic Analysis of Bacterial Strain

The 16S rRNA sequences of all bacterial strains were aligned with reference sequences showing sequence homology from the NCBI database using the multiple sequence alignment program of MEGA 4.0 (Tamura et al., 2007). Phylogenetic analysis of gene sequence data were conducted using the neighbor-joining (NJ) method.

RESULTS AND DISCUSSION

RESULTS AND DISCUSSION

Seagrasses are one of the key plant community found in the Marine Biome. More Seagrass meadows play critical role in sustaining several marine species. More than their ecological significance, seagrasses are rich sources of untapped resources. The seagrass associated microbiome is one among them. They are associated with endo-ecto bacteria and fungi. By studying microbiota of sea grasses it is possible to generate the knowledge of the diversity of associated microbes and the microbes' functional and evolutionary roles.

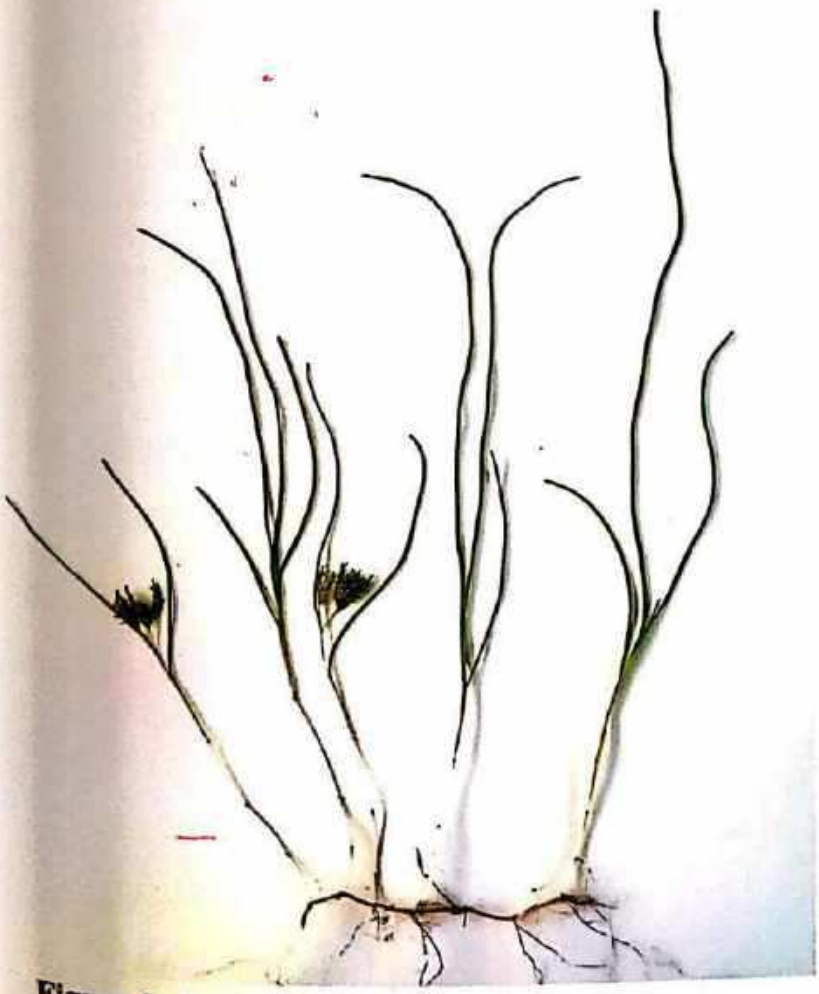

Figure 1: ***Syringodium isoetifolium*** **habit**

Endophytic bacteria are those bacteria lives inside the plant body by establishing a symbiotic relationship with the host. Garcias-Bonet *et al.*, (2020) analyses the endophytic and total bacterial communities of leaves, rhizomes, and roots of six Red Sea seagrass species. These microbes provide certain level of immunity to the host. Tarquino *et al.* (2019) report the presence of microbes in seagrass is important for the development of seagrasses from seed and protect them against pathogens and saprophytes. The seagrass-bacterial relationship is highlighted in their review on the importance of marine eukaryotic microorganisms in ecosystems. Venkatachalam *et al* (2015) reported that colonization frequency of the endophytic fungi was more for the rhizome than for the leaves. Li *et al.*, (2011) investigated the structure of endophytic bacteria in seagrass roots and identified these endophytic bacteria are capable of fixing nitrogen and improve plant growth. They identified these endophytic bacteria used in the process of phytoremediation for the restoration of eutrophic systems. Cho *et al.*, (2007) identified the roots of ginseng associated with endophytic bacteria. The isolated bacteria contain_potential activity as biocontrol agents against phytopathogenic fungi.

Isolation and identification of Gram staining response

The endophytic bacteria were isolated from sterilised leaf, rhizome and roots of *S. isoetifolium* (Figure 1) and plated on LB-agar containing fungicide as described in the materials and method chapter. The colonies were appeared around 16hrs after platting. The colonies on nutrient agar were white with regular margin in 12 hours of growth. But the colony became dirty white with irregular margin (like the egg on a pan) in 48hrs. The root and stem displayed more colonies compared to the leaf (Figure 2). A similar result is presented by Garcias-Bonet *et al* (2012) in *Posidonia oceanic*. They also reports that the Operational Taxonomic Units found in roots different from of rhizomes and leaves. The endophytic bacterium in the roots and rhizome may be capable of fixing nitrogen and

improve plant growth (Li *et al.*, 2011). Bacteria grown were gram stained and violet colour was visible under the microscope indicating the gram positive behaviour.

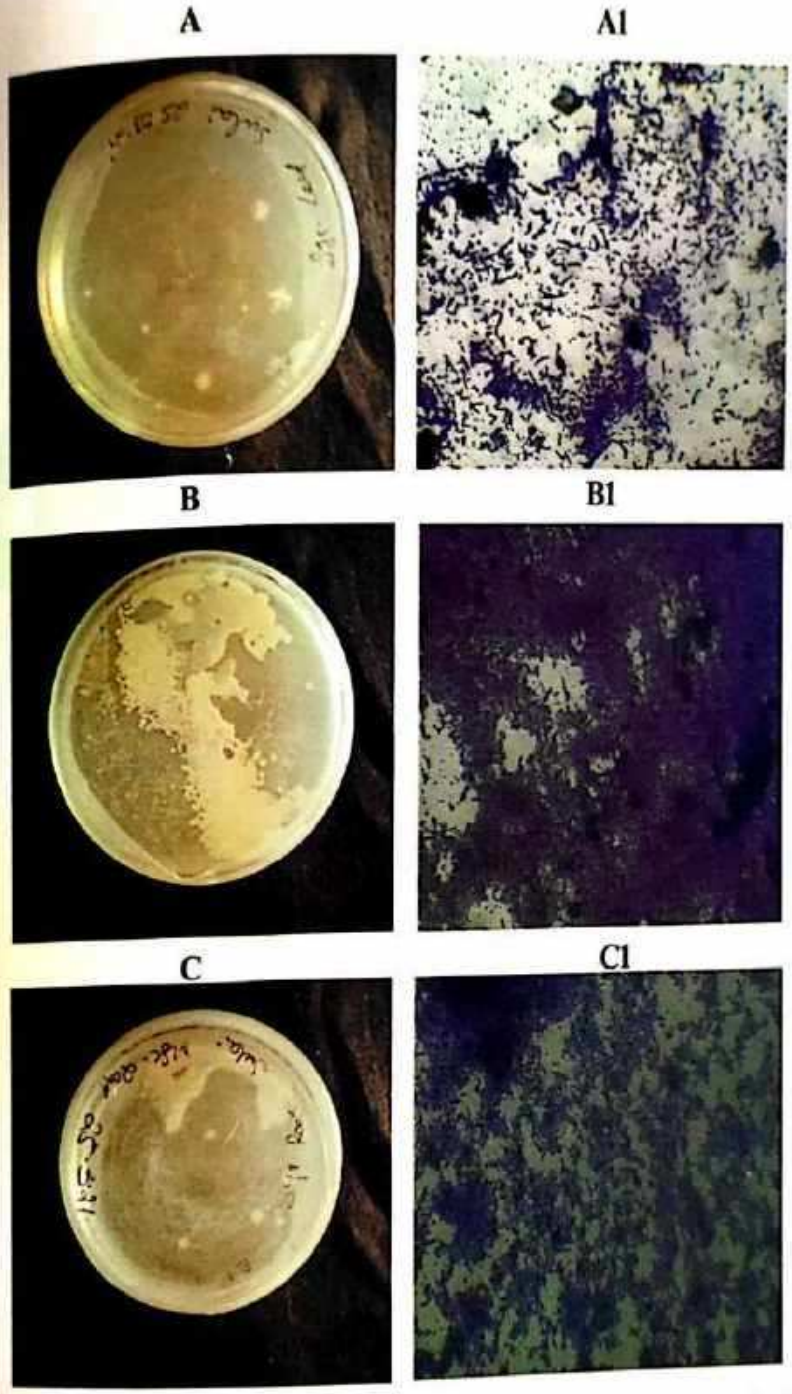

Figure 2: Endophytic bacteria isolated from *S. isoetifolium*. A-C. Bacteria from leaf, rhizome and root. A1 - C1. Endophytic bacteria from each isolate after gram staining

Amplification of DNA fragment of 16S rRNA

16S rRNA fragment was amplified from the total genomic DNA isolated from the selected endophytic bacterium. The DNA was isolated from the cultured bacteria and PCR amplified the specific 16S fragment as described in the Materials and method chapter. The amplified PCR product was run in 1% agarose gel (Figure 3). The amplicon size is around 700Kb. The amplicon is pointed by an arrow in the Figure 2. The bands were excised and isolated the DNA using Agarose gel electrophoresis kit, GeNei (Bangalore, India) according to the manufacture's instructions. The eluate was reamplified using gene specific primers of 16S rRNA. The purified reamplification product was used for sequencing.

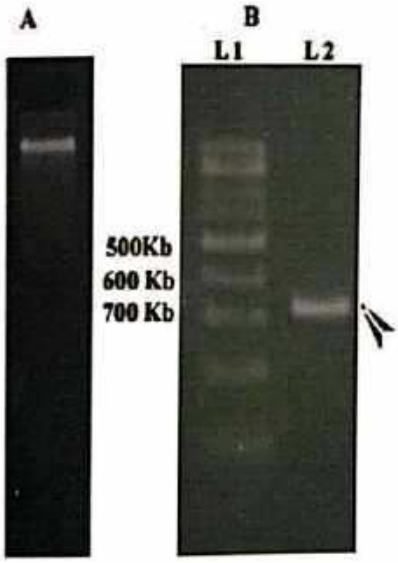

Figure 3: DNA isolated and PCR amplicon. A. The DNA isolated is run in Agarose gel. B. 16S rRNA amplified using specific primers. L1 - 500 kB marker, L2 - Amplicon

Similar sequences appeared in BLAST

The sequence obtained was blasted in NCBI and confirmed the bacterium is a *Bacillus sp.* The result displayed five sequence with maximum score 1203with percentage of identity 99.7% and E-value 0.0 (Figure 4A). The graphic summary is presented in Figure 4B.

According to the analysis *Bacillus aerius, Bacillus aerophilus, Bacillus stratosphericus and Bacillus altitudinis* were exhibiting maximum similarity with the query sequence.

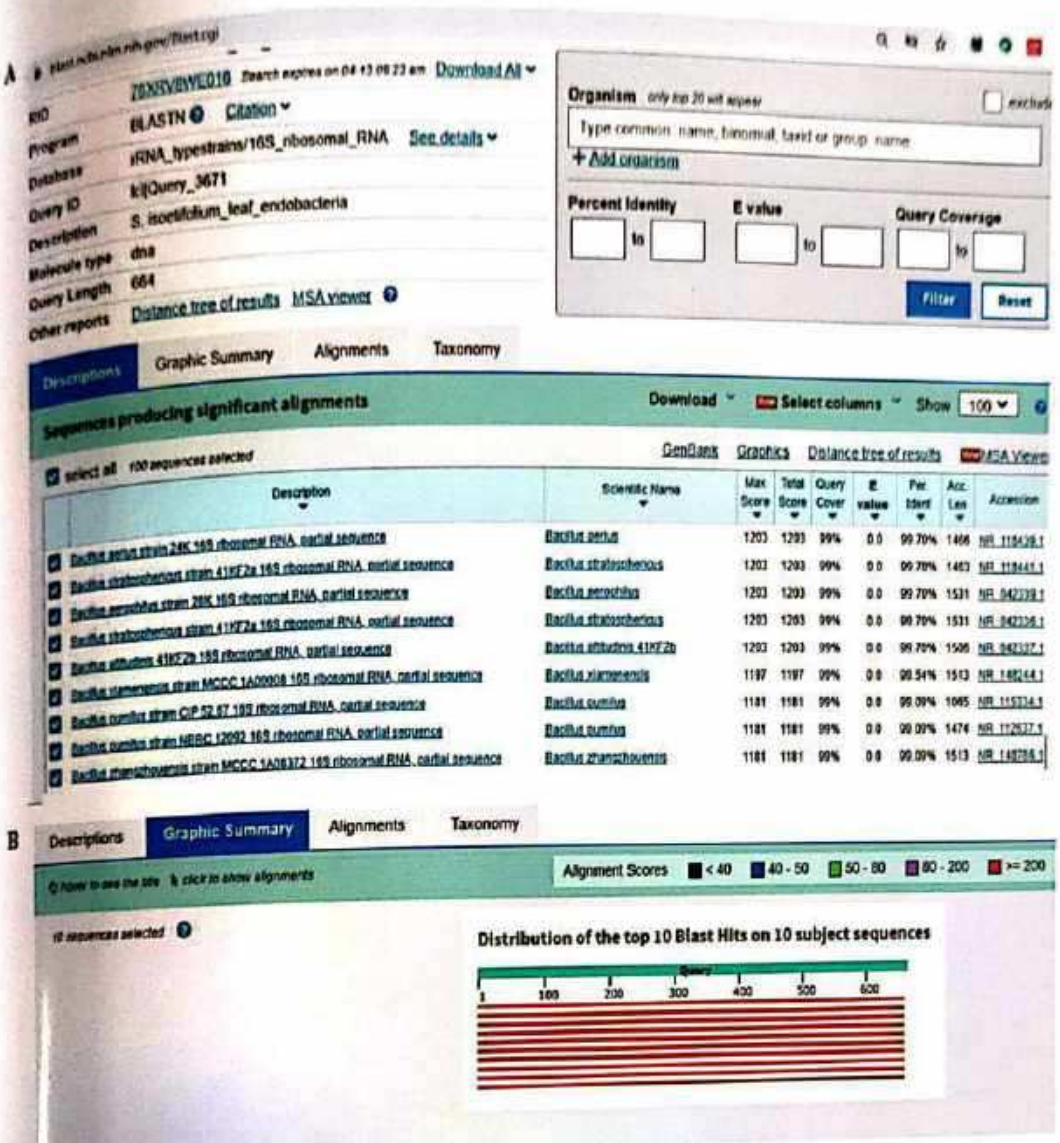

Figure 4 : Similarity search results in BLAST. A. Descriptionof the maximum similar sequences. B. Graphic summary of the sequence generated

Gene identification in BLAST

Sequence comparison with the databases was performed using Basic Alignment Search Tool (BLAST) through the National Centre for Biotechnological Information (NCBI), USA, and server. Five sequencs were showing 99.7% similarity. They were *Bacillus aerius* NR_118439.1, *Bacillus aerophilus* NR_042339.1, *Bacillus stratosphericus* (NR_118441.1 and NR_042336.1), and *Bacillus altitudinis* NR_042337.1.

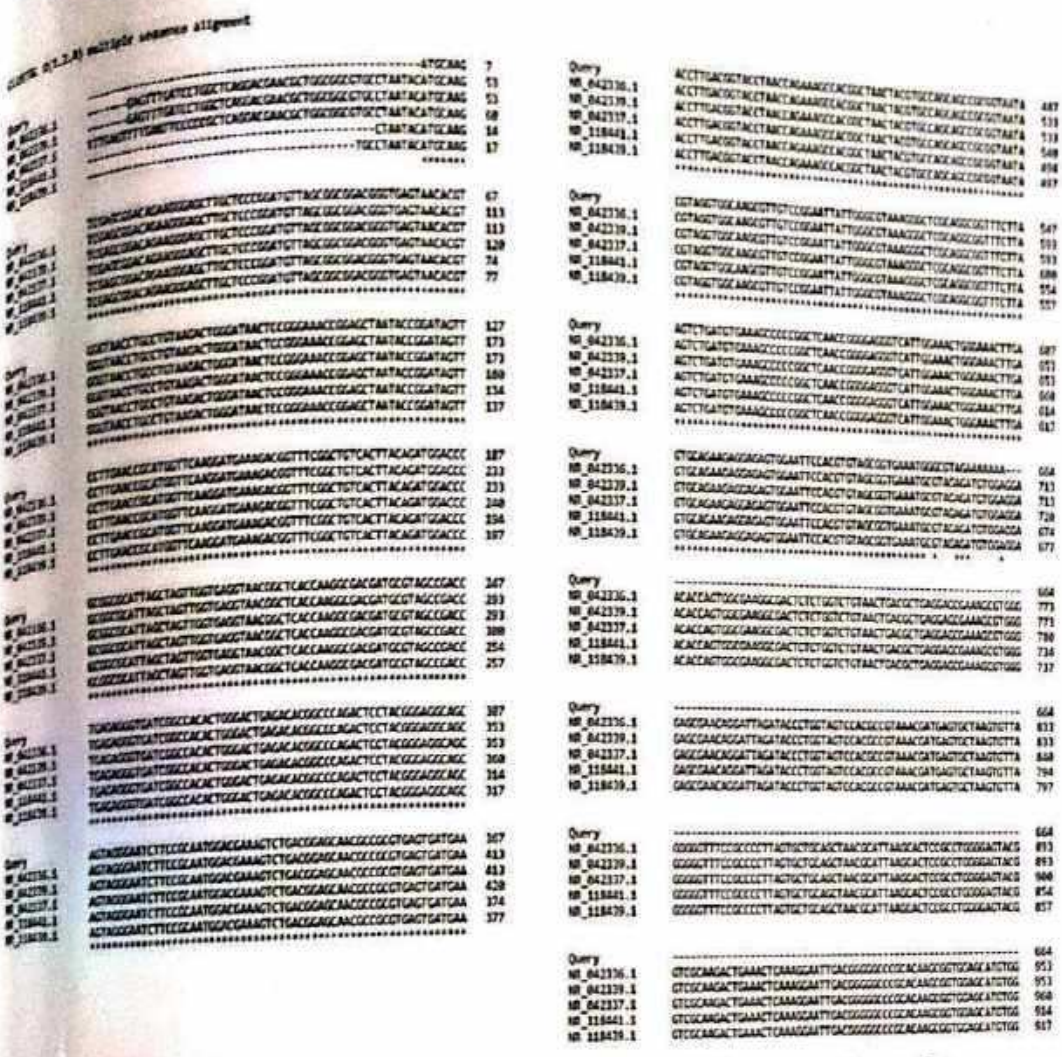

Figure 5: Multiple sequence alignment in Clustal Omega. Multiple sequence alignment for the best five similar sequences appeared in BLAST against the test sequence.

Phylogenetic similarity

For further clarification in the best similar sequences appeared in BLAST were aligned together against the test sequence in CLUSTAL omega Figure 5. *Bacillus aerius* NR_118439.1, *Bacillus aerophilus* NR_042339.1, *Bacillus stratosphericus* (NR_118441.1 and NR_042336.1), and *Bacillus altitudinis* NR_042337.1 were aligned in CLUSTAL.

All the four species exhibit extremely close phylogenetic relationship. The *Bacillus* sp. identified in the present study may be a fifth species which is a close relative of the above species. The bacteria isolated from the leaves are similar to the bacteria which are

reported from harsh habitat. All the four bacterial strains are isolated from cryotubes that are used to collect air at altitudes between 24 and 41 km during a balloon flight from Hyderabad, India (Shivaji S., *et al* 2006). But the names were *Bacillus aerius* sp. nov., *Bacillus aerophilus* sp. nov., and *Bacillus stratosphericus* sp. nov rejected by the JCICSP (Judicial Commission of the International Committee of Systematics of Prokaryotes). Therefore these species no longer exists in international nomenclature system. The related species *Bacillus altitudinis* has came up in several studies after its first discovery.

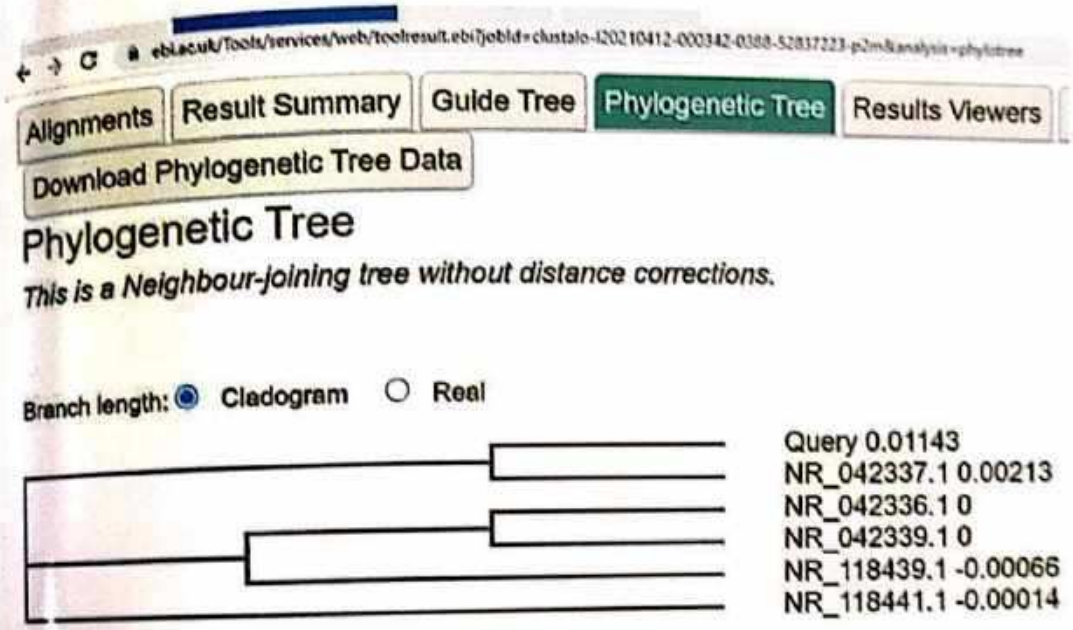

Figure 6: The Clustal Omega Phylogenetic Tree.

Bacillus altitudinis is a Gram-positive, rod-shaped aerobic bacterium classified in the phylum Firmicutes. It was first reported to be isolated from extreme UV-stressed air samples collected in the stratosphere (Shivaji *et al*). Since then, *B. altitudinis* has been reported in diverse habitats, including the southern Indian Ocean (Halder U., *et al*, 2017), deep freshwater of Manasbal Lake (Shafi S et al., 2017), soil (Vijay KE et al., 2011), and silt (Mao S. et al, 2013).

The *Bacillus altitudinis* SORB11 strain is isolated from the Indian sector of the Southern Ocean at a depth of 3.8 km is tolerant to UV radiation (Halder *et al.*, 2017). Sunar *et al.*, (2013) studied that biocontrol efficacy and plant growth promoting activity of *Bacillus altitudinis*. The bacterial isolates are obtained from the rhizosphere of *Sechium edule* growing in the lower foothills of Darjeeling, India. This study clearly suggest that *B. altitudinis* is a potential PGPR which can be used as efficient microorganism for enhancement of plant growth and suppression of fungal disease. Kumar et al., (2017) obtained bacterial isolates from rice phyllosphere are investigated for their plant growth promoting activities and role in alleviation of drought stress in rice. The rice treated with *Bacillus altitudinis* increased proline content, phenolics content, catalase activity and reduced malondialdehyde (MDA) content in plants. They found that the ethylene emission is significantly reduced by *B. altitudinis* FD48 inoculation under drought condition when compared with control. This study suggests that the isolate Bacillus altitudinis FD48 can be used at field level to mitigate drought stress.

Though all the bacterial colonies appeared in the present study was not analysed completely, this study generates a culture collection of bacteria which gives the knowledge of the diversity of *S. isoetifolium* associated microbes and functional and evolutionary roles of microbial eukaryotes associated with seagrasses (Ettinger and Eisen, 2020). The identified endophytic bacteria may be used in the process of phytoremediation (Li *et al.*, 2011). But the clear role needs to be investigated.

SUMMARY AND CONCLUSION

SUMMARY AND CONCLUSION

Seagrasses are one of the key plant community found in the Marine Biome. Microbial communities are found associated the sea grasses. By studying microbiota of sea grasses it is possible to generate the knowledge on their diversity and also the functional and evolutionary roles of this microbiome. Many bioactive compounds beneficial to pharmaceuticals, environment, agriculture, and industries are produced by endophytes. Due to their great importance to plants, scientists have already started exploiting them very much for newer compounds.

The bacterial isolates from leaf, rhizome and leaves were gram positive. One of the colonies appeared in the leaf extract treated plate was characterized. The 16S rRNA amplified exhibits around 700Kb. The BLAST analysis revealed that the bacteria has 99.7% similarity with *Bacillus aerius* (NR_118439.1), *Bacillus aerophilus* (NR_042339.1), *Bacillus stratosphericus* (NR_118441.1 and NR_042336.1), and *Bacillus altitudinis* NR_042337.1 in the NCBI database. All the four species exhibit extremely close phylogenetic relationship. The Bacillus sp. identified in the present study may be a fifth species which is a close relative of the other species. The related species *Bacillus altitudinis* has came up in several studies after its first discovery. B. altitudinis is a potential PGPR which can be used as efficient microorganism for enhancement of plant growth and suppression of fungal disease.

The results reported here provide a pioneering step towards the characterization of the endophytic bacteria associated with leaf of *Syringodium isoetifolium*. This study generates a culture collection of bacteria which gives the knowledge of the diversity of *S.*

zostifolium associated microbes and functional and evolutionary roles of microbial eukaryotes associated with seagrasses. The identified endophytic bacteria may be used in the process of phytoremediation.

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PHYTOCHEMICAL, ANTIOXIDANT, FT-IR, GC-MS ANALYSIS AND ANTIBACTERIAL ACTIVITY OF CORCHORUS AESTUANS L. AND TRIUMFETTA RHOMBOIDEA Jacq.

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By

VIMALA C

Reg.No.19APBO16

DEPARTMENT OF BOTANY

ST. MARY'S COLLEGE (Autonomous)

THOOTHUKUDI -628001

2020-2021

CERTIFICATE

This is to certified that this dissertation entitled **'PHYTOCHEMICAL, ANTIOXIDANT, FT-IR, GC-MS ANALYSIS AND ANTIBACTERIAL ACTIVITY OF CORCHORUS AESTUANS L. AND TRIUMFETTA RHOMBOIDEA Jacq.'** submitted by **Vimala C Reg.No. 19APBO16** to ST. MARY'S COLLEGE (Autonomous) Thoothukudi - 628001 in partial fulfillment for the award of the degree of **'Master of Science in Botany'** is done by her under my supervision. It is further certified that this dissertation or any pâre of this has not been submitted elsewhere for any other degree.

B. Mary Sheeba

GUIDE

M. Glory

HEAD OF THE DEPARTMENT

HOD of Botany
St. Mary's College (Autonomous)
Thoothukudi- 628 001.

M Sheeba

EXAMINAR

M.

Lucia Rosa

PRINCIPAL

St. Mary's College (Autonomous)
Thoothukudi - 628 001.

DECLARATION

I do here by declare that this dissertation entitled **"PHYTOCHEMICAL, ANTIOXIDANT, FT-IR, GC-MS ANALYSIS AND ANTIBACTERIAL ACTIVITY OF CORCHORUS AESTUANS L. AND TRIUMFETTA RHOMBOIDEA Jacq."** submitted by me in partial fulfillment for the award of the degree of **'Master of Science in Botany'**, in the result of my original and independent work carried out under the guidance of **Dr. Mrs. B. Maria Sumathi M.Sc, M.Phil., Ph.D.** Assistant Professor. Department of Botany, St. Mary's College (Autonomous) Thoothukudi and it has not been submitted elsewhere for the award of any other degree.

Station: Thoothukudi

Date: 15/4/21

C. Vimala

(Vimala C)

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CONTENTS

„Every plant is a medicinal herb". So, the plant kingdom is a treasure house of potential drugs and used for prevention and treatment of ailments. The animals including human beings always depends plants for their food, shelter and medicine. It always plays an individual role in its habitat. Our ancient people praise the plants because they knew the medicinal properties of plants. But nowadays we don't know whether plant contains medicinal compounds or not. Due to the lack of knowledge about plants, we couldn"t able to conserve plants. But we forgot that without plants we will lose our planet. The phytochemical examination of plants opens the research of medicinal field. The secondary metabolites of each plant shows unique, consistent and mysterious medicinal and antimicrobial activity. To promote the use of medicinal plants as potential sources of antimicrobial compounds, it is important to thoroughly investigate their composition and activity and thus validate their use. Some phytochemicals produced by plants have antimicrobial activity and used for the development of new antimicrobial drugs.

Plant materials remain as an important resource to combat serious diseases in the world. The traditional medicinal methods, especially the use of medicinal plants are still play a vital role to cover the basic health needs in the developing countries. The medicinal value of these plants lies in some chemical active substances that produce a definite physiological action on the human body. The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids and phenolic compounds. GC-MS and FT-IR has played an important role in pharmaceutical analysis in recent years.

Many infective diseases are treated with chemotherapeutic agent, such as antibiotics, that selectively inhibit or kill the pathogen with little or no effect on the host. Ideally, antimicrobial

agents disrupt microbial processes or structures that differ from those of the host. They may damage pathogen by hampering cell wall synthesis, inhibiting microbial protein and nucleic acid synthesis, disrupting microbial membrane structure and function or blocking metabolic pathways through the inhibition of key enzymes (Prescott *et al*., 2008). The conventional antimicrobial agents face a lot of resistant problems in recent times as microorganisms are losing sensitivity to some of these drugs. Recently, concern has been expressed about the rising prevalence of pathogenic microorganisms which are resistant to the old generation, and to the newer or modern antibiotics that have been produced in the last decades (Cohen, 1992; Nascimento *et al*., 2000; Okesola and Makanjuola, 2009). Also the problem posed by the high cost; adulteration and increasing toxic side effects of these synthetic drugs coupled with their relative inadequacies in disease treatment especially in the developing countries portend serious limitations (Shariff, 2001). Consequently, the continuous acute need of novel and effective antibiotics for antimicrobial chemotherapy is clearly evident. Phytochemicals derived from plants have shown great promise in the treatment of intractable infectious diseases (Nascimento *et al*., 2000; Rios and Recio, 2005) with lesser side effects compared to the synthetic drug agent (Iwu *et al*., 1999).

In traditional herbal practice, indigenous medicinal plants have been employed in the treatment of several important infections (Fennell *et al*., 2004, Taylor *et al*., 2001). Also, plant-based extractives have equally served as source of lead compounds for further developments of future antimicrobial agents. Therefore, evaluation of a candidate medicinal plant may lead to identification of very effective herbal antimicrobial treatments or provide leads for further development into novel antimicrobial agents.

Phytochemicals are known to work as immune modulators and may have anti-inflammatory, anticancer and antimicrobial activities. All these properties of the phytochemicals

are attributed to its effective antioxidant mechanisms against the endogenously produced harmful free radicals. Our body has effective antioxidant defence systems, which constitute enzymes, such as superoxide dismutase (SOD), catalyse and compounds, such as ascorbic acid, tocopherol, and glutathione. But all these endogenous antioxidants are not sufficient in protecting the body against oxidative stress. Therefore, dietary supplementation through natural antioxidants in place of synthetic antioxidants is necessary for strengthening the antioxidant system of the body by inhibiting free radical generation and thus preventing chronic diseases. Recently, much attention has been directed towards exploring natural antioxidants because they are natural products that are considered to be a safe source (Abdul Sadat *et al*., 2017).For the present study, the two taxa - *Corchorus aestuans* L. and *Triumfetta rhomboidea* Jacq. were selected.

Corchorus aestuans L. (Tiliaceae) is an erect to procurement of annual herb grow up to 20 cm long. Capsules are 1.5-2.7 cm long. Widely distributed in the tropics. Biologically *Corchorus* species are used as diuretic, chronic cystitis, gonorrhoea, antihistaminic, anti-inflammatory, antimicrobial, cardio tonic, and also to increase the viscosity of the seminal fluid (D.Ramadevi and S.Kanapathi. 2012). The whole plant of *Corchorus aestuans* is used in the treatment of fever and stomach ache while seeds are used in the treatment of gonorrhoea and tonic.

Triumfetta rhomboidea Jacq. (Tiliaceae) is a perennial herb having important role in ancient therapy. It is a tropical weed. It is very widespread in continental Africa, including South Africa. Various Parts of this plant used therapeutically are fruit, flower, leaves, bark and root. Root is tonic styptic, aphrodisiac, cooling, useful in dysentery and as diuretic. Pounded roots are given in the treatment of intestinal ulcer. Leaves, Flowers and Fruit are mucilaginous demulcent, astringent, and also used in gonorrhoea and against leprosy. In the present study, active

constituents of the plants were analyzed and evaluated for antibacterial activity (V.P.Devmurari *et al*., 2010).

The current study of phytochemical analysis, antioxidants and antimicrobial analysis will contribute the knowledge of medicinal properties of selected plants and it will create a path to travel the researchers. Through this research I could shares the therapeutic uses of these plants to my society.

SCOPE AND OBJECTIVES

The aspiration of current study was to assess the biochemistry and bioactivities of the plants extract (*Corchorus aestuans* and *Triumfetta rhomboidea*). In this work the following objectives are focused.

i. Collection of *Corchorus aestuans* and *Triumfetta rhomboidea* plants for extract preparation.

ii. To qualitatively screen the presence phytochemicals by using different solvents (acetone, methanol, ethanol,) and aqueous extracts of *Corchorus aestuans* and *Triumfetta rhomboidea.*

iii. To quantitatively analyses and compare the total phenols, flavonoids, vitamin C, Tannin and vitamin E of *Corchorus aestuans* and *Triumfetta rhomboidea.*

iv. To identify and compare the functional group of *Corchorus aestuans* and *Triumfetta rhomboidea* by Fourier transform infrared spectroscopy (FTIR) analysis.

v. To identify the bioactive compounds of *Corchorus aestuans* and *Triumfetta rhomboidea* plants extract using GC-MS analysis.

vi. To assess the antioxidant potential of *Corchorus aestuans* and *Triumfetta rhomboidea* using aqueous extract against DDPH radical scavenging activity.

vii. To evaluate the anti-bacterial potential of acetone, methanol, ethanol and aqueous extracts of *Corchorus aestuans* and *Triumfetta rhomboidea.*

REVIEW OF LITERATURE

Phytochemicals are chemical compounds formed during the plants normal metabolic processes. These chemicals are often referred to as *"*Secondary metabolites*"* of which there are several classes including alkaloids, flavonoids, coumarins, glycosides, polysaccharides, phenols, tannins, terpenes and terpenoids (Okwu, 2004). In addition to these substances, plants contain other chemical compounds. These can act as agents to prevent unconsiderable side effects of the main active substances or to assist in the assimilation of the main substances. Many herbaceous and medicinal plants contain important photochemical and vitamins such as alkaloids, flavonoids, tannins, cyanogenic glycosides, phenolic compounds, saponins, lignins, vitamin C, vitamin E and carotenoids, which are utilized both by humans and animals as important components of diets (Hussain *et al.,* 2011). The medicinal effects of plants are considered to be due to metabolites, especially secondary compounds, produced by plant species. Phytochemical analysis suggests that the presence of various biologically active compounds [alkaloids, phenols, flavanoids, proteins-lectin, carbohydrates, indigo, steroids etc.] and could be correlated to various therapeutic purposes (Vinoth *et al.,* 2011). Plants have an almost limitless ability to synthesize aromatic substances, mainly secondary metabolites of which 12,000 have been isolated, a number estimated to be less than 10% of the total (Mallikharjuna *et al.,* 2007).

The phytochemical screening of different parts of the *Jatropha curcus* revealed the presence of tannins, saponins, carbohydrates, sterols, diterpenes, alkaloids, flavanoids and various enzymes. Root contains di-terpenoid, Jatrophol and Jatropholones A and B, taraxerol b-sito-sterol. The bark contains tannins, resins, saponins, reducing sugar and traces of a volatile oil.

Leaves contain Steroid, alkaloids triterpene (Rajore and Batra, 2004). Musa *et al*., (2000) studied the phytochemistry of powdered leaves of *Acalypha recemosa* (Euphorbiaceae). This study revealed the presence of alkaloid, tannin, flavanoid and terpenes.

Sivaraj *et al.*, (2011) conducted preliminary phytochemical screening using five different solvents extracts of *Aegle marmelos, Ruta graveolens, Opuntia dillenii, Euphorbia royleana*and *Euphorbia antiquorum*. Phytochemical profiling of *Mimosa pudica*was carried out by Sriram *et al.*, (2011). Sukumaran *et al*., (2011) identified the phytochemical constituents of methanol extract of flower of *Peltophorumpterocarpum*. Phytoconstituents found in *Tridax procumbens* were isolated and characterized by Surendra and Talele (2011).

Nwokocha *et al.,* (2011) studied the comparative phytochemical screening of *Jatropha curcus*, *Jatrophagossypifolia, Jatropha multifida* and *Jatropha podagrica* on leaf, stem root and seeds and the results revealed that tannins were found to be the most abundant followed by saponins and flavanoids and phenols. Vindhya K *et al*., (2014) conducted the preliminary phytochemical study in *Gardenia latifolia* and *Gardenia gummifera*, using different solvents. The petroleum ether extract of both the plants were found to contain glycosides, phytosterols, fats and oils, resins, phenols and triterpenes. Flavonoid was found to be present in *Gardenia latifolia* and not in *Gardenia gummifera*. Alkoloids, carbohydrates, saponins, tannins, proteins, amino acids and diterpenes were absent in both the plants. Ethyl acetate extracts of the plant was found to contain glycosides, phytosterols, resins, phenols, flavonoids and triterpenes. Alkoloids, carbohydrates, saponins, fats, oils, tannins, proteins, amino acids and diterpenes were absent in both the plants.

Ved Prakash *et al,* (2015) investigated phytochemical screening and antioxidant activity of *Adina cordifolia* leaf. The plant extracts were screened for presence of flavonoids, carbohydrate, alkaloid, saponin, phenol, tannins, phlobatannins, terpenoids, and cardiac glycosides. Total flavonoid content, phenols content was estimated. Antioxidant activity was determined using nitric oxide scavenging assay, DPPH assay, hydrogen peroxide scavenging and ferric reducing methods, also MIC was calculated against a set of bacteria (*S. aureus, B. subtilis, E. coli, V. cholarae*). Ravindranath (2003) has been isolated a novel macrocyclic diterpene– Jatrophenone from the whole plant of *Jatropha gossypifolia*. This compound possesses significant antibacterial activity.

FTIR

A large number of medicinal plants are used as alternate medicine for diseases of man and other animal since most of them are without side effects when compared with synthetic drugs. Identification of the chemical nature of phytochemical compounds present in the medicinal plant will provide some information on the different functional groups responsible for their medicinal properties. Iqbal Ahamed *et al*., (2006) detected major groups of compounds as the most active fraction of four plants extract by infrared spectroscopy.

Ramamoorthi and Kannan (2007) screened the bioactive group of chemicals in the dry leaf powder of *Calotropis gigantea* by FTIR analysis Kareru*et al.*, (2008) detected saponins in crude dry powder of 11 plants using FTIR spectroscopy.

Muruganantham *et al.*, (2009) carried out the FTIR spectroscopic analysis in the powder samples of leaf, stem and root of *Eclipta alba* and *Ecliptaprostratea*. The FTIR analysis of aqueous methanolic leaf extracts of *Bauhinia racemosa* for phytochemical compounds was

done by Gauravkumar *et al*., (2010). Ragavendran *et al*., (2011) detected the functional groups in various extracts of *Aervalanata* using spectroscopic method .

Thangarajan Starlin *et al*., (2012), analyzed the ethanolic extracts of *Ichnocarpus frutescens*, by FTIR, revealed the presence of functional group components of amino acids, amides, amines, carboxylic acid, carbonyl compounds, organic hydrocarbons and halogens. Parag A. Petnekar and Bhanu Raman (2013) carried out the FTIR spectroscopic analysis of methanolic leaf extract of *Ampelo cissuslantifolia* for antimicrobial compounds.

FTIR analysis for five selected green leafy vegetables (GLVs) viz., *Hibiscus cannabinus* , *H. sabdariffa* , *Basella alba* , *B. rubra* L. and *Rumex vesicarius* confirmed the presence of free alcohol, intermolecular bonded alcohol, intramolecular bonded alcohol, alkane, aromatic compounds, imine or oxime or ketone or alkene, phenol and amine stretching (Sravan Kumar and Manoj., 2015).

The functional group identification is made by FTIR analysis and the active components based on the peak value in the region of infrared radiation. The ethanolic flower extract of *Erythrina variegata* L. is passed into the FTIR spectroscopy and the functional groups of the components are separated based on the peak ratio. The results of FTIR analysis confirm the presence of functional groups such as non-bonded, O-H stretch, carboxylic group, acidic, H bonded, C-H stretch, asymmetric stretching of –CH (CH2) vibration, C=N (stretch), carbon-carbon triple bond, multiple bonding, carbonyl compound frequency, C=O stretch, C=C stretch, O-H bend, alcoholic group, C-N stretch, C-O stretch, PO3 stretch, =C-H bending and C-Cl (Priyanga S *et al.,* (2017).

GC-MS

The chemical composition of the essential oils from leaves and wood of *Ocotea brenesii* growing wild in Costa Rica was determined by capillary GC/FID and GC-MS. From the leaves, 64 compounds were identified, corresponding to 85.9% of the oil, and from the wood 57 compounds were identified corresponding to 69.0% of the oil (Carlos and Jose, 2005). The chemical compositions of the essential oils of *Ocimum basilicum*L. *cv*. purple and *Ocimum basilicum*L. *cv*. green cultivated in Iran were investigated by GC-MS (Seyed, 2006).

GC-MS analysis of *Jatropha curcas* leaves revealed the presence of 16 compounds. The most abundant components were 22, 23-dihydro-stigmasterol (16.14%) alpha-tocopherol (15.18%), beta amylin (7.73%) and dotriacontanol (7.02%) The content of gamma tocopherol reached 2.88% and Vitamin E reached 18.06% in the extract (Wang *et al*., 2009). The GC-MS analysis of *Strobilanthes crispus* oil revealed the presence of 28 components. The main constituents were found to be phytol, α-cadinol, Megastigmatrienone, 2,3-dihydrobenzofuranand eugenol (Asmah*et et al*., 2006).

Nithya Narayanaswamy and Balakrishnan (2011) evaluated the antioxidant properties of 13 important medicinal plants and it showed that *Ocimum basilicum* leaf, *Alpinia calcarata* leaf, *Jatropha mulitifida* flower,*Hyptissua veolens* leaf, *Solanum indicum* leaf and *Clitoria ternatea* leaf and flower possessed higher DPPH scavenging activity. Moussa *et al*., (2011). The aqueous leaf extracts of 124 Egyptian plant species belonging to 56 families were investigated and compared for their antioxidant activity by DPPH scavenging assay. Safi *et al*. (2012) studied the biological activities of aqueous extract of the root of *Jatropha curcas* like antimicrobial and free radical scavenging activities. In the evaluation of DPPH free radical scavenging activity. Olabinri *et al.,* (2013) investigated *in vitro* antioxidant and nitric oxide radical scavenging capabilities of *Jatropha gossypifolia* extract.

Sermakkani M. And V. Thangapandian (2012) evaluated GC-MS analysis of *C. italica* leaves revealed the presence of seventeen compounds. The identified compounds possess many biological properties. For instance, 9,12,15-Octadecatrienoic acid, (Z,Z,Z)- Linolenic acid (R/T 20.06) possesses anti-inflammatory, insectifuge, hypocholesterolemic, cancer preventive, nematicide, hepatoprotective, antihistaminic, antieczemic, antiacne, 5-alpha reductase inhibitor, antiandrogenic, antiarthritic and anticoronary properties. n-Hexadecanoic acid - palmitic acid (R/T 17.25) can be an antioxidant, hypocholesterolemic, nematicide, pesticide, lubricant activities.

Fenghuan Wei *et al*., (2015) identified thirty compounds in *Jasminum grandiflorum* by using GCMS. The major volatile components of the flower were 3,7,11,15- tetramethyl-2-hexadecen-1-o（phytol） (25.77 %), 3,7,11- trimethyldodeca -1,6,10-trien-3-ol (12.54 %) and 3,7,11,15- tetramethyl -1-Hexadecen-3-ol (12.42 %). The results show that phytol is the major volatile component of *Jasminum grandiflorum.*

Praveen Kumar P *et al.,* (2018) studied the identification of bioactive compounds from the Neem sap by Gas chromatography and Mass spectroscopy (GC-MS). The GC-MS analysis of the Neem sap revealed the presence of 30 volatile compounds. Among the 30 compounds, the most predominant compounds are fatty acids like Hexadecanoic acid and Pentadecanoic acid. Hence, this current attempt forms a basis for the biological characterization and importance of the compounds which could be exploited for future development of drugs.

Seventy six kinds of chemical compounds were found in methanol extract of *E.cephalotes* including aldehydes (7.9%), phenols (7.5%), fatty acids (5.8%) and furfural (5.4%)

and 86 kinds of chemical compounds found in *M.anisodan* extract. Furfural, steroids, vitamin B and flavonoids are the main compounds of *M.anisodan* by S. Mohammadi*et al*., (2019).

Antioxidant activity

Antioxidant compounds in food play an important role as a health protecting factor. Primary sources of naturally occurring antioxidants are whole grains, fruits and vegetables. Natural antioxidants can also be replaced by commercially available, synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), which are quite unsafe to use and is restricted due to their carcinogenic effect (Velioglu*et al*., 1998). Natural antioxidants or phytochemical antioxidants are the secondary metabolites of plants (Walton and Brown, 1999). Carotenoids, flavonoids, cinnamic acids, folic acid, ascorbic acid, tocopherols, tocotrienols *etc*., are some of the antioxidants produced by this plant for their sustenance. Beta-carotene, ascorbic acid and alpha tocopherol are the widely used as antioxidants (McCall and Frei, 1999).

Flavonoids are polyphenolic compounds, which are ingredients of many vegetables and fruits. They are classified into flavanols, flavanones, flavones, iso-flavones, catechins, anthocyanins, proanthocyanidins, etc. (Huy*et et al*., 2008). They are among the most bioactive plant secondary metabolites which outperform well-known antioxidants.

Natural antioxidants are known to exhibit a wide range of biological effects including antibacterial, antiviral, anti-inflammatory, anti-allergic, anti-thrombic and vasodilatory activities. Antioxidant activity gives rise to anti-carcinogenicity, anti- immunogenicity and anti-aging activity (Gulcin*et al*., 2010).

Flavonoids serve as ROS scavengers by locating and neutralizing radicals (Gill and Tuteja, 2010). Bioactive properties such as free radical scavenging, inhibition of hydrolytic and oxidative enzymes and anti-inflammatory action of flavonoids is known (Njoku *et al*., 2011). The antioxidant activity of the dietary phenolics considered to be superior to that of the essential vitamins and is ascribed to their high redox potential, which allows them to interrupt free radical mediated reactions by donating hydrogen from the phenolic hydroxyl groups (Beevi*et al.*, 2010).

Phenolics are secondary metabolities that behave as antioxidants due to the reactivity of the phenol moiety (hydroxyl substituent on the aromatic ring). The antioxidant activities of phenolic compounds are also attributed to their ability to chelate transition metal ions, such as those of iron and copper, which have been proposed as the catalyst for the initial formation of ROS (Knezevic*et al*., 2011).

Ascorbic acid (vitamin C) is a vital component in human diet with the highest concentrations in animal organs like the liver, leukocytes, and anterior pituitary. It is used for its antioxidant effect (Ensafi*et al*., 2010). Vitamin C is a major ubiquitous non-enzymatic, water soluble antioxidant (Ueta*et al*., 2003). It acts as ROS scavenger, thus potentially protecting cells from harmful oxidative products (Fossati *et al*., 2011). Vitamin C functions in enzyme activation, oxidative stress reduction, and immune function. There is considerable evidence that vitamin C protects against respiratory tract infections and reduces risk for cardiovascular disease and some cancers (Schlueter and Johnston, 2011).

Tannins are group of polymeric phenolic substances. Consumption of tannin containing beverages, especially green teas and red wines can cure or prevent a variety of illness including heart related diseases (Van-Burden and Robinson, 1981).

Swamy *et al*., (2004) tested the leaf extracts of medicinal plant, *Leptadenia reticulata* for AgNPs production and antioxidant activity studies. He observed that, 500 µg/ml of green synthesized silver nanoparticles showed maximum (64.81 %) radical scavenging activity. The silver nanoparticles were synthesized using aqueous *Piper longum* fruit extract and the aqueous *P. longum* fruit extract and the green synthesized silver nanoparticles showed powerful antioxidant properties *in vitro* antioxidant assays. Haes *et al*., (2002).

Pourmorad *et al*., (2006) carried out a comparative study on the antioxidant potentials of some selected Iranian medicinal plant extracts. The antioxidant properties of 25 edible tropical plants were studied by Wong *et al.*, (2006). Badami and Channabasavaraj (2007) studied the *in vitro* antioxidant activities of thirteen medicinal plants collected from Western Ghats of India.

Ademiluyi and Oboh (2008) studied the antioxidant activity of methanol leaf extract of *Viscum album* by using linolenic acid peroxidation and DPPH methods. Effat *et al.*,(2008) screened thirteen medicinal plant extracts for antioxidant activity. MoniRani *et al.,*(2008) evaluated antioxidant activities of methanol extract of *Ixora coccinea* by DPPH free radical scavenging activity, reducing power and total antioxidant activity assays.

Gayatri *et al*., (2011) observed that the piperine, an alkaloid found naturally in *Piper nigrum* and *Piper cubeba*. It is widely used in various herbal cough syrups and anti-inflammatory, antimalarial, anti-leukemiatreatement. Ethanol extract of *Piper cubeba* showed high antioxidant activity.

Inbathamizh *et al.,* (2013) studied in vitro evaluation of antioxidant and anticancer potential of *Morindapubescens* synthesized silver nanoparticles. The decolorization from purple DPPH radical to yellow DPPH molecule by the sample in a dose-dependent manner

with an IC50 value of 84±0.25 µg/ml indicated the sample‟s high radical scavenging activity, which was closer to that of the standard whose IC50 value was found to be 80±0.69 µg/ml.

Niraimathi *et al.,* (2013) investigated on biosynthesis of silver nanoparticles using *Alternanthera sessilis* (Linn.) leaf extract and determined antioxidant activities. Free radical scavenging activity of the AgNPs on DPPH radical was found to increase with increase in concentration, showing a maximum of 62% at 500 µg/ml. The standard gallic acid, however, at this concentration exhibited 80% inhibition. The IC50 value was found to be 300.6 µg/ml.

The silver nitrate extract of *Annona squamosa* and *Sapium macrocarpum* showed two times more DPPH scavenging activity than the commercial antioxidant butylated hydroxyl anisole. (Ruiz *et al.*, 2008). The silver nitrate extracts of *Melissa officinalis*, *Matricaria recuttia* and *Cymbopogan citrates* were found to possess DPPH scavenging activity. (Pereira *et al*., (2009). Sowndharajan *et al*., (2010) studied the antioxidant capacity and total phenolic contents present in the silver nitrate extracts of leaves, stem, and roots of *Melothria maderaspatana* were evaluated. Sathisha *et al*., (2011) determined antioxidant potentials in silver nitrate extract of some plants, *Curcuma longa, Coffea Arabica*, *Tribulus terrestris*, *Bacopa monnieri* and *Trigonella foenumgraceum* using various *in vitro* assays.

Iwalewa *et al*., (2005) studied the pro and antioxidant effects of silver nitrate extracts of nine edible vegetables in southwest Nigeria using 1, 1-diphenyl-2-picrylhydrazyl free radical assay. The silver nitrate extract of *Helichrysum plicatum* had been reported to have antioxidant activity using two *in vitro* methods, namely DPPH and -carotene linoleic acid assays . (Tepe *et al*.,(2005)

The silver nitrate extracts of *Chlorophytum borivilianum* had been shown to scavenge DPPH radical and decrease TBRAS (Thiobarbituric Acid Reactive Substances), revealing that it is a promising anti-stress agent as well as a potential antioxidant. (Kenjale *et al*., 2007).

Antibacterial Activity

Musa *et al*., (2000) studied the phytochemistry of powdered leaves of *Acalypha recemosa* (Euphorbiaceae). This study revealed the presence of alkaloid, tannin, flavanoid and terpenes. Antimicrobial activities of cold water, hot water and methanolic extracts were studies against *Staphylococcus aureus* was more than *Escherichia coli* but *Candida albicans* was completely resistant to the extracts. The cold water extracts showed activity with MIC range from 3.0 mg/ml (against *S. aureus*) to 4.0 mg/ml *Escherichia coli* for cold water and 7.0 mg/ml for the two isolates (methanolic extract). The MBC of cold water extract (6.0 mg/ml) was able causes 2 log cycle reduction of cell population in 90 minutes. Prema (2004) studied the antibacterial activity in eleven medicinal plants. The acetone extract of *Acalypha indica* was more effective against *Staphylococcus aureus*. Ethanol extract of *A. indica* and *Eucalyptus globulus* were highly sensitive to *S. aureus* and *P. Aeruginosa*.

Poonkothai *et al*., (2005) worked on antibacterial activity of chloroform, ethanol and aqueous extracts of the leaves of *Gymnema sylvestre* on *Bacillus subtilis, Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa, Escherichia coli* and *Salmonella typhi* on Muller Hindon agar plates. Commercially available chloramphenicol disc (30 mg) was used as control and discs impregnated with DMSO were also used in this technique. *Klebsiella pneumoniae* was resistant to both chloroform and ethanol extracts exhibiting a zone of inhibition of 12 and 11 mm respectively. *Pseudomonas aeruginosa* (16 and 21mm) and *Salmonella typhi* (17 and 19mm) were found to be sensitive to both the extracts. This indicates that gymnemic

acid, an active component of *Gymnemasylvestre* double in both chloroform and ethanol was found to have a strong antibacterial activity. There was no significant effect of aqueous extract because there was no zone of inhibition.

Akinpelu *et al*., (2009) studied the medicinal plants *Jatropha curcas*and *Newboulda laevis*. Methanolic leaf extract of *J. curcas, N. laevis* exhibited antibacterial activity against 8 of the thirteen tested bacterial isolates at a concentration of 20 mg/ml. The zones of inhibition exhibited by *J. curcas*ranged between 18 and 17mm. *N. laevis* varies between 10 and 23 mm.

Dhale and Birari (2010) studied the antimicrobial effect of *Jatropha gossypifolia* leaf extracts on gram positive species *Staphylococcus spp.* and *Bacillus spp*. and gram negative species like *Escherichia spp*. and *Pseudomonas spp.,* in solvents like petroleum ether, alcohol and chloroform. The method employed was disc diffusion method, standard was Amphicillin, the alcoholic extract of leaves showed maximum antibacterial activity.

Dipankar Choudhury *et al*., (2011) studied phytochemical screening and antimicrobial activity of extracts from leaves and stem of *Ecbolium linnean* .The bacterial pathogens were strongly inhibited by leaf extracts but acetone extracts of stem have failed to inhibit the growth of *Staphylococcus aureus* and *Pseudomonas aeruginosa* even at the highest concentration. The results revealed that leaf extracts were found to be more effective than stem extracts. *E. linneanum* possesses antimicrobial activity against most commonly encountered human pathogens.

Yusha‟u*, et al*., (2011) studied antibacterial activites of ethanolic extracts of *Annona squamosal (L.)* leaves were studied against clinical respiratory tract isolates of *Klebsiella pnemoniae, Proteus species, Pseudomonas species, Staphylococcus aureus, Streptococcus*

pnemoniae and - haemolytic *Streptococci* using disc diffusion and micro broth dilution techniques. Sensitively test results showed that water fraction of the plant was active on *Stephylococcus aures and Streptococcus pnemoniae* (10mm) at 50µg/disc concentration while ethanolic extract of the plant was active , *Streptococcus pnemoniae*and *Proteus species* at 200µg/disc concentration with zone diameter formed by *Klebsiella pnemoniae*(11mm) being wider than that formed in response to standard Augmentin disc (06mm).

Nidhi uttamkumar and sumitkumar (2013) evaluated antibacterial activity of rhizome of *Barleria prionitis*. The methanol extract showed antibacterial activity against two Gram‟s positive *(S. aureus and B. cereus*) and two Gram‟s negative (*E. coli and S. typhi*) bacteria. The antibacterial potential was measured by agar disc plate method. The active phytocomponents of *Barleria prionitis* were revealed using Gas chromatography with mass spectrophotometric detector and 27 constituents identified, Phthalazine was the most abundant phytocompound in methanol extract. All the results supported that the extract can be used to prevention of bacterial infection and may have role in pharmaceutical medicine evolution.

Nayan R. Bhalodia and V.J.Shukla (2014) reported extracts obtained from *Cassia fistula* show strong activity against most of the tested bacterial and fungal strains. The results were compared with standard antibiotic drugs. The results show that the activity of hydroalcohol extracts of *Cassia fistula* shows significant antibacterial and antifungal activities.

Niveditapatel *et al*., (2014) reported phytochemical analysis and antibacterial activity of *Moringa oleifera*. The result showed that the plant leaves are very good nutrient supplement for malnutrition and also used as an antibiotic. To evaluate the antibacterial activity of *Moringa oleifera* leaf extracts, *Escherichia coli, Pseudomonas aeroginosa, Staphylococcus aureus, Proteus vulgaris, Streptococcus mutans, Bacillus subtilus,* and *Staphylococcus*

epidermidis bacteria were used. Phytochemical analysis of the leaf in solvents of varying polarity; viz., aqueous, ethanol were also carried out. The phytochemical screening indicated the presence of flavonoids, tannins, steroid, alkaloid, saponins etc. in the both extracts. Well diffusion method was used to assess the antibacterial effect of the extracts on micro-organisms. The ethanolic and aqueous extract were active against all strains but the ethanol leaf extract showed maximum activity against *Streptococcus mutant* and aqueous extract shows maximum activity against *Proteus vulgaris*.

Hassan Waseem *et al*., *(*2016) detected the antimicrobial activity of *C. tamala* against a number of organisms. The Plant extract from *C. tamala* was found to have antimicrobial activity against only one tested bacterium, *S. aureus* (ATCC 25293). They found different degrees of antimicrobial activity against all tested gram positive and gram negative bacteria contrary to our result where only *S. aureus* was found to be effective.

Anthelmitic activity

Anthelmintic effects of various extracts of the whole plant of *Enicostemma littorale*, resulted helminthiasis of the worm in following order, ethanol > ethyl acetate > chloroform > hexane. In particular the ethanol extract exhibited an increased paralytic as well as anthelmintic effect over albendazole. This may be due to the increased level of extraction of tannins in ethanol followed by ethyacetate> water > chloroform > hexane extracts (Vidyadhaer *et al*., 2010).

Seema and Amrish (2012) studied anthelmintic activity of colloidal solution of silver nanoparticles using *Saraca indica* leaves extract. Anthelmintic activity of colloidal solution of silver nanoparticles was more than the aqueous extract. Overall the anthelmintic activity revealed the concentration-dependent nature of the aqueous extracts and colloidal solution of silver

nanoparticles. Different concentration of aqueous extracts and colloidal solution of silver nanoparticles were tested against adult Indian earthworms (*Pheretimaposthuma*) as test worms. The bioassay involved determination of the time of paralysis and time of death control. Piperazine citrate 23 (10 mg/ml) was used as a standard reference drug. Normal saline was used as a control.

Anthelmintic activity of various concentrations (25-500mg/ml) of hot and cold hydroalcohoic extracts of *Gymnema sylvestre* were evaluated that involving determination of time of paralysis (p) and time of death (D) of the worms. Albendazole was used as standard anthelmintic drug and distilled water was used as control. The hydro alcoholic extracts significantly exhibited the paralysis in worm and also caused death of worms in dose dependent manner, among which hot maceration extract showing more significant results when compared with the cold maceration extract (Reddy *et al*., 2013).

Anthelmintic activity of the alcoholic and aqueous bark extracts of the *Holarrhenaanti dysentrica* was assessed by Satpute *et al*.,(2014). The effect was dose dependent and shortest time taken for paralysis and observed in case of alcohol extract at 40mg/ml concentration with potent activity against Indian adult earthworms (*Pheretima poshuma*).

Dora Babu *et al*., (2018) evaluates anthelmintic activity of methanol extract of *Buchanania axillaris* Desr on Indian adult earthworms, *Pheretima posthuma* (annelid).Bark was extracted by using soxhlet apparatus. Phytochemical screening of crude extracts showed the presence of steroids, alkaloids, tannins, flavonoids, carbohydrates and glycosides. Various concentrations (25, 50, 100mg/ml) of crude extracts were tested for Anthelmintic activity. The activity was compared with standard piperazine citrate. The methanolic extract shows significant

activity when compared to the standard piperazine citrate. The paralysis and death time is 50, 31, 17 and 76, 52, 34 minutes respectively at concentrations 25, 50 and 100mg/ml. whereas these are 31, 18, 10 and 63, 41, 22 minutes for piperazine citrate.

Narasimha Rao, Y *et al*., (2018) investigated anthelmintics activity of ethanolic extract of *Potrulaca quadrifida* whole plant using earthworms (*Pheretima posthuma*), various concentrations (50 and 100 mg/ml) of plant extract were tested. Piperzine citrate (10 mg/ml) was used as reference standard drug whereas distilled water as control. Determination of paralysis time and death time of the worms were recorded. Extract exhibited significant anthelmintics activity at the concentration of 100 mg/ml. The result shows that aqueous extract possesses vermicidal activity and found to be effective as anthelmintics.

Somnath De *et al*., (2019) studied anthelmintic activity of methanol and aqueous extract of *Calotropis gigantea*. Both are showed different paralysis and death time at similar concentrations. Albendazole was used as reference standard drug. As expected control (0.5% CMC) does not show any positive results. But standard drug (albendazole 20mg/ml), methanol and aqueous plant extract (50mg/ml and 100mg/ml) showed significant results of paralysis and death time of each worms.

Ruby Philip *et al*., (2019) was investigated for anthelmintic potential from extracts of *Jasminum sessiliflorum* using earthworms, *Pheretima posthuma*. Different concentration of plant extracts were used for the evaluation. Albendazole (10 mg/ml) was used as reference standard drug. The method employs the determination of paralysis time and death time of the worms and these results were recorded. Extracts showed significant activity. The ethanolic extract was found to be most efficient.

PLANT MATERIALS

Botanical name: *Corchorus aestuans* L.

SYSTEMATIC POSITION

Class : Dicotyledons

Sub class : Dilleniidae

Order : Malvales

Family : Tiliaceae

Subfamily : Grewioideae

Plate -1

DISTRIBUTION:

Corchorus aestuans L. is a Prostrate or ascending herb, widely distributed in the tropics. The whole plant is used in the treatment of fever and stomach ache while seeds are used in the treatment of gonorrhea and tonic.

BOTANICAL DESCRIPTION:

The plants are tall, usually annual herbs, reaching a height of 2–4 m, unbranched or with only a few side branches. The leaves are alternate, simple, lanceolate, 5–15 cm long, with an acuminate tip and a finely serrated or lobed margin. The flowers are small (2–3 cm diameter) and yellow, with five petals; the fruit is a many-seeded capsule.

PLANT MATERIAL

Botanical name: *Triumfetta rhomboidea* Jacq.

SYSTEMATIC POSITION

Class : Dicotyledons

Sub class : Dilleniidae

Order : Malvales

Family : Tiliaceae

Subfamily : Grewioideae

Plate -2

DISTRIBUTION:

Triumfetta rhomboidea Jacq. is a tropical weed. It is very widespread in continental Africa, including South Africa. It is introduced and naturalized in Cape Verde, Madagascar, Seychelles, Reunion and Mauritius and in Australia.

BOTANICAL DESCRIPTION:

Erect, much-branched under shrubs; stem hairy, stellate hairs mixed with simple hairs. Leaves 2.5-7 x 2.5-6 cm, generally rhomboid-ovate, base rounded or cordate, margins irregularly serrate, apex acute or acuminate, stellate-pubescent to glabrescent; basal ones palmately 3-lobed; petioles up to 4 cm long; stipules 3-4 mm long, subulate. Flowers in terminal or leaf-opposed cymes, 5-6 mm across, shortly pedicellate. Sepals 4-5 mm long, lanceolate. Petals 4-5 mm long,

Stamens 8- 15. Ovary 1.5 mm long, ovoid. Capsules 4-5 mm across, subglobose, stellate hairy outside, setose; setae c. 2 mm long, hooked at tip.

COLLECTION AND IDENTIFICATION OF PLANT MATERIALS

The fresh plant materials of *Corchorus aestuans* L. and *Triumfetta rhomboidea* Jacq. are collected from St. Mary‟s College campus, Thoothukudi. The collected samples were cut into small fragments and shade dried until the fracture is uniform and smooth. The dried plant material was granulated or powdered by using a blender, and sieved to get uniform particles by using sieve No. 60. The final uniform powder was used for extraction of active constituents of the plant materials.

QUALITATIVE ANALYSIS

Water soluble extractive

Two gram of the shade dried powder of *Corchorus aestuans* and *Triumfetta rhomboidea* was macerated with 50 ml water in a closed flask for 24 hours. Shaking frequently during first 6 hours and allowed to stand for 18 hours. It was filtered using a muslin cloth and used for phytochemical analysis.

Methanol soluble extractive

Two gram of the shade dried powder of *Corchorus aestuans* and *Triumfetta rhomboidea* was macerated with 50 ml methanol in a closed flask for 24 hours. Shaking frequently during first 6 hours and allowed to stand for 18 hours. It was filtered using a muslin cloth and used for phytochemical analysis.

Acetone soluble extractive

Two gram of the shade dried powder of *Corchorus aestuans* and *Triumfetta rhomboidea* was macerated with 50 ml acetone in a closed flask for 24 hours. Shaking frequently during first 6 hours and allowed to stand for 18 hours. It was filtered using a muslin cloth and used for phytochemical analysis.

Ethanol soluble extractive

Two gram of the shade dried powder of *Corchorus aestuans* and *Triumfetta rhomboidea* was macerated with 50 ml ethanol in a closed flask for 24 hours. Shaking frequently during first 6 hours and allowed to stand for 18 hours. It was filtered using a muslin cloth and used for phytochemical analysis.

Test for tannins (Ciulei I.)

To 1 ml of the extract, 2 ml of 5% $FeCl_3$ was added. A dark blue or green -black indicates the presence of tannins.

Test for saponins (Harbronejb)

Foam test

The crude extract is mixed with 5 ml of distilled water and shaken vigorously, resulting in the formation of stable foam which is a positive indication for saponins.

Test for Flavonoids (Savithramma*et al* and selvaraj *et al.*,)

For identification of flavonoids, 2ml of plant extract, 1ml of 2N sodium hydroxide (NaOH) was added. Formation of yellow colour indicates the presence of flavonoids.

Test for Coumarins (Harbrone JB)

For identification of coumarins, 1ml of plant extract, 1ml of 10% NaOH was added. Formation of yellow colour indicates the presence of coumarins.

Test for Terpenoids (Harbrone JB)

For identification of terpenoids, 0.5 ml of the plant extract, 2ml of chloroform along with concentrated Sulphuric acid. Formation of red brown colour at the interface indicates the presence of Terpenoids.

Test for Quinines (P. D. Egwaikhide and C. E. Gimba)

A small amount of extract was treated with concentrated HCl and observed for the formation of yellow colour precipitate.

Test for Alkaloids (E. C. G. Clarke)

Wagner's test

A fraction of extract was treated with Wagner‟s reagent (1.27 g of iodine and 2 g of potassium iodide in 100 ml water) and observed for the formation of reddish brown colour precipitate. There was a formation of reddish brown colour confirming the presence of alkaloid.

Test for Sterols (P. D. Egwaikhide and C. E. Gimba)

Extract (1 ml) was treated with chloroform, acetic anhydride and drops of H_2SO_4 was added and observed for the formation of dark pink or red colour. No dark pink or red colour precipitate, absence of sterols.

Test for Carbohydrate (Harbrone JB)

Fehling's test

5 ml of Fehling‟s solution was added to 0.5 mg of extract and boiled in a water bath. The formation of yellow or red precipitate indicates the presence of reducing sugars.

Test for Glycosides (E. C. G. Clarke)

0.5 mg of extract was dissolved in 1 ml of water and then aqueous NaoH solution was added. Formation of yellow colour indicates the presence of glycosides.

Test for Protein (Harbrone JB)

Ninhydrin test:

0.5 mg of extract was taken and 2 drops of freshly prepared 0.2% ninhydrin reagent was added and heated. The appearance of pink or purple colour indicates the presence of proteins, peptides or amino acids.

Test for phenol (Harbrone JB)

To 1 ml of the extract, 2 ml of distilled water was added and followed by few drops of 10% aqueous ferric chloride. Appearance of blue or green colour indicates the presence of phenols.

Quantitative analysis of antioxidant

Total phenolic content :(Duan *et al*., 2006)

Reagents

- 50%Folin – ciocalteau reagent

- 20%sodium – carbonate
- Gallic acid – standard

Procedure

100mg of samples was homogenate with 10 ml of distilled water and filtered through a muslin cloth. 1ml of the filtrate was added to 1.5 ml of deionised water and 0.5 ml of 50% folinciocalteau reagent and the contents were mixed thoroughly. After 1min, 1 ml of 20% sodium carbonate solution was added mixed the control contained all the reagents except the sample. After 30 minutes of incubation at 37^{o}c, the absorbance was measured at 750nm. Total phenolics were calculated as Gallic acid equivalent (GAE) per gram fresh weight.

Total flavonoid content (Zhinshen *et al*., 1999)

Reagents

- 5% sodium nitrate (NaNo2)
- 10% Aluminium chloride (Alcl3,H2O)
- 1N sodium hydroxide (NaoH)
- Quercetin standard

Procedure

100mg of plant material was homogenized with 10ml of distilled water and filtered through a muslin cloth. 0.5 ml of the extract was added with 2.5 ml distilled water and mixed. After 6 minutes 0.15 ml NaOH, was added and again after 6min 0.3 ml of 10% $Alcl^{3}$ was added. After 5 minutes 1ml of 1M NaOH and 0.5 ml of water were added. Following through mixing of

the solution the absorbance against blank were recorded at 510nm. Quercetin was used as standard and the results were expressed as my quercetin equivalents (QE) 1g fresh weight.

Vitamin C [Ascorbic acid] (Baker and Frank, 1968)

Reagents

- 5% of TCA
- Indophenols reagent
- 20mg of dichlorophenol indophenols was dissolved in 10ml of warm distilled water
- DT reagent 2g of 2, 4 dinitraphenyl hydrazine and 1g of thiourea were dissolved.
- 85% sulphuric acid
- L-ascorbic acid - standard

Procedure

100 mg of plant material was homogenized with 10ml of 5% Trichloro acetic acid (TCA). The homogenate was centrifuged. To 2 ml of indophenols reagent and 0.5ml of DT reagent was added and incubated at 10c for 1hour and then cooled in ice bath and 2.5 ml of 85% sulphuric acid was added and shaken well for 30 minutes (until) red colour appeared. The absorbance was measured at 540nm. 1-ascorbic acid was used as standard and the results were expressed as mg/1g/FW.

Estimation of Tannin (Julkunen-Titto, 1985)

Procedure

100 mg of sample homogenized with 10 ml of distilled water and filtrated through a muslin cloth. 1ml of aliquot of aqueous extract was mixed with 1.5ml of 4% vanillin (prepared

with methanol) and 750 µl of concentrated HCL was added the solution was shaken vigorously and left to stand at room temperature for 20 minutes in darkness the absorbance against blank was read at 500nm using UV-Visible spectrophotometer. Results were expressed as mg catechin equivalent (CE) 1g tissue.

Vitamin E (Tocopherol): Rosenberg, 1992

Procedure

The plant sample (2.5g) was homogenized in 50ml of 0.1 N sulphuric acids and allowed to stand overnight the content in the flask was shaken vigorously and filtered through what man No.1 filter paper. Aliquots of the filtrate were used for estimation.

In stoppered centrifuge tubes 3ml of extract and 3ml of water were pipette out separately. To both the tubes, 3ml of ethanol and 3ml of xylene were added, mixed well and centrifuged. Xylene (2.0ml) layer was transferred into another stoppered tube. To each tube, 2.0 ml of dipyridyl reagent was added and mixed well, the mixture (3ml) was pipette out into a cuvette and the extinction was read at 460nm. Ferric chloride solution (0.66 ml) was added to all the tubes and mixed well. The red colour developed was read exactly after 15min at 520nm. Tocopherol was used as standard.

FT-IR analysis

A little powder of plant specimen was mixed with KBr salt, using a mortar and pestle, and compressed into a thin pellet. Infra -red spectra were recorded as KBr pellets on a Thermo Scientific NicotiS5ID1 transmission, between 4000-400 cm^{-1} (Kareru et al., 2008).

GC-MS Analysis:

Extract Preparation

The 50g tuber powder of *Corchorus aestuans* and *Triumfetta rhomboidea* was serially extracted with 250 ml of Methanol with the help of Soxhlet apparatus. The extraction procedures were continued for 3-4 hours at 60°C -80°C[15]. These extracts were concentration under reduced pressure evaporator and stored in air tight vials at 4°C for further study.

Phytochemical analysis by GC-MS

Gas chromatography-Mass spectrometry (GC-MS) analysis of the ethanolic extracts was performed by using a GC-MS (Model; QP2010 series, Shimadzu, Tokyo, Japan) equipped with a VF-5ms fused silica capillary column of 30 m length, 0.25 mm dia.and0.25µm film thickness. For GC-MS detection, an electron ionization system with ionization energy of 70 eV was used. Helium gas (99.99%) was used as a carrier gas at a constant flow rate of 1.51 ml/min. Injector and mass transfer line temperature was set at 200 and 240°C respectively. The oven temperature was programmedfrom70to220°Cat10°C/min, held isothermal for 1 min and finally raised to 300°C at 10°C/min. 2 µ1 of respective diluted samples was manually injected in the split less mode, with split ratio of 1:40 and with mass 18 scan of 50-600 amu. Total running time of GC-MS is 35min. The relative percentage of the each extract constituents was expressed as percentage with peak area normalization.

Identification of phytochemical components

The identity of the components in the extracts was assigned by the comparison of their retention indices and mass spectra fragmentation patterns with those stored on the computer library and also with published literatures. NIST08s. LIB and WILEY 8. LIB library sources

were used for matching the identified components from the plant material.

ANTIOXIDENT ACTIVITY

Crude samples extracts were prepared by pouring 100ml of distilled water in a conical flask containing 10g of each samples separately in the ratio of 10:1 (V/W). After 24 hours, the mixture was filtrated through whatman no: 1 filter paper and the filtrate were evaporated to dryness. Crude (aqueous) extracts of all samples (1mg/ml) were used for the determination of free radical scavenging activity.

Free radical scavenging assays (Hatano *et al.,* 1998).

Free radical scavenging assay was measured by 2-2 Diphenyl, 1-picryl hydrazine (DPPH) method proposed by with slight modifications. 1ml of aliquot of test sample was added to 3ml of 0.004% DPPH solution prepared in methanol. The mixture was vortexes for 1min and kept at room temperature for 30 minutes in darkness the absorbance was read at 517 nm. Allow absorbance of the reaction mixture indicated a high free radical scavenging activity. Ascorbic acid was used as standard.

DPPH scavenging activity (%)

A control –A test / A control * 100

Where, a control is the absorbance of the DPPH solution without test solution. A test is the absorbance of DPPH with the test solution. Aqueous extract was used as blank.

Antibacterial studies

Extraction of plant materials

The plant powder was extracted with methanol, ethanol, acetone and water.25 gms of plant powder was extracted with methanol, acetone and water solution individually in soxhelt apparatus continuously for about 4-6 hours, which was again concentrated till it become semi solid. It was evaporated to dryness and stored at 0 C, until the time of the experiment.

Bacterial strains used

The test organisms were obtained from the Department of Microbiology; St. Mary‟s College (Autonomous), Thoothukudi. The one gram positive bacteria viz; *Bacillus subtilis* G-ve MTCC 1133 and four gram negative bacteria *Escherichia coli*, G-ve, MTCC 50, *Staphylococcus* G-ve, 737. *Vibrio cholera* G-ve MTCC 3906, were used in the present study.

Broth Medium:

- Nutrient broth Himedia MOO1
- Nutrient broth 1.3 gm
- Distilled water 100 ml

2-3 ml of sterilized broth medium was taken in the culture tube. The inoculating loop was flamed and after a few minutes a loopful bacterial colony was transferred to the broth medium. This microbe culture was incubated at room temperature for 24 hours.

Agar medium:

- Nutrient broth Himedia MOO1
- Nutrient broth 1.3 gm
- Distilled water 100 ml

To prepare the agar medium all the above ingredients were dissolved and sterilized.

Disc diffusion method

Anti- bacterial activity was evaluated by agar disc diffusion method (Kirby –Bauer *et al*., 1986). Test solution was prepared with known weight of methanol, ethanol, acetone and water extracts dissolved in 5% dimethyl sulphoxide (DMSO). What man No.1 filter paper disc (5mm) was impregnated with 20 of these extracts and allowed to dry at room temperature. The spread plates were prepared by proper concentration of inoculate. Each sample loaded discs was placed in the seeded agar plate. 24-48 hours of + 37^0c incubation, the diameter of the inhibition zone was for positive control, amoxicillin discs (100g/ml) was used, whereas for negative control; respective solvents loaded on the sterile discs.

RESULT AND DISCUSSION

Plant have been major sources of bioactive principle employed in drug formulations both modern and traditional medicine. According to World Health Organization 80% of the people living in rural areas depend on medicinal herbs as primary health care system. (Sakarkar and Deshmukh, 2011).

Corchorus aestuans and *Triumfetta rhomboidea* are the important medicinal plants in Tiliaceae. Both the plants are selected for the present study. *Corchorus aestuans* is used as diuretic, gonorrhoea, anti-inflammatory, antimicrobial, (D.Ramadevi and S.Kanapathi, 2012). *Triumfetta rhomboidea* is used for tonic, intestinal ulcer, cooling, useful in dysentery and diuretic. Leaves, Flowers and Fruits of *Triumfetta rhomboidea* is used in gonorrhoea and leprosy. In the present study, active constituents of the plants were analyzed and evaluated (V.P.Devmurari et al., 2010).

QUALITATIVE ANALYSIS

Preliminary phytochemical analysis of the various solvent extracts of *Corchorus aestuans* and *Triumfetta rhomboidea* showed different results. The alkaloids, phenols, tannin, saponins, glycosides, quinones, flavonoids, terpenoids and coumarins were predominantly present in solvent extracts. Table: (1&2).

Johnson *et al*., (2012) reported the methanol extracts of some medicinal plants contain tannin, saponin, flavonoid, phenol, betacyanin and coumarin. Sukumaran *et al*., (2011) reported the presence of alkaloids, flavonoids, tannins, saponins, phenol and terpenoids in *Peltrophorum pterocarpum* flowers.

QUANTITATIVE ANALYSIS

The total phenol, flavonoid, tannin, vitamin C and vitamin E were analysed in plant extract of *Corchorus aestuans* and *Triumfetta rhomboidea* belongs to the family Tiliaceae. Table (3-7)

TOTAL PHENOL

Phenolics are the most wide spread secondary metabolites and are believed to be responsible for antioxidant activity. The total phenol contents of *Triumfetta rhomboidea* (7.2143 mg GAE/g) were higher than that *Corchorus aestuans* (6.6703 mg GAE/g) (Table -3). Phenolic compounds are as class of antioxidant agents act as free Terminators (Shahidi and Wanasundara, 1992). Phenolic compounds have a variety of beneficial activities. They have potential antioxidants and free radical scavenger. (Meenakshi et al., 2012). The antimicrobials (most of the phenolics) may provide a microbe-free environment within the body.

TOTAL FLAVANOID

Flavonoids are secondary metabolites and has responsible for antioxidant activity in medicinal field. The total flavonoids contents of *Triumfetta rhomboidea* (3.0113 mg GAE/g) were higher than that *Corchorus aestuans* (0.8185 mg GAE/g) (Table -4). Flavonoids are potent antioxidants and epidemic Studies indicate that high flavonoid in take is correlated with decreased risk of lifestyle diseases like diabetes and cardiovascular diseases (Kaur *et al*., 2008). Flavonoids are potent water-soluble antioxidants and free radical scavengers, which prevent oxidative cell damage and have strong anti- cancer activity (Havsteen, 2002).

TOTAL VITAMIN -C

Table -5 shows *Triumfetta rhomboidea* (17.0613mg/g) and *Corchorus aestuans* (3.66mg/g) contain significant amount of vitamin C. Vitamin C is a vital component in human

diet with the highest concentrations in animal organs. Vitamin-C is a non-enzymatic, water soluble antioxidant (Ueta *et al*., 2003). Vitamin C functions in enzyme activation, oxidative stress reduction, and immune function. It protects against respiratory tract infection and reduces risk for cardiovascular disease and cancer.

TOTAL TANNINS

(Table-6) shows *Triumfetta rhomboidea* (1.3833mg/g) and *Corchorus aestuans* (1.1847mg/g) contain significant amount of tannin (Table -6). Tannins are present primarily in the leaves of trees growing in stress conditions. They are accumulated in the vacuoles, especially those of the epidermal layer and the palisade mesophyll. Tannins are useful in treating inflammation, ulcers, and remarkable activity in cancer prevention and anticancer activities (Li *et al*., 2003; Akinpelu*et al*., 2009).

TOTAL VITAMIN –E

Total vitamin E content in *Corchorus aestuans* (16.1443mg/g) is highest and *Triumfetta rhomboidea* (4.636mg/g) is lowest (Table-7). Vitamin E is a fat-soluble nutrient found in many foods (Jacob, 1995). In the body, it acts as an antioxidant, helping to protect cells from the damage caused by free radicals. Free radicals are compounds formed when our bodies convert the food we eat into energy (Havsteen, 1983).

FTIR

Fourier Transform Infrared spectroscopy was used to analyse the functional group present in *Triumfetta rhomboidea* and *Corchorus aestuans.* The FTIR spectroscopy analysis of *Corchorus aestuans* obtained peaks at 3419.56 $cm^{-1,}$ 2920.99 $cm^{-1,}$ 1796.57 cm^{-1}, 1645.17 cm^{-1},

1551.63 cm^{-1}, 1432.05 cm^{-1}, 1373.22 cm^{-1}, 1243.04 cm^{-1}, 819.69 cm^{-1}, 702.04 cm^{-1}, 668.29 cm^{-1}, 602.71 cm^{-1}, 517.85 cm^{-1}. These absorption peaks are known to be associated with the stretching vibration for O-H in Aromatic amines, H-C-H in Alkane, C=O in Ester, C=N in Guanidine, N=O in Nitro group, C-H in Alkane, C–O in Hydroxyl groups, C-O-C in Aralkyl, S-O in Sulphinic group, N-O in Nitrate, N-H in Aromatic amines, , C-I in Iodo compound, C-Br in Aromatic compound, Fig: 6, Table: (8).

The FTIR spectroscopy analysis of *Triumfetta rhomboidea* obtained peaks at 3423.41 cm^{-1} , 2920.99 cm^{-1}, 1737.74 cm^{-1}, 1383.83 cm^{-1}, 1318.25 cm^{-1}, 1249.79 cm^{-1}, 1165.89 cm^{-1} , 1030.88 cm^{-1}, 781.12 cm^{-1} , and 516.89 cm^{-1} . These absorption peaks are known to be associated with the stretching vibration for N-H in Aromatic amines, H-C-H in Alkane, C=O in Esters, C=C in Aromatic, N=O in Nitro group, C-N in Aryl amine, C-O-C in Acetates, C–O in Ether groups, C-H in Alkane, C-Br in Aromatic compound, Fig: 7, Table: (9).

From the spectral data presence of O-H, H-C-H, C=O, C=N, N=O, C-H, C–O, C-O-C, S-O, N-O, N-H, C-I, C-Br, C=C, C-N were identified. These bonding are responsible for the presence of amine group, aldehyde group, Aromatic group, nitro group, ether, sulphinic acid, carboxylic group, aliphatic group and iodo group. Carboxylic acid present in the medicinal plant serves as main pharmaceutical product in curing ulcer, jaundice, head ache, stomatitis, hemicranias, fever, pain in lever, treatment of rheumatic joint pain. Amides, amine and amino acid are the main groups which are involved in protein synthesis. The study revealed that the whole plant of *Triumfetta rhomboidea* and *Corchorus aestuans* contain a considerable amount of secondary metabolites and it may considered in future to be used human disease management.

GC-MS Analysis

The GC-MS analysis of ethanol plant extract of *Corchorus aestuans* is confirmed the presence of 21 compounds with retention time. Interpretation of mass spectrum of GC-MS was using the data base of NIST and WILEY libraries. Out of this 21 compounds 8 compounds were majority present in the plant extract of *Corchorus aestuans* respectively 1-Methyl-3-Phenyl indole (-0.21%), Silicic acid, diethyl bis ester, (1.12%), Arsenous acid, tris ester (1.12%), 1,2-Bis (trimethyl silyl) benzene (0.15%), 1,2-Benzisothiazole-3-amine (3.52%), Tris arsane (3.52%), 5-Methyl-2-Phenylindolizine (10.82%), 1,4-Bis benzene (10.82%).

The thirteen minor compounds such as Hepta methyl trisilo (-0.21%), Methyl 3-bromo-1-ada mantane acetate (-0.21%), Octasiloxane (1.12%), Dodecahydropyrido (1,2-b) isoquinoline -6-one (1.12%), 2- Methyl-7-phenyl indole (1.12%), 9H fluorene 2-carboxylic acid, 9-oxo amide (0.15%), 2,4-cyclohexadien-1-one, 3,5- bis-4-hydroxy-(0.15%) (E)-2- bromobutyloxychalcone (32.79%), Methyl tris silane (13.80%), 1H Indole, 1- methyl-2-phenyl (13.47), cyclotrisiloxane, hexamethyl (13.47%) were also reported from the ethanolic plant extract of *Corchorus aestuans*. The chemical constituents analysis results of *Corchorus aestuans* plant was reported in table 10 and their GCMS chromatogram is presented in fig 8 Table 10.

The first compound identified with less retention (14.596) min was Heptamethyltrisilo, Methyl 3- bromo-1-adamantaneacetate, whereas 1H-Indole, 1-Methyl-2-phenyl, cyclotrisiloxane, and hexamethyl was the last compound which took longest retention time (18.520 min) to identify. At (17.338 min) retention time 1, 4-Bis benzene (E) -2-bromobutyloxychalcone was found to be high (32.79%) and the lowest percentage (-0.21%) was found to be Heptamethyltrisilo, Methyl 3- bromo-1-adamantaneacetate.

The GC-MS analysis of ethanolic plant extract of *Triumfetta rhomboidea* is confirmed the presence of 16 compounds with retention time. Interpretation of mass spectrum of GC-MS was using the data base of NIST and WILEY libraries. Out of this 16 compounds 7 compounds were majority present in the plant extract of *Triumfetta rhomboidea* respectively cyclotrisiloxane, hexamethyl (19.78 %), 1H Indole , 1 Methyl-2-phenyl (6.27%), Benzo (h) Quinoline, 2, 4-dimethyl (5.32%), 2- Ethylacridine (15.06%), 1,2-Bis benzene (11.97%), 2- Methyl-7-phenylindole (15.06%).

The nine minor compounds such as 3,Methoxy-2,4,5-trifluorobenzoic acid, Nona decyl ester (15.06%), Tris butyl dimethyl silyloxy arsane (5.32), 3-Quinoline carboxylic acid, 6,8-difluoro -4- hydroxy - ethyl ester (6.27%), 2,4- cyclohexadien (19.78%), 2-(Acetoxymethyl)-3-Methoxycarbonyl) biphenylene (15.39%), 1-Methyl-3-phenylindole (15.39%), Trimethylsilyl (5.74%), 5-Methyl -2- phenylindolizine (17.3%), 1,2-Benzisothiazole-3- amine (17.30%) were also reported from the ethanolic plant extract of *Triumfetta rhomboidea*. The chemical constituents analysis results of *Triumfetta rhomboidea* plant were reported in table 11 and their GCMS chromatogram is presented in fig 9 Table 11.

The first compound identified with less retention (16.223) min was 2- Ethylacridine, 2 Methyl -7- phenylindole whereas 5 Methyl-2-phenyl indolizine and 1, 2-Benzisothiazole-3-amine was last compound which took longest retention time (20.922 min) to identify. At (17.008 min) retention time cyclotrisiloxane, hexamethyl,2,4-cyclohexadien -1-one, 3,5 bis -4-hydroxy-was found to be high (19.78) and the lower percentage (3.22%) was found to be 2-Ethylacridine, 1H- indole, 1- Methyl-2-phenyl. The above mentioned isolated compounds from the ethanolic extract of *Triumfetta rhomboidea* plant have a medicinal important.

Benzo (h) quinoline in the ethanolic plant extract of *Triumfetta rhomboidea* is a main antiviral compound (WWW.pharmaexpert.ru/pass online predict, php.). Quinolines are important compounds because of their bioactive properties and medicinal uses such as antimalarial (Larsen *et al*., 1996), anti-inflammatory (Chen *et al*., 2001), antiasthmatic (Roma *et al*., 2000), antibacterial (Dube *et al*., 1998) and tyrosine inhibiting agent (Billker *et al*., 1998).

2-Ethylacridine is found in ethanolic plant extract of *Triumfetta rhomboidea* is a main antimicrobial and antitumor compound. 1H Indole, 1-Methyl, 2-phenyl is found in both the ethanolic plant extract. Which is used for Antineoplastic (breast cancer) (J.Vijayakumari and T.Leon Stephan Raj., 2019). Cyclohexadien and Benzisothiazole identified in both the ethanolic plants extracts of *Corchorus aestuans* and *Triumfetta rhomboidea* is a main antioxidants compound that helps remove harmful toxins and free radicals in the human body. The presence of the identified bioactive components present in *Corchorus aestuans* and *Triumfetta rhomboidea* plants extract could be responsible for the antioxidant and antimicrobial effects of the plants. Identification of these compounds in the plants that serves as the basis in determining the possible health benefits of the plant leading to further biological and pharmacological studies.

ANTIOXIDANT ACTIVITY

An antioxidant is a molecule capable of showing or preventing the oxidation of other molecules. In a biological system, they protect cells from the damage caused by unstable molecules known as free radicals. Antioxidants terminate the chain reactions by removing free radical intermediates, and inhibit other oxidation reactions by being oxidized themselves. They

are believed to play a role in preventing the development of chronic diseases like cancer, heart disease, storke, AD, RA and cataracts (chakra borty *et al.,* 2010).

Antioxidant chemicals found in nature inhibit or prevent oxidation of substrate leading to the formation of reactive oxygen species and reactive nitrogen species and thus protect the biological system (Hwang *et al.,* 2007).

Fruits and vegetables are endowed with antioxidants and consumption of these, prevent and protect from oxidative stress related diseases, inflammatory diseases viz., arthritis, autoimmune disease, carcinogenesis, neurodegenerative diseases, inflammatory diseases, cardiovascular disorders etc. Several food industries use butylated hydroxynol, butylated hydroxyl toluene and tertiary butyl hydroquinone, the common synthetic antioxidants for preventing lipid oxidation in food products while processing and storage. These synthetic antioxidants have been suspected to be carcinogenic and hence their use as food ingredients has been prohibited (hung and Wang 2004). Natural antioxidants comprised non-detrimental chemical combinations are considered to be rather safer for use in food products. Further, uncared wastes are if exploited as resource of antioxidants, will be more beneficial to human kind and protecting the environment. Flavonoids are water soluble polyphenolic molecules with antioxidant activity which has many beneficial effects on the cardiovascular system (Evans, 1989). Vitamin C acts as ROS scavenger, thus potentially protecting cells from harmful oxidative products (Fossati *et al.,).* Vitamin E supplement elevates the activities of antioxidant enzymes (Kiron *et al.,* 2004).

DPPH FREE RADICAL SCAVENGING ACTIVITY

The DPPH is a stable free radical and is widely used to assess the radical scavenging activity of a specific compound or plants extracts (Wei et al., 2012). DPPH solution hows a strong absorption band at 517nm appearing as a deep violet colour. The absorption vanishes and the resulting decolourization is stoichiometric with respect to degree of reduction. The plants extract of *Corchorus aestuans* and *Triumfetta rhomboidea* was able to reduce stable DPPH radical to yellow colour diphenyl picryl hydrazine. The degree of reduction in absorbance is the reflection of radical scavenging power of the compound.

The antioxidant activity aqueous extracts of *Corchorus aestuans* and *Triumfetta rhomboidea* was evaluated by using DPPH scavenging assay Fig (10). Aqueous extract using *Triumfetta rhomboidea* has higher scavenging activity (89.18%) followed by *Corchorus aestuans* (82.94%), as shown Figure (10) and Table (14).

This result indicated aqueous extract of *Triumfetta rhomboidea* plant shows higher scavenging activities. It has been reported that the antioxitant activity of aqueous extract of *Corchorus aestuans* and *Triumfetta rhomboidea* was due to presence of phenolics and it is responsible for redox properties, which allow them to act reducing agent, hydrogen donors and singlet oxygen quenchers. (Arasali and Kadimi 2009).

ANTIBACTERIAL ACTIVITY

In the present study, antibacterial activity of different solvents (acetone, ethanol methanol and water) using *Corchorus aestuans* and *Triumfetta rhomboidea* were tested against five human pathogenic bacteria (*Bacillus substilis, Escherichia coli, Staphylococcus, Vibrio cholerae*) presented in Table (15&16). The diameter of the inhibition zones against these spceies ranged from (3 to 11).

The different solvents (acetone, ethanol, methanol and water) extracts of *Corchorus aestuans* exhibited maximum activity against different bacterial species, *E.coli* (3-8mm), *Bacillus substilis* (3-6mm), *Vibrio cholerae* (3-11mm), *Staphylococcus* (3-5mm) inhibition zone. (Plate: 3), (Fig 11).

The different solvents (acetone, ethanol, methanol and water) extracts of *Triumfetta rhomboidea* exhibited maximum activity against different bacterial species, *E.coli* (3-9mm), *Bacillus substilis* (3-10mm), *Vibrio cholerae* (3-6mm), *Staphylococcus* (5-8mm) inhibition zone. (Plate: 4), (Fig 12).

The maximum activity was found to be 11mm zone of inhibition obtained by ethanol extract of *Corchorus aestuans* against *Vibrio cholerae*. The ethanol extract of *Corchorus aestuans* exhibited high antibacterial activity against *Vibrio cholerae*, the diameter of inhibition zone was 11mm. The ethanol extract of *Corchorus aestuans* exhibited more or less same zone of inhibition compared to standard antibiotics Amoxicillin. Maximum bacterial effect was found in *Vibrio cholerae* for ethanol extracts of *Corchorus aestuans.*

The maximum activity was found to be 10mm zone of inhibition obtained by methanol extract of *Triumfetta rhomboidea* against *Bacillus substilis*. The methanol extract of *Triumfetta rhomboidea* exhibited high antibacterial activity against *Bacillus substilis*, the diameter of inhibition zone was 10mm. The methanol extract of *Triumfetta rhomboidea* exhibited more or less same zone of inhibition compared to standard antibiotics Amoxicillin. Maximum bacterial effect was found in *Bacillus substilis* for methanol extracts of *Triumfetta rhomboidea.*

The antibacterial activity of *Corchorus aestuans* and *Triumfetta rhomboidea* plants extract were nearly similar to Amoxicillin. Maximum bacterial effects were found *Vibrio cholerae* in

Corchorus aestuans and *Bacillus substilis* in *Triumfetta rhomboidea* plant extract. The effects were significant in *Corchorus aestuans* and *Triumfetta rhomboidea*. The antibacterial activities of *Corchorus aestuans* and *Triumfetta rhomboidea* may be due to presence of various phytochemicals which are known to be synthesized by plants in response to microbial infection (Cowan, 1999). The mechanism of action of saponins as antimicrobial agents may be due to membranolytic properties, rather than simply altering the surface tension of the extracellular medium (Killeen, 1998). In our study *Corchorus aestuans* and *Triumfetta rhomboidea* showed the extracellular of saponins. The antimicrobial activity of these plants may be due to the presence of saponons. The presences of tannins were also reported in *Corchorus aestuans* and *Triumfetta rhomboidea*.

The antibacterial activity of tannins may due to their intercalation with enzymes, cell envelope transport proteins and also complex with cell wall polysaccharides (Ya *et al*., 1998). Hence these plants stand as a potential candidate as a source of ingredients in drug formulation for the treatment of bacterial infection.

SUMMARY AND CONCLUSION

Plants have been an important source of medicine for thousands of year. Medicinal plants are a source of great economic value. The *Corchorus aestuans* and *Triumfetta rhomboidea* were collected near St.Mary‟s college, Thoothukudi, Tamil Nadu. for current study. *Corchorus aestuans* and *Triumfetta rhomboidea* are known plants of family Tiliaceae. The shrub *Triumfetta rhomboidea* is used by practitioners of herbal medicines to treat burns, skin and wound infections, diarrhoeal diseases, gastrointestinal and upper respiratory tract infections. *Corchorus aestuans* is used for the treatment of diabetes and gonorrhoea. In ayurvedic systems of medicine, herbal extracts but not purified compounds have been used from centuries because of many constituents are considered to be beneficial. It is also used to reduce fever and sore throat pain. The macerated juices of the young fresh leaves are used to treat eye infections and parasitic diseases (Dalziel, 1937). The medicinal effects of plants are considered to be due to metabolites, especially secondary compounds, produced by plants. The phytochemical study revealed the presence of steroids, flavonoids, alkaloids, saponoins, terpenoids, phenol and tannins. The preliminary phytochemical tests are helpful in finding chemical constituents in the plants materials that may lead to their quantitative estimation and also in locating the source of pharmacologically active chemical compound. The information obtained from preliminary phytochemical screening will be finding out the genuinity of the drug.

In this study, total phenol, flavonoid, tannin, vitamin C and vitamin E content were quantitatively analysed in *Corchorus aestuans* and *Triumfetta rhomboidea* using spectrophotometric methods. The results of this study showed that the *Triumfetta rhomboidea*

have significant amount of phenol, flavonoids, tannins, vitamin C and vitamin E and ascorbic acids compared to *Corchorus aestuans*.

The FTIR spectrum of *Corchorus aestuans* showed strong IR bands characteristics of amines (3419.56 cm^{-1}), Alkane (2920.99 cm^{-1}), Ester (1744.49 cm^{-1}), Guanidine (1645.17 cm^{-1}), Nitro group (1551.63 cm^{-1}), Hydroxyl groups (1373.22 cm^{-1}), Aralkyl (1243.04 cm^{-1}), Sulphinic group (819.69 cm^{-1}), Iodo compound (602.71 cm^{-1}), functional groups and in *Triumfetta rhomboidea* showed strong IR bands characteristics of Aromatic (516.89 cm^{-1}), Alkane (2920.99 cm^{-1}), Carbonyl Esters (1737.74 cm^{-1}), Nitro group (1383.83 cm^{-1}), Amide (1318.25 cm^{-1}), Aromatic compound (516.89cm^{-1}), functional group. From the spectral data, presence of O-H, H-C-H, C=O, C=N, N=O, C-H, C–O, C-O-C, S-O, N-O, N-H, C-I, C-Br, C=C, C-N were identified. These bonding are responsible for the presence of alkyl group, aldehyde group, nitro group, sulphinic acid, ether, carboxylic group, aliphatic group and iodo group. Carboxylic acid present in the medicinal plant serves as main pharmaceutical product in curing ulcer, jaundice, head ache, stomatitis, hemicranias, fever, pain in lever, treatment of rheumatic joint pain. Amides, amine and amino acid are the main groups which are involved in protein synthesis.

The GC-MS analysis of ethanolic plant extract of *Corchorus aestuans* is confirmed the presence of 21 compounds with retention time. Out of these 21 compounds 8 compounds were majority and fifteen minor compound present in the plant extract of *Corchorus aestuans.* The GC-MS analysis of ethanol plant extract of *Triumfetta rhomboidea* is confirmed the presence of 16 compounds with retention time. Out of these 16 compounds 7 compounds were majority and nine minor compound present in the plant extract of *Triumfetta rhomboidea*. The above mentioned isolated compound from the ethanol extract of *Corchorus aestuans* and *Triumfetta*

rhomboidea have a medicinal important. Cyclohexadien and Benzisothiazole identified in the ethanol extract of *Corchorus aestuans* and *Triumfetta rhomboidea* is a main antioxidants compound that helps remove harmful toxins and free radicals in the human body.

Benzo (h) quinoline in the ethanol plant extract of *Triumfetta rhomboidea* is a main antiviral compound (WWW.pharmaexpert.ru/pass online predict, php.). Quinolines are important compounds because of their bioactive properties and medicinal uses such as anti malarial (Larsen *et al*., 1996), anti-inflammatory (Chen *et al*., 2001), anti asthmatic (Roma *et al*., 2000), antibacterial (Dube *et al*., 1998) and tyrosine inhibiting agent (Billker *et al*., 1998).

2-Ethylacridine is found in plant extract of *Triumfetta rhomboidea* is a main antimicrobial and antitumor compound. 1H Indole, 1-Methyl, 2-phenyl is found in both the ethanol plants extract. Which is used for Antineoplastic (breast cancer) (J.Vijayakumari and T.Leon Stephan Raj., 2019).The presence of the identified bioactive components present in *Corchorus aestuans* and *Triumfetta rhomboidea* plant extract could be responsible for the antioxidant and antimicrobial effects of these plants.

The antioxidant or free radical scavenging activity of plant extracts of these selected medicinal plants are investigated by using methods like DPPH scavenging activity. The plant extracts of *Corchorus aestuans* and *Triumfetta rhomboidea* show maximum antioxidant activity and these extracts are further subjected for antimicrobial studies.

The different solvent extracts of *Corchorus aestuans* and *Triumfetta rhomboidea* and Amoxicillin were used for antibacterial studies against human pathogenic bacteria, *Bacillus substilis, Escherichia coli, Staphylococcus, Vibrio cholerae.* These extract showed ranging degree of antibacterial activity. The maximum activity was found to be 11mm zone of inhibition

obtained by ethanol extract of *Corchorus aestuans* against *Vibrio cholera.* The maximum activity was found to be 12mm zone of inhibition obtained by methanol extract of *Triumfetta rhomboidea* against *Bacillus substilis*. The methanol extract of *Triumfetta rhomboidea* exhibited more or less same zone of inhibition compared to standard antibiotics Amoxicillin. The effects were significant in *Corchorus aestuans* than *Triumfetta rhomboidea*. The antibacterial activity of various phytochemicals which are known to be synthesized by plants in response to microbial infection.

Table 1: Preliminary phytochemical screening and distribution of secondary constituents in *Corchorus aestuans*

Phytochemical Tests	Acetone	Methanol	Ethanol	Water
Alkaloids	+	+	+	_
Flavanoids	+	+	_	+
Sterols	_	_	+	_
Carbohydrates	+	+	+	+
Glycosides	+	+	+	+
Saponin	_	+	+	+
Protein	+	_	+	+
Quinone	−	−	−	−
Phenol	−	+	+	−
Coumarin	−	−	−	+
Tannin	+	+	+	+
Terpenoid	+	+	+	_

(+ = Present), (- = Absent)

Table 2: Preliminary phytochemical screening and distribution of secondary constituents in *Triumfetta rhomboidea*

Phytochemical Tests	Acetone	Methanol	Ethanol	Water
Alkaloids	+	+	+	+
Flavanoids	+	+	+	+
Sterols	+	+	+	+
Carbohydrates	+	+	–	+
Glycosides	–	+	+	+
Saponin	–	+	+	+
Protein	+	–	+	+
Quinone	+	+	+	+
Phenol	+	+	+	–
Coumarin	+	+	–	–
Tannin	+	+	+	+
Terpenoid	–	+	+	–

(+ = Present), (- = Absent)

TABLE: 3

TOTAL PHENOL CONTENT OF CORCHORUS AESTUANS AND TRIUMFETTA RHOMBOIDEA	
Samples	**Amount of phenol mg (GAE)/g**
Corchorus aestuans	6.6703± 0.00152
Triumfetta rhomboidea	7.2143± 0.0028

Values are the mean of triplicates ± standard deviation. Dry sample were used for analysis. Garlic acid equivalent (1 mg/ml) was used as standard.

TABLE: 4

TOTAL FLAVANOIDS CONTENT OF CORCHORUS AESTUANS AND TRIUMFETTA RHOMBOIDEA	
Samples	**Amount of flavanoids mg (GAE)/g**
Corchorus aestuans	0.8185± 0.0004
Triumfetta rhomboidea	3.0113± 0.004

Values are the mean of triplicates ± standard deviation. Dry sample were used for analysis. Quercetin acid equivalent (1 mg/ml) was used as standard.

TABLE: 5

TOTAL VITAMIN-C CONTENT OF CORCHORUS AESTUANS AND TRIUMFETTA RHOMBOIDEA	
Samples	**Amount of Vitamin-C mg (GAE)/g**
Corchorus aestuans	17.0613± 0.0023
Triumfetta rhomboidea	3.66± 0.0017

Values are the mean of triplicates ± standard deviation. Dry sample were used for analysis. Vitamin-C equivalent (1 mg/ml) was used as standard.

TABLE: 6

TOTAL TANNIN CONTENT OF CORCHORUS AESTUANS AND TRIUMFETTA RHOMBOIDEA	
Samples	**Amount of tannin mg (GAE)/g**
Corchorus aestuans	1.1847± 0.0011
Triumfetta rhomboidea	1.3833± 0.0012

Values are the mean of triplicates ± standard deviation. Dry sample were used for analysis. Catechin equivalent (1 mg/ml) was used as standard.

TABLE: 7

TOTAL VITAMIN-E CONTENT OF CORCHORUS AESTUANS AND TRIUMFETTA RHOMBOIDEA	
Samples	**Amount of Vitamin-E mg (GAE)/g**
Corchorus aestuans	16.1443± 0.04
Triumfetta rhomboidea	4.636± 0.04

Values are the mean of triplicates ± standard deviation. Dry sample were used for analysis. Vitamin-E equivalent (1 mg/ml) was used as standard.

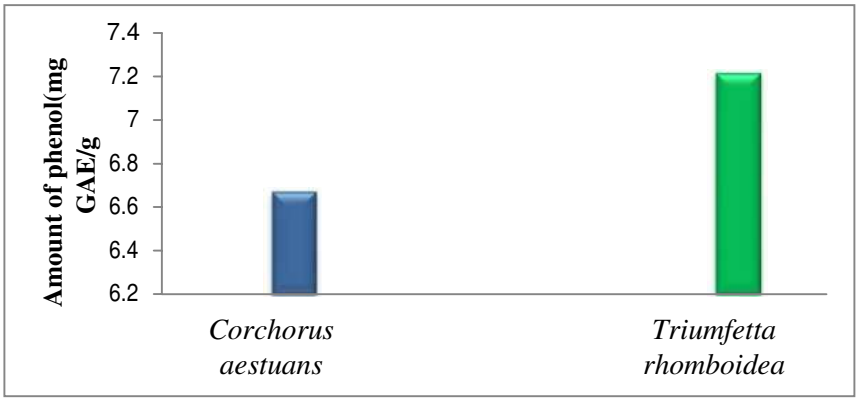


Fig 1: TOTAL PHENOL CONTENT OF CORCHORUS AESTUANS AND TRIUMFETTA RHOMBOIDEA

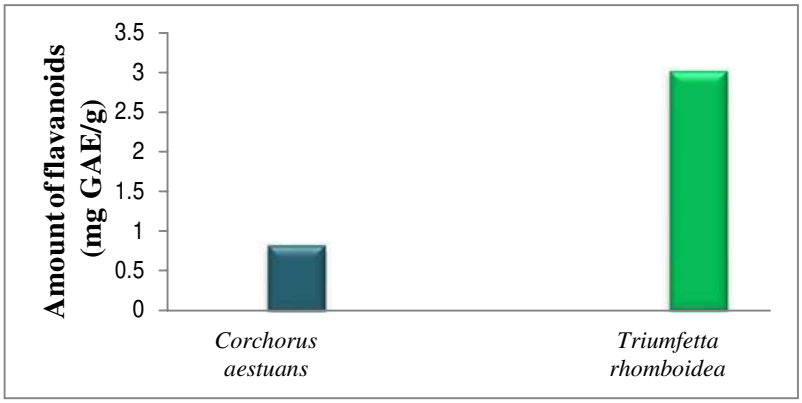


Fig 2: TOTAL FLAVANOID CONTENT OF CORCHORUS AESTUANS AND TRIUMFETTA RHOMBOIDEA

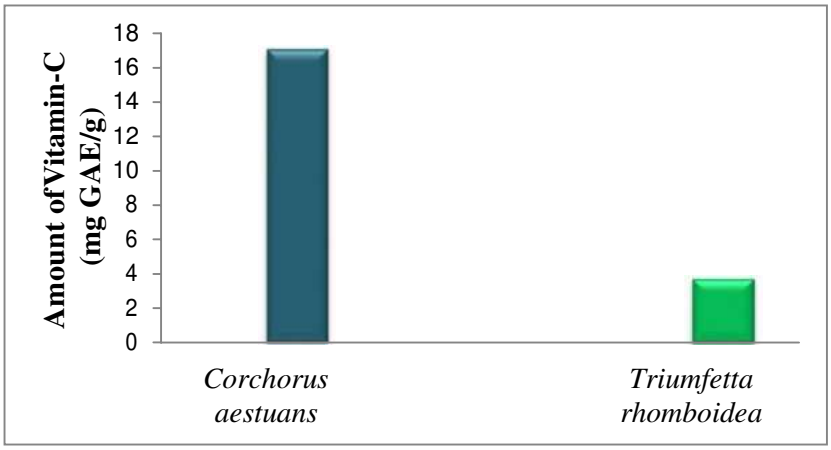


Fig 3: TOTAL VITAMIN-C CONTENT OF CORCHORUS AESTUANS AND TRIUMFETTA RHOMBOIDEA

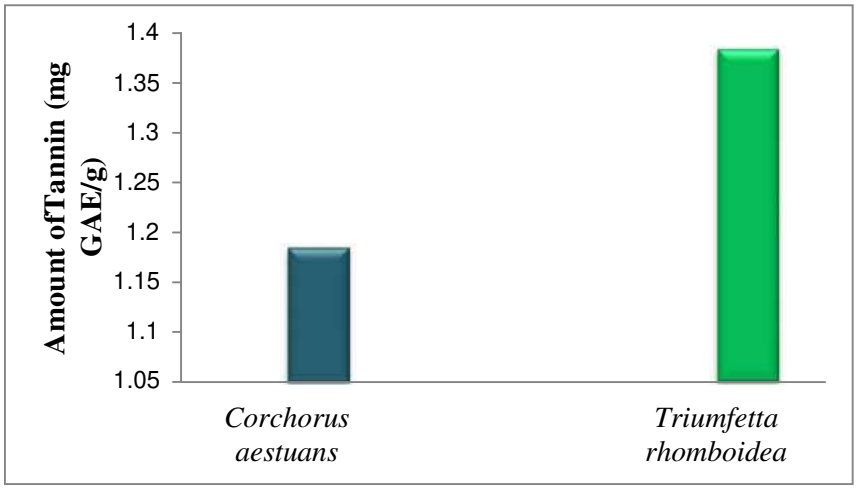


Fig 4: TOTAL TANNIN CONTENT OF CORCHORUS AESTUANS AND TRIUMFETTA RHOMBOIDEA

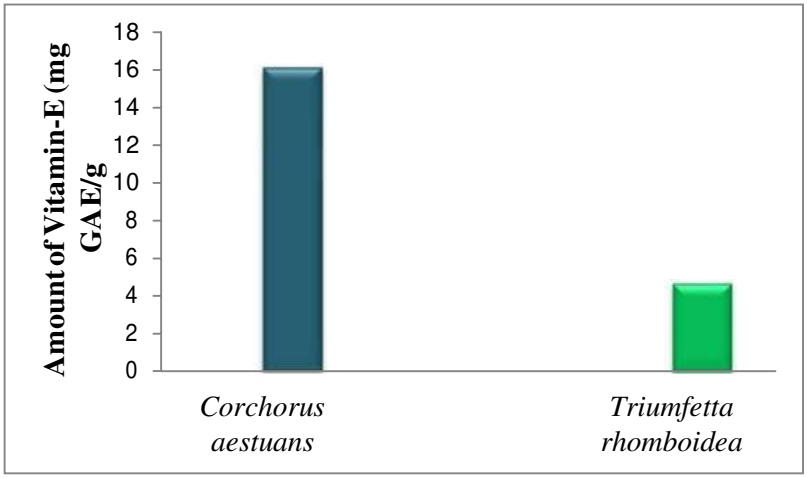


Fig 5: TOTAL VITAMIN-E CONTENT OF CORCHORUS AESTUANS AND TRIUMFETTA RHOMBOIDEA

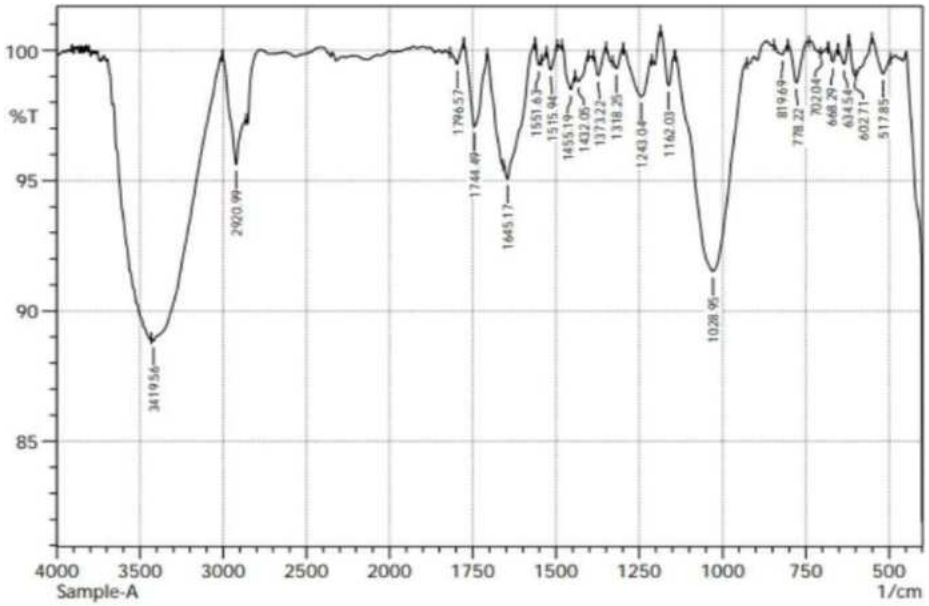


Figure 6: FTIR spectroscopy analysis of *Corchorus aestuans*

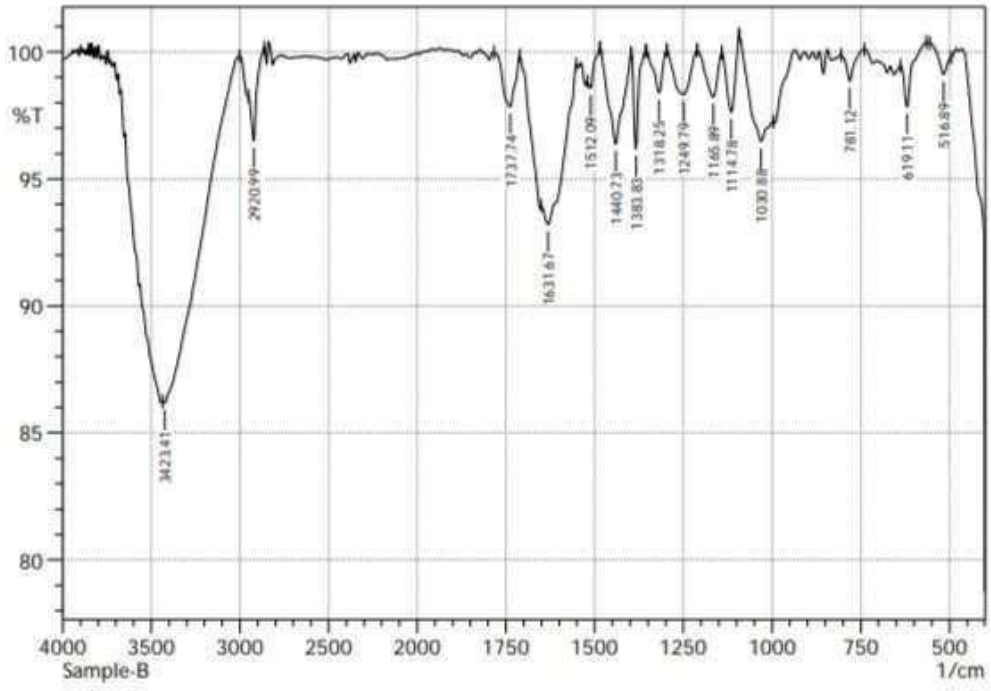


Figure 7: FTIR spectroscopy analysis of *Triumfetta rhomboidea*

Table: 8 FTIR spectroscopy analyses of *Corchorus aestuans*

PEAK VALUE	BOND	FUNCTIONAL GROUP
3419.56	MEDIUM	O-H
2920.99	MEDIUM,ASYMMETRIC STRETCH	H-C-H
1796.57	STRONG STRETCH	C=O
1744.49	STRONG, ESTER	C=O
1645.17	STRONG, AROMATIC	C=N
1551.63	ASYMMETRIC STRETCH	N=O
1515.94	AROMATIC, ASYMMETRIC	N=O
1455.19	STRONG, AMINES	N=O
1432.05	MEDIUM	C-H
1373.22	STRONG, HYDROXYL GROUP	C-O
1318.25	STRONG, HYDROXYL GROUP	C-O
1243.04	STRONG,ARALKYL ASYMMETRIC	C-O-C
1162.03	VERY STRONG, ESTERS GROUP	C-O
1028.95	STRONG, ETHER	C-O
819.69	STRONG, SULPHINIC ACID GROUP	S-O
778.22	STRONG, HYDROGEN ATOMS	C-H
702.04	WEAK,NITRATE GROUP	N-O
668.29	STRONG, PRIMARY AMINES	N-H
634.54	STRONG,VINYL AMIDES	C-H
602.71	STRONG, IODO COMPOUND	C-I
517.85	STRONG, AROMATIC	C-Br

Table: 9 FTIR spectroscopy analysis of *Triumfetta rhomboidea*

PEAK VALUE	BOND	FUNCTIONAL GROUP
3423.41	MEDIUM, URETHANES	N-H
2920.99	MEDIUM,ASYMMETRIC STRETCH	H-C-H
1737.74	STRONG, ESTERS	C=O
1631.67	MEDIUM, PRIMARY AMIDES	N-H
1512.09	WEAK, SECONDARY AMIDE	N-H
1440.73	VERY STRONG, ALKANES	H-C-H
1383.83	STRONG, SYMMETRIC NITRO COMPOUND	N=O
1318.25	STRONG, ARYL TERTIARY AMINE	C-N
1249.79	STRONG, ACETATES	C-O-C
1165.89	STRONG, SYMMETRIC STRETCH	C=C
1114.78	STRONG, SYMMETRIC STRETCH	C-O-C
1030.88	VERY STRONG, RING STRETCH	C-O
781.12	STRONG, HYDRO COMPOUND	C-H
619.11	STRONG,PRIMARY AMINES	N-H
516.89	STRONG, AROMATIC	C-Br

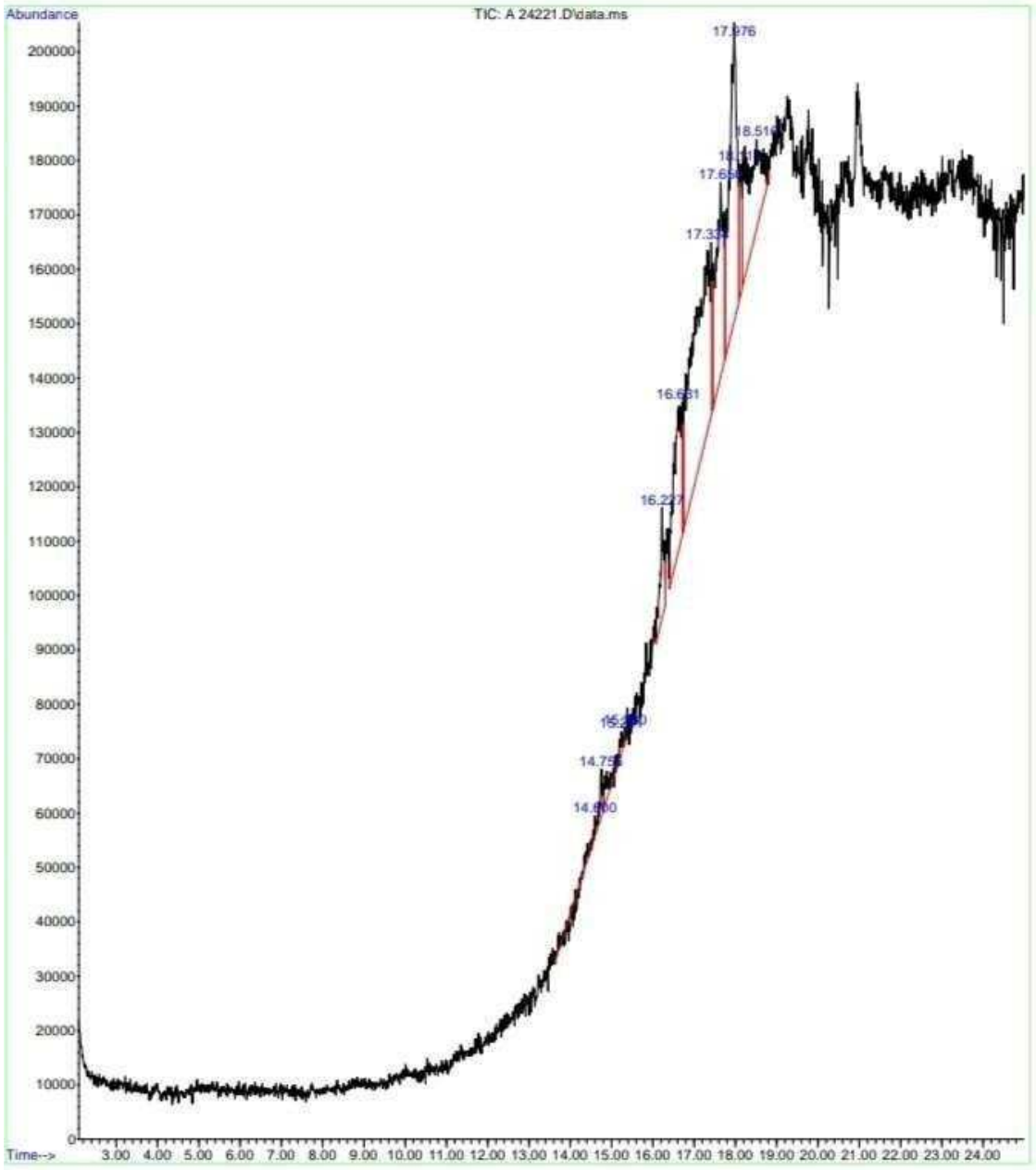
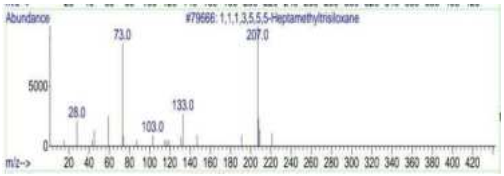
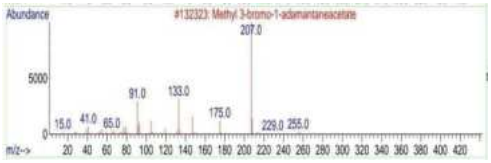
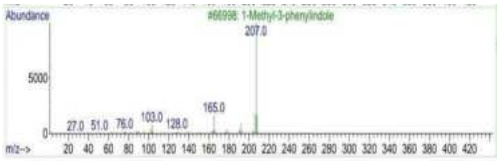
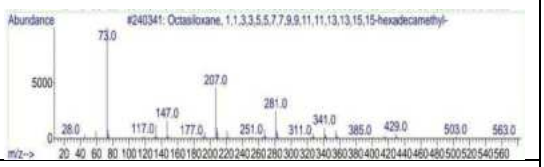
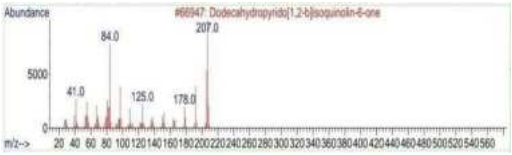
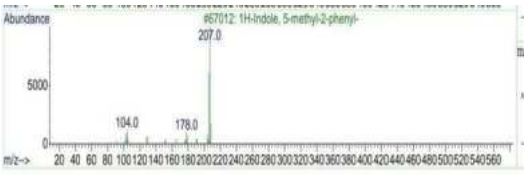
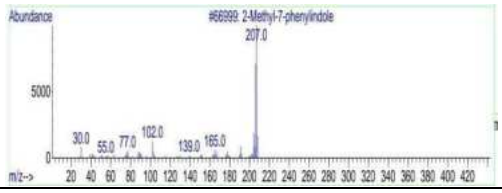
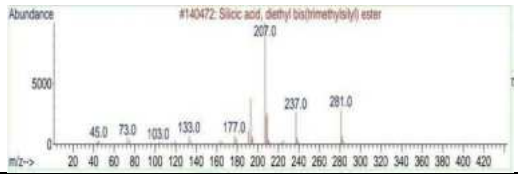
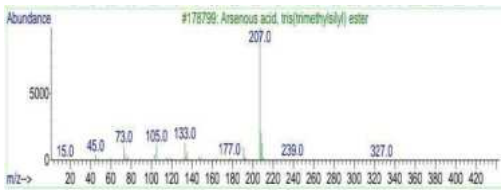
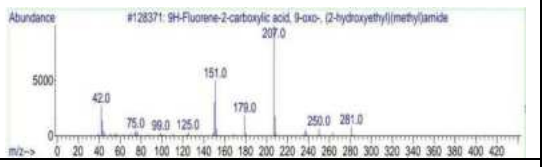
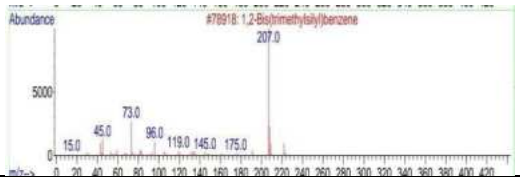
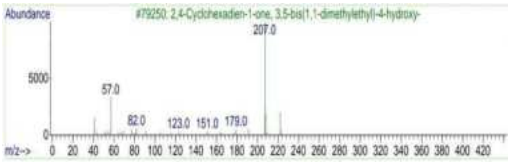
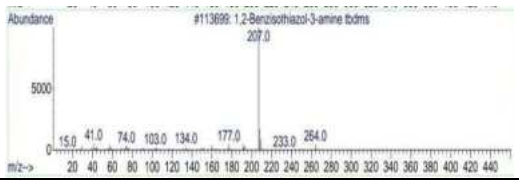
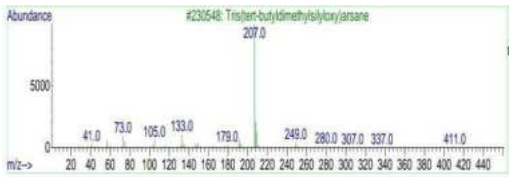
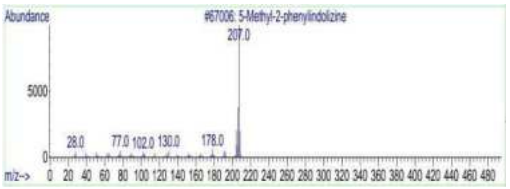
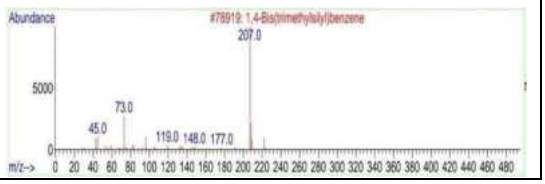
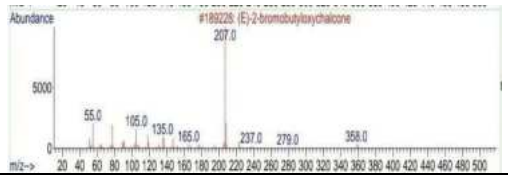
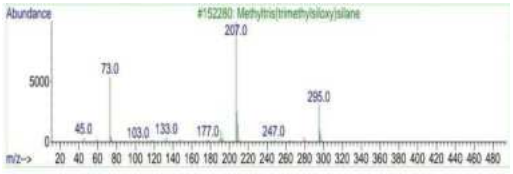
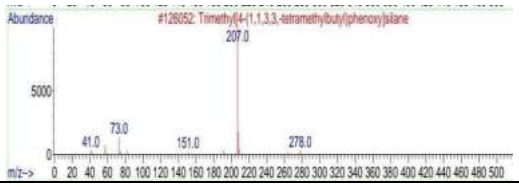
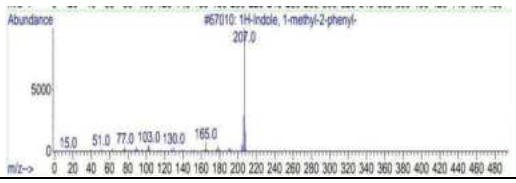
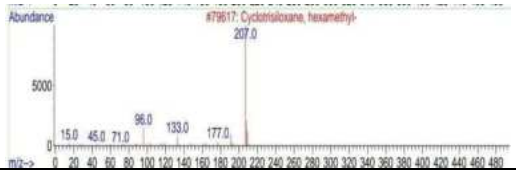


Figure 8: GC-MS Chromatogram of plant extracts (Ethanol) ***Corchorus aestuans***

Table 10. ***Corchorus aestuans*** **Mass spectrum**

S.No.	RT	Name of the Compound	Area (%)	Mass spectrum
1.	14.596	1,1,1,3,5,5,5 Hepta methyl trisilo	-0.21	
2	14.596	Methyl 3- bromo-1-adamentane acetate	-0.21	
3.	14.596	1-Methyl-3-Phenylindole	-0.21	
4.	14.757	Octasiloxane	1.12	
5.	14.757	Dodeca hydropyrido [1,2- b] iso quinolin-6- one	1.12	
6.	14.757	1 H - Indole, 5-Methyl -2 phenyl	1.12	
7.	15.249	2- Methyl-7-phenylindole	1.57	
8.	15.249	Silicic acid, diethyl bis (trimethylsilyl) ester	1.57	

9.	15.249	Arsenous acid tris (trimethylsilyl) amide	1.57	
10.	15.334	9-H- Fluorence- 2-carboxylic acid, 9-oxo (2- hydroxy ethyl) (methyl) amide	0.15	
11.	15.334	1,2- Bis (trimethylsilyl) benzene	0.15	
12.	15.334	2,4-Cyclohexadien-1-one,3,5-bis (1,1-dimethyl ethyl -4 hydroxy	0.15	
13.	16.232	1,2- Benzisothiazole -3- amine	3.52	
14.	16.232	Tris (tert-butyl dimethyl silyloxy arsane	3.52	
15.	16.629	5- Methyl - 2- Phenyl indolizine	10.82	
16.	16.629	1,4- Bis (trimethylsilyl) benzene	10.82	

17.	17.338	(E) -2- bromo butyl oxychal one	32.79	
18.	17.650	Methyl tris (trimethylsilyl siloxyl silane)	13.80	
19.	18.113	Trimethyl [4- (1,1,3,3- tetra methyl butyl) phenoxy] silane	2.83	
20.	18.520	1H Indole,1- methyl,2- phenyl	13.47	
21.	18.520	Cyclotrisiloxane,hexa methyl	13.47	

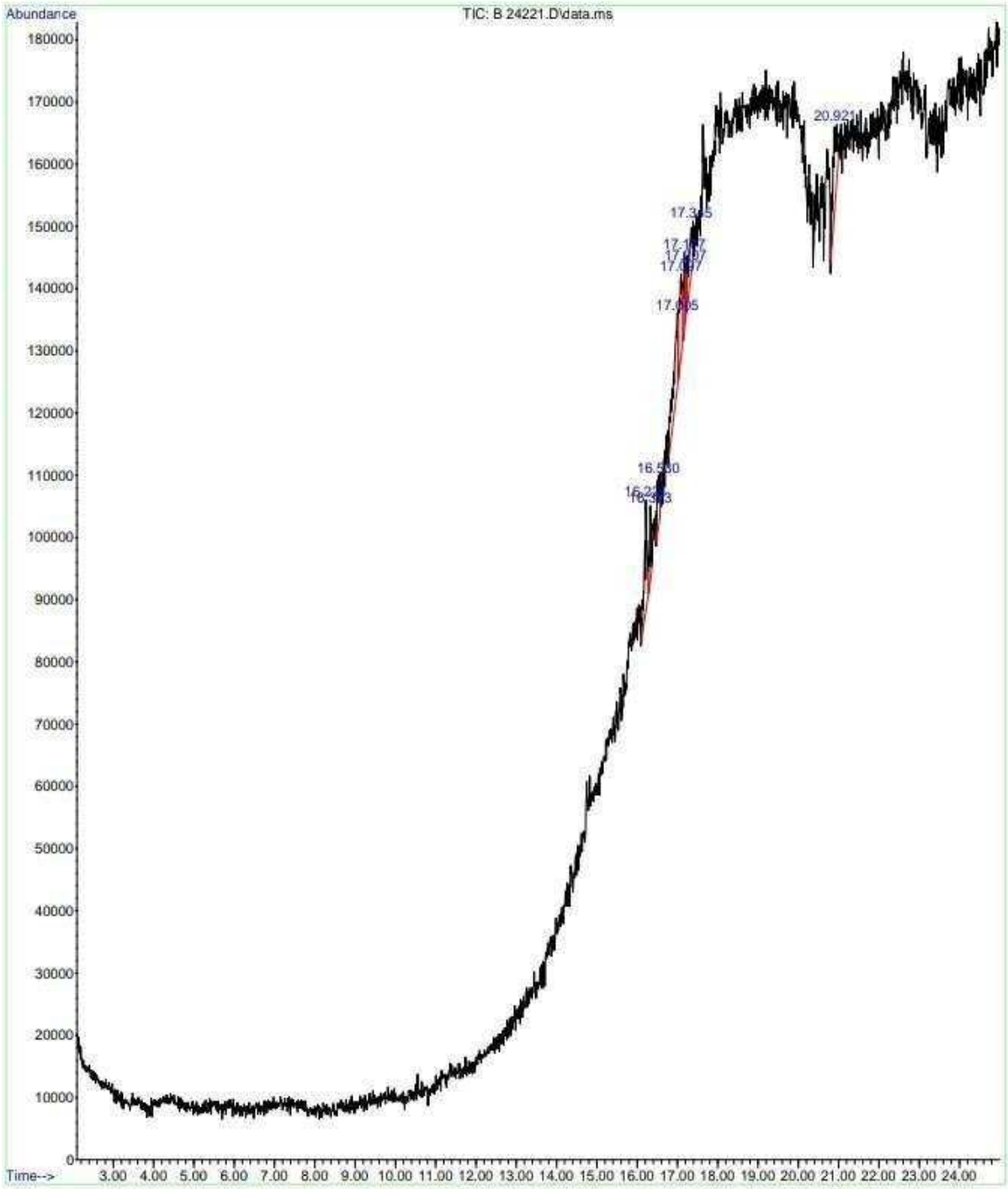
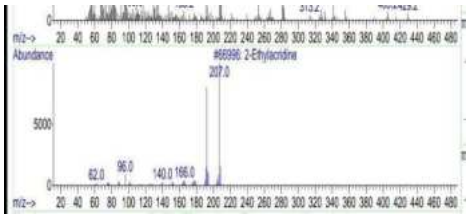
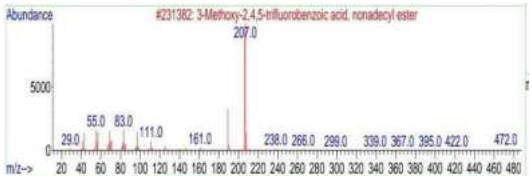
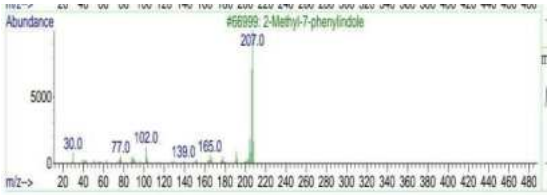
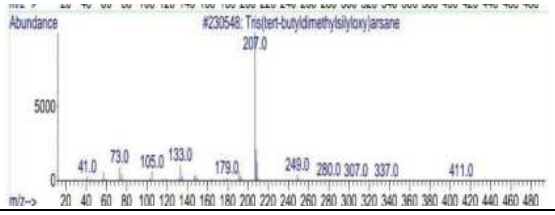
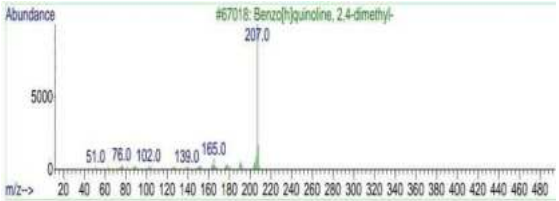
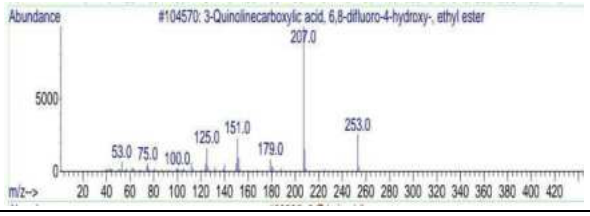
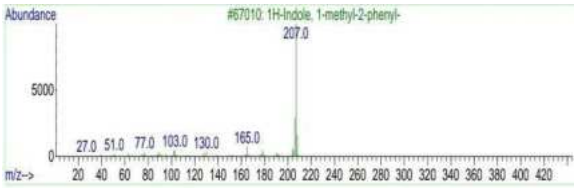
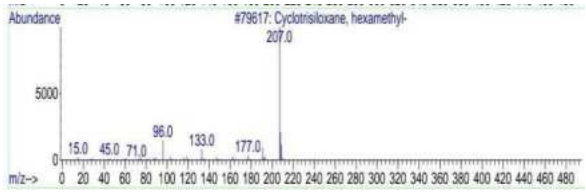
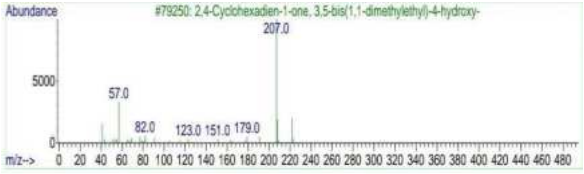
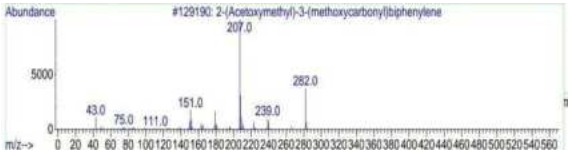
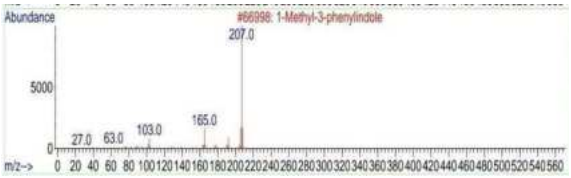
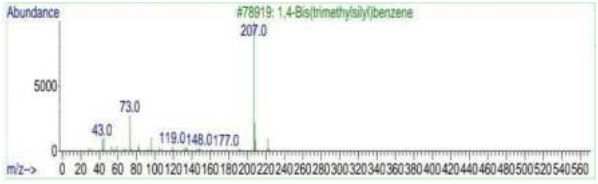


Figure 9: GC-MS Chromatogram of plant extracts (Ethanol) ***Triumfetta rhoboidea***

Table: 11 *Triumfetta rhomboidea Mass* spectrums

S.No.	RT	Name of the Compound	Area (%)	Mass spectrum
1.	16.223	2-Ethyl acridine	15.06	
2.	16.223	3-Methoxy-2,4,5-Trifluoro benzoic acid, nonadecyl ester	15.06	
3.	16.223	2-Methyl-7-Phenyl indole	15.06	
4.	16.336	Tris (tert butyl dimethyl silyloxy) arsane	5.32	
5.	16.336	Benzo [h] Quinoline,2,4-dimethyl	5.32	

6.	16.526	3-Quinoline carboxylic acid,6,8-difluoro-4- hydroxy ethyl ester	6.27	
7.	16.526	1H Indole,1-Methyl-2-Phenyl	6.27	
8.	17.008	Cyclotrisiloxane hexamethyl	19.78	
9.	17.008	2,4,-cyclohexadien-1-one,3,5,-bis(1,1-dimethyl ethyl) 4- hydroxy	19.78	
10.	17.093	2-(Acetoxymethyl) 3-(methoxy carbonyl) biphenylene	15.39	
11.	17.093	1-methyl3-phenylindole	15.39	
12.	17.093	1,4Bis(trimethylsilyl) benzene	15.39	

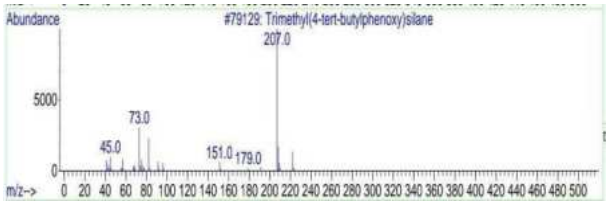
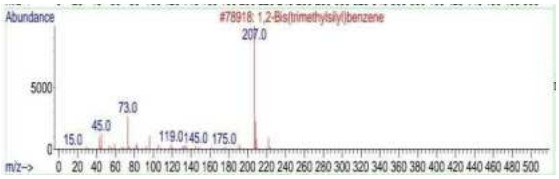
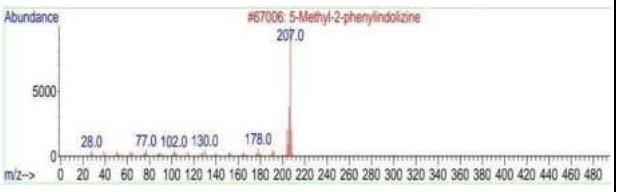
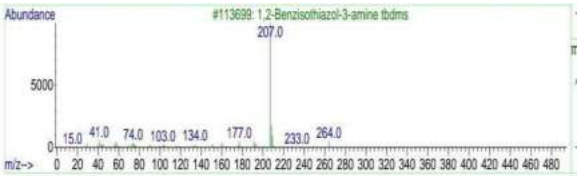
13.	17.178	Trimethyl (4-tert-butyl phenoxy) silane	5.74	
14.	17.178	1,2-Bis (trimethylsilyl) benzene	5.74	
15.	20.922	5- Methyl-2 phenylindolizine	17.30	
16	20.922	1,2,- Benziso thiazole- 3-amine	17.30	

Table: 12 List of chemical compounds identified from ethanol extract of *Corchorus aestuans* through GC-MS analysis

S.No.	RT	Name of the Compound	Area (%)	Biological activity
1.	14.596	1,1,1,3,5,5,5 Hepta methyl trisilo	-0.21	Antifungal, Antiviral, Anti parkinsonia activities
2	14.596	Methyl 3- bromo-1-adamentane acetate	-0.21	Anti-inflammatory, Anti diabetic, Antiobesity, Antineoplastic, Antiviral activities
3.	14.596	1-Methyl-3-Phenylindole	-0.21	Antineurotic, Skeletal muscle relaxant stimulant, protein synthesis activities
4.	14.757	Octasiloxane	1.12	Antithematotoxic, Antineoplastic, Antiviral, Anti rickettsial, Antibacterial activities
5.	14.757	Dodeca hydropyrido [1,2-b] iso quinolin- 6- one	1.12	Antiseborrheic, Anticonvulsant, Antiviral, Antiprotozoal, Antianemic, Antiemetic activities
6.	14.757	1 H - Indole, 5- Methyl -2 phenyl	1.12	Anti hypoxic, Kidney function stimulant, Anti neurotic activities
7.	15.249	2- Methyl-7- phenylindole	1.57	Skeletal muscle relaxant stimulant, Antiviral, Menopausal disorder treatment, Anti alcoholic activities
8.	15.249	Silicic acid, diethyl bis (trimethylsilyl) ester	1.57	Antineoplastic, kidney function stimulant, Antidyskinetic, Antiviral, Antibacterial,Anti parasitic, Anti fungal activities
9.	15.249	Arsenous acid tris (trimethylsilyl) amide	1.57	HIV - 1 reverse transcriptase inhibitors, Antineoplastic, Antiviral, Antibiotic, Anti-inflammatory activities
10.	15.334	9-H- Fluorence- 2-carboxylic acid, 9- oxo (2-hydroxy ethyl) (methyl) amide	0.15	Antineurotic, Antidyskinetic, Cholesterol antagonist, Antiviral, Antipsoriatic activities

11.	15.334	1,2- Bis (trimethylsilyl) benzene	0.15	Antineoplastic, Insecticide
12.	15.334	2,4-Cyclohexadien-1-one,3,5-bis (1,1- dimethyl ethyl -4 hydroxy	0.15	Eye, skin irritation inhibitors, Antioxidant, thyroid hormone alpha agonist activities
13.	16.232	1,2- Benzisothiazole -3-amine	3.52	Anti-inflammatory, Antipyretic, Antioxidant,Anti infective activities
14.	16.232	Tris (tert-butyl dimethyl silyloxy arsane	3.52	Antineurotic,Antinociceptive, Antiviral,Anti infective activities
15.	16.629	5- Methyl - 2- Phenyl indolizine	10.82	Anti-inflammatory, Antipyretic,Anti infective, Anti-smoking activities
16.	16.629	1,4- Bis (trimethylsilyl) benzene	10.82	Antiarthritic activities
17.	17.338	(E) -2- bromo butyl oxychal one	32.79	Kidney function stimulant, skin irritation inactivity
18.	17.650	Methyl tris (trimethylsilyl siloxyl silane)	13.80	Keratoses actinic (solar) treatment, Antineoplastic, Antiviral (HIV) activities
19.	18.113	Trimethyl [4-(1,1,3,3- tetra methyl butyl) phenoxy] silane	2.83	Anti infective, Antiviral, Keratoses, actinic treatment, Antiparasitic activities
20.	18.520	1H Indole,1- methyl,2-phenyl	13.47	Antineoplastic (breast cancer), Antineurotic activity
21.	18.520	Cyclotrisiloxane,hexa methyl	13.47	Antineoplastic, Antiseborrheic, Antimyopathies, HIV 1 integrate inhibitor activities

Table: 13 List of chemical compounds identified from ethanol extract of *Triumfetta rhomboidea* through GC-MS analysis

S.No.	RT	Name of the Compound	Area (%)	Biological activity
1.	16.223	2-Ethyl acridine	15.06	Antimicrobial, Anti tumor activities
2.	16.223	3-Methoxy-2,4,5-Trifluoro benzoic acid, nonadecyl ester	15.06	Antidiabetic, Antiseborrheic activities
3.	16.223	2-Methyl-7-Phenyl indole	15.06	Antidyskinetic, Antineurotic, Antiviral activities
4.	16.336	Tris (tert butyl dimethyl silyloxy) arsane	5.32	HIV 1 reverse transcriptase inhibitor, Antiviral, Antineoplastic,Antivira (Picora virus) activities
5.	16.336	Benzo [h] Quinoline,2,4-dimethyl	5.32	Antiseborrheic, Antineurotic, Antiprotozoal (Amoeba), Antiviral activities
6.	16.526	3-Quinoline carboxylic acid,6,8-difluoro-4- hydroxy ethyl ester	6.27	Antihypoxic, Antiviral, Anti infective, Anti tuberculosis, Anti mycobacterial, Antinephritic activities
7.	16.526	1H Indole,1-Methyl-2-Phenyl	6.27	Antineoplastic (Breast cancer), Antineurotic activities
8.	17.008	Cyclotrisiloxane hexamethyl	19.78	Antineoplastic, Antiseborrheic,HIV 1 reverse transcriptase inhibitor activities
9.	17.008	2,4,-cyclohexadien-1-one,3,5,-bis(1,1-dimethyl ethyl) 4- hydroxy	19.78	Eye, skin irritation inactivity, Antioxidant, Thyroid hormone alpha agonist, Antithematotoxic activities
10.	17.093	2-(Acetoxymethyl) 3-(methoxy carbonyl) biphenylene	15.39	Antieczematic, Anti hypertensive, Anti-inflammatory, Antibiotic activities
11.	17.093	1-methyl3-phenylindole	15.39	Antileprosy, Antineurotic, Aantinociceptive, Antipsychotic, Anti rickettsial activities

12.	17.093	1,4Bis(trimethylsilyl) benzene	15.39	Antiarthritic activities
13.	17.178	Trimethyl (4-tert-butyl phenoxy) silane	5.74	Anti infective,Anti viral, Insecticide, Antiparasitic activities
14.	17.178	1,2-Bis (trimethylsilyl) benzene	5.74	Antineoplastic, Antioxidant activities
15.	20.922	5- Methyl-2 phenylindolizine	17.30	Anti-inflammatory, Antipyretic,Antiinfective, Antioxidant
16	20.922	1,2,- Benziso thiazole- 3-amine	17.30	Antiviral, Anti ulcerative, Anti infective,Anti rickettsial, activities

Table 14. Antioxidant activity in aqueous extract of *Corchorus aestuans* and *Triumfetta rhomboidea*

S.NO	AQUEOUS EXTRACT	DPPH free radical assay (%)
1.	***Corchorus aestuans***	82.94
2.	***Triumfetta rhomboidea***	89.18

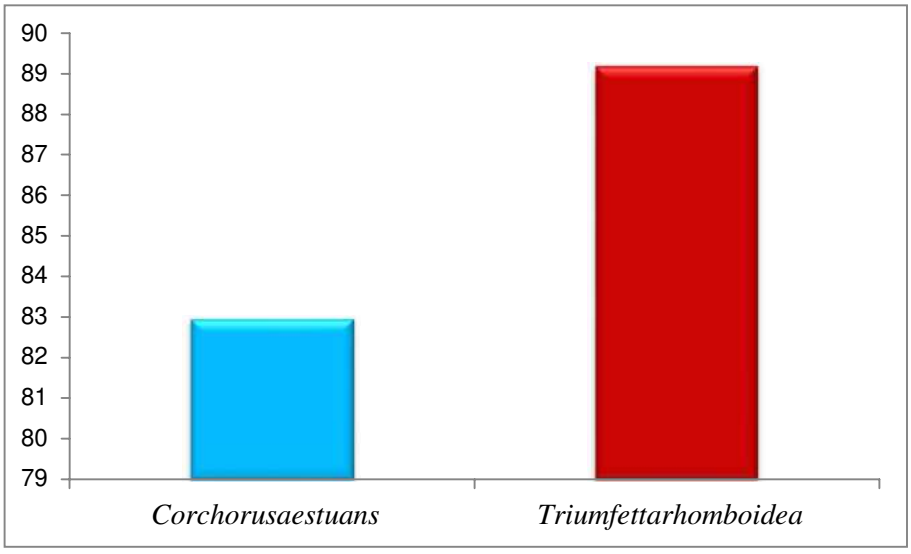


Figure 10: Antioxidant activities in aqueous extract of *Corchorus aestuans* and *Triumfetta rhomboidea*

Plate 3

Antibacterial activity of different solvent extracts of *Corchorus aestuans* against human pathogen

Plate (a) *Escherichia coli*

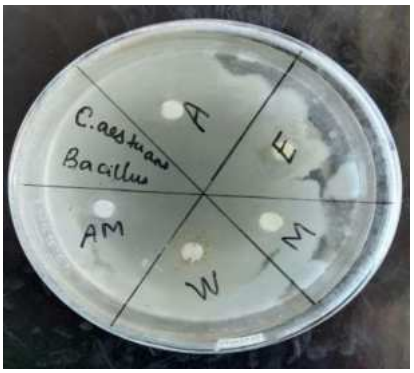


Plate (b) *Bacillus substilis*

Plate (c) *Vibrio cholerae*

Plate (d) *Staphylococcus sp.*

Plate 4

Antibacterial activity of different solvent extracts of *Triumfetta rhomboidea* against human pathogen

Plate (a) *Bacillus substilis*

Plate (b) *Staphylococcus sp.*

Plate (c) *Vibrio cholerae*

Plate (d) *Escherichia coli*

Table 15: Antibacterial activity of *Corchorus aestuans* plant extract with different solvent against human pathogen

Samples	*Corchorus aestuans*				
Microorganisms	**Acetone**	**Ethanol**	**Methanol**	**Water**	**Amoxicillin**
E.coli	**3**	**7**	**8**	**6**	**9**
Bacillus subtilis	**4**	**3**	**3**	**5**	**6**
Vibrio cholerae	**9**	**11**	**7**	**3**	**10**
Staphylococcus	**3**	**3**	**4**	**3**	**5**

Table 16: Antibacterial activity of *Triumfetta rhomboidea* plant extract with different solvent against human pathogen

Samples	*Triumfetta rhomboidea*				
Microorganisms	**Acetone**	**Ethanol**	**Methanol**	**Water**	**Amoxicillin**
E.coli	**3**	**4**	**7**	**5**	**8**
Bacillus subtilis	**4**	**6**	**10**	**3**	**11**
Vibrio cholerae	**3**	**5**	**4**	**4**	**6**
Staphylococcus	**3**	**4**	**5**	**3**	**5**

Fig 11: Antibacterial activity of *Corchorus aestuans* plant extract with different solvent against human pathogen

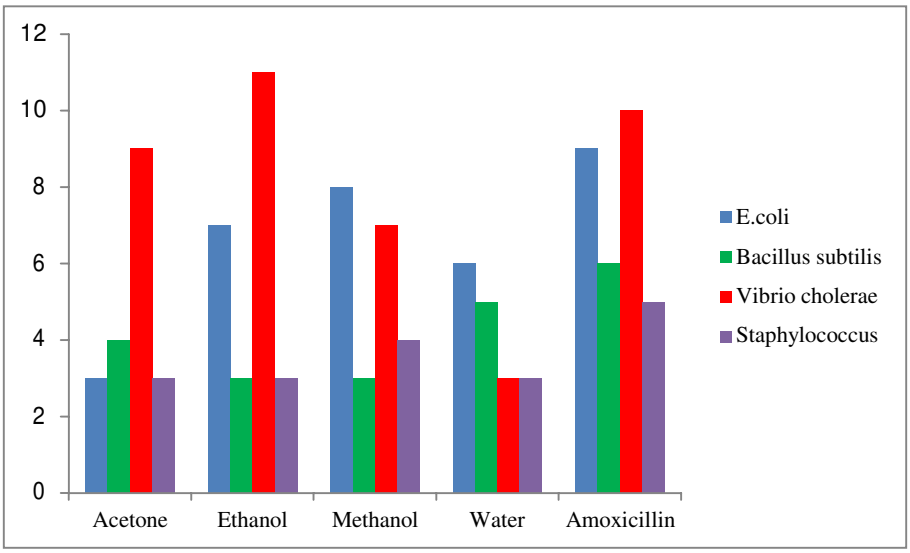
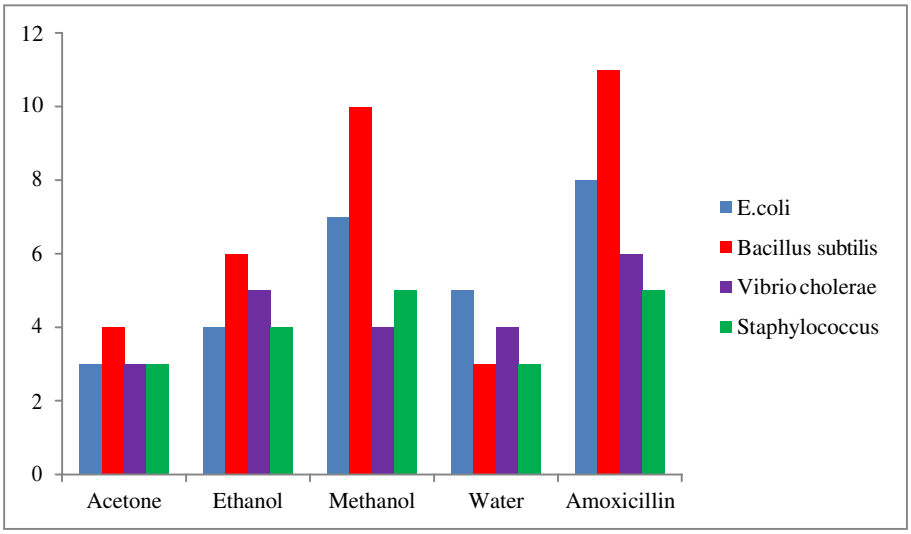


Fig 12: Antibacterial activity of *Triumfetta rhomboidea* plant extract with different solvent against human pathogen



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AN ETHNOBOTANICAL SURVEY OF MEDICINAL PLANTS USED IN NADUVAKURICHI VILLAGE, SAWYERPURAM TOWN PANCHAYAT IN THOOTHUKUDI DISTRICT, TAMIL NADU, INDIA.

A dissertation submitted to

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Master of Science in Botany

By

VINCY AGNES.M

Reg.No.19APBO17

DEPARTMENT OF BOTANY

ST. MARY'S COLLEGE (Autonomous)

THOOTHUKUDI -628001

2020-2021

CERTIFICATE

This is to certified that this dissertation entitled **AN ETHNOBOTANICAL SURVEY OF MEDICINAL PLANTS USED IN NADUVAKURICHI VILLAGE, SAWYERPURAM TOWN PANCHAYAT IN THOOTHUKUDI DISTRICT, TAMIL NADU, INDIA.** Submitted by VINCY AGNES.M Reg.No.19APBO17 to St.Mary's College (Autonomous) THOOTHUKUDI - 628001in partial fulfillment for the award of the degree of **'Master of Science in Botany'** is done by her under my supervision. It is further certified that this dissertation or any pare of this has not been submitted elsewhere for any other degree.

GUIDE 15/04/2021

M. Glory
HEAD OF THE DEPARTMENT

HOD of Botany
St. Mary's College (Autonomous)
Thoothukudi- 628 001.

EXAMINAR

Lucia Rose
PRINCIPAL

St. Mary's College (Autonomous)
Thoothukudi - 628 001.

DECLARATION

I do here by declare that this dissertation entitled **AN ETHNOBOTANICAL SURVEY OF MEDICINAL PLANTS USED IN NADUVAKURICHI VILLAGE, SAWYERPURAM TOWN PANCHAYAT IN THOOTHUKUDI DISTRICT, TAMIL NADU, INDIA.** Submitted by me in partial fulfillment for the award of the degree of **'Master of Science in Botany'**, in the result of my original and independent work carried out under the guidance of **Dr. Mrs. S. Beulah Jerlin M.Sc, M.Phil., Ph.D.** Assistant Professor. Department of Botany, St.Mary's College (Autonomous) THOOTHUKUDI and it has not been submitted elsewhere for the award of any other degree.

M. Vimy Agnes

Station : THOOTHUKUDI

Date : 15.04.2021

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CONTENTS

INTRODUCTION

INTRODUCTION

Ethnobotany is the study of medicinal plants used by local people, with a particular importance on old-style tribal beliefs and information. The World Health Organization (WHO 2002) estimates that unevenly, 80% of the people from developed and developing countries depend on traditional medicines, especially on plant-based medicine in primary healthcare. The current pharmacopoeia has at least 25% drugs derived from medicinal herbs and others which are synthetic compounds isolated from herbs. The day to day demand for herbal plants is growing both in developed and developing countries, the recognition of natural harvests is growing, due to the fact that they have small side-effects and are easily accessible at reasonable prices. They are the main source in primary healthcare of poor people. The Indian people have incredible desire for herbal plants and use them in the wide range of applications from cold to mortal diseases (Araveeti Madhusudhana Reddy *et al*., 2019)).

According to the World Health Organization (2008), the term "traditional medicine" is to be understood as the sum total of the knowledge, skills and practices based on theories, beliefs and experiences indigenous to different cultures that are used to maintain and improve health, as well as to prevent, diagnose, and treat physical and mental illnesses. The World Health Organization has a keen interest in documenting the use of medicinal plants by native peoples from different parts of the world (Buragohain, 2011). Across the world and throughout the ages plants have traditionally played a major role in the treatment of human diseases (Thirumalai et al., 2009).The use of herbal remedies as an adjunct or alternative to conventional medicine is also becoming increasingly popular all over the world. It is

estimated that 80% of the South African population will use a traditional remedy at some stage during their lifetime (Lewu and Afolayan, 2009).

According to Cheikhyoussef et al. (2011), there are several advantages for people in rural areas in opting for traditional medicine: traditional healers are usually to be found within relative close proximity to their homes, they are familiar with the patient's culture and environment, and the costs associated with such treatments are generally negligible.

Ethnobotany and ethnomedical studies are today recognized as the most effective method of identifying new medicinal plants or refocusing on those plants reported in earlier studies for the possible extraction of beneficial bioactive compounds (Thirumalai et al., 2009). The need for continued ethnobotanical research to find and document important medicinal plants cannot be over-emphasised (Wintola and Afolayan, 2010).

Ethnobotany is the study of the relationship between plants and people: From “ethno” – study of people and “botany” – study of plants. Ethnobotany is considered a branch of ethnobiology. Ethnobotany studies the complex relationship between plants and cultures. The focus of ethnobotany is on how plants have been used managed and perceived in human societies and includes plants used for food, medicine, divination, cosmetics, dyeing, textiles, for building, tools, currency, clothing, social life and music.

Ethnobotany is a multidisciplinary science defined as the interaction between plants and people. The relationship between plants and human cultures is not limited to the use of plants for food, clothing and shelter but also includes their use for religious ceremonies, ornamentation and healthcare. (K. Choudhary, *et al* 2008).

Medicinal plants are used for curing and healing throughout the history of human beings and have been transferred from generation to generation (Pieroni&Quave, 2005; Perumal&Ignacimuthu, 2000; Napar*et al*., 2012; Jan et al., 2015; Qasim*et al*., 2016). About eighty percent population of the word relies on plant remedies for their primary health (RiazUllah*et al*., 2010). Medicinal plants are main sources to cure most of the diseases practiced by herbal pharmaceuticals (Hamayun 2005; Rehecho*et al*., 2011). Various surveys have been conducted in different communities of the world (Kargoglu, *et al*., 2008; Ratnam&Raju 2008; Jamila&Mostafa, 2014).

Herbal remedies are the oldest form of health care known by mankind. Prior to the development of modern medicine, traditional medicine systems that have evolved over the centuries among various communities, were still maintained as a great traditional knowledge basis in herbal medicines (Mukherjee and Wahil, 2006). This knowledge has been passed on orally from generation to generation without any written document (PerumalSamy and Ignacimuthu, 2000) and is still retained by various indigenous groups around the world. During the last few decades there has been an increasing in the study of medicinal plants and their traditional use in different parts of the world (Lev, 2006).

SCOPE AND OBJECTIVES

Ethnobotanical survey is highly needed for the conservation of plants and represents the preliminary information required for future phyto-chemical investigation. There is very limited information available regarding medicinal plants used by traditional healers and general people in villages, for treating common ailments and diseases. It is very urgent need for identifying and documenting these valuable resources before they become inaccessible and extinct. Medicinal plants are accessible and cheap so 80% of people in developing countries used these for treating many health problems (Anup, 2014). The use of plants and plant products for different purposes such as food, fodder, medicine, fiber, etc., could be traced as far back as to the beginning of human civilization (Bhattarai, 2016). The indigenous system of medicine namely Ayurvedic, Siddha and Unani have been in existence for several centuries. The most common way of preparing remedies from herbs is decoctions, by boiling plant parts in a large amount of water until this is considerably reduced and colored by plants phytochemicals.

Objectives:

- To conduct a detailed botanical survey for documenting the diversity of plants.
- To discover the medicinal value of plant in that region.
- To assess and document the traditional and local knowledge of plants use among traditional healer and local community member on the use of medicinal plants.
- To analyse socio economic survey of the area with special emphasis on population structure and the proportion using medicinal plants for different purpose.

REVIEW OF LITERATURE

India has a geographic area of 329 million hectares. The large size of the country and variety of habitat conditions have contributed to the great biological diversity particularly to the flora of India. Taxonomists have estimated that 2,50,000 to 3,00,000 vascular plants are present in this earth. These range from the tiniest genus *Wolfia* to the giant tree genera such as *Sequoia dendron* and *Eucalyptus* (Narasimhan, 2001).

David J Simbo (2010)The survey identified and recorded 107 plants species from 54 plant families, 98 genera used for treating diseases in Babungo. The Asteraceae was the most represented plant family while herbs made up 57% of the total medicinal plants used. The leaf was the most commonly used plant part while concoction and decoction were the most common method of traditional drug preparation. Most medicinal plants (72%) are harvested from the wild and 45% of these have other non medicinal uses. Knowledge of the use of plants as medicines remains mostly with the older generation with few youth showing an interest

RatihNilaPamungkas and Luchman Hakim (2013).were collected the data through depth and semi structured interviews. Information gathered was about traditional uses of wild plants as well as cultivated plants. All the collected data was filled into an analytical table and,for the ease of analyses, was grouped into ten categories continued with detailed information on uses. Data was calculated using synthetic indexes, namely Relative Frequency of Citation (RFC) and Cultural Importance Index (CI). One hundred and one plants were cited by informants as being traditionally used in the area. These 99 ethnospecies belong to 45 families. From the 10 categories, most of them are used for alimentary, medical,

and economical plants. For alimentary plants, 316 citations, 58 species, and 5 different uses were recorded. For medical plants, there were 63 citations, 22 species, and 4 different uses of categories. A few species of plants belong to others categories, like erosion control.

AbidaBano et al. (2014) reported total of 50 medicinal plants belonging to 25 families were reported to be used against 33 different ailments in the valley. The maximum reported medicinal plant families were Asteraceae (7 report species), Lamiaceae (6) , Polygonaceae (4) and Rosaceae (4), the most dominant life form of the species includes herbs (38) followed by shrubs and subshrubs (12), the most frequent used part was leaves (41%) followed by root (26%), flower (14%), fruit (9%), seeds (8%), bulb (1%) and bark (1%), the most common preparation and administration methods were infusion (32%), decoction (26%), paste (18%), herbal juice (17%) and powder drug (7%). The Pearson correlation coefficient between RFC and UV was 0.732 showing highly positive significant association.

KhajoeiNasab. F. and Ahmad Reza Khosravi.(2014). Many plants collected have medicinal properties and have been used by local people to treat various ailments. Of these plants, nineteen families, 37 genera and 43 species belonged to medicinal plants. Among them, Lamiaceae with 8 species and Malva L. with 3 species were the largest medicinal plant families and genera, respectively. These plants are often used as decoction (28%) and as powder (21%). Also, the fruit of these plants are used most often. Besides being used as medicinal plants, they have other uses such as food, fuel, etc. *Malva sylvestris* has the largest value of relative frequency of citation and cultural importance indices. The most ailment categories have the highest level of informant agreement (mean FIC¼0.92).

Mushtaq Ahmad *et al* (2014).Their research work reports total of 50 plant species belonging to 48 genera of 35 families from Chail valley. *Origanum vulgare, Geranium wallichianum* and *Skimmia laureola* have the highest values of relative frequency of citation (RFC) and are widely known by the inhabitants of the valley. The majority of the documented plants were herbs (58%) followed by shrubs (28%), trees (12%) and then climbers (2%). The part of the plant most frequently used was the leaves (33%) followed by roots (17%), fruits (14%), whole plant (12%), rhizomes (9%), stems (6%), barks (5%) and seeds (4%). Decoction was the most common preparation method use in herbal recipes. The most frequently treated diseases in the valley were urinary disorders, skin infections, digestive disorders, asthma, jaundice, angina, chronic dysentery and diarrhea.

Rakesh Samar *et al* (2015). The study was focused on identifying medicinal plants, disease treated, part of the plant used, methods of preparation, route of administration, ingredients added etc. The data was collected using interview and questionnaires by selecting 16 healers using purposive sampling method. A total of 32 medicinal plant species were collected and identified from the study area for treating various human aliments. The paper enumerates these medicinal plant species belonging to 26 genera and 18 families.

Koto-te-NyiwaNgboluaet *al* (2016). Surveys were conducted from February to April 2014 among 50 medicinal plant vendors, in five markets (Limete, Makala, Matete, Mont-Ngafula, and Ngaba). The education level of the majority of informants was secondary school. The age of the informants ranged between 20 and 68 years. Cited plant species were collected and identified at the herbarium of the Faculty of Science, University of Kinshasa. Their ecological status was also determined. The 50 informants used 32 plant species (belonging to 22 families and 30 genera) in traditional medicine in Kinshasa. Their herbal

remedies were administered as aqueous decoctions against 38 different diseases. It was found that ligneous, savanna, phanerophyte, and pantropical-type plant species were predominant both in numbers of species as well as in citations. Roots were the most used plant part, and malaria and haemorrhoids were the most treated diseases.

AbderrahmaineKatiri, *et al* (2017) This study was carried out to identify the medicinal plants traditionally used in human therapy to treat diabetes in the Tizin'Test region, and contribute to safeguarding knowledge and local expertise in traditional herbal medicine. A total of 280 interviews were conducted with traditional health practitioners and knowledgeable villagers. Data were collected by semi structured and structured questionnaires. Indices on Fidelity Level (FL), Use Value (UV) and Relative Frequency of Citation (RFC) were calculated. The ethno botanical survey has identified 39 species representing 24 families. The most encountered medicinal plant families were Asteraceae and Lamiaceae. The following plant species were showed the high significant FL, UV and RFC: *Artemisia herba-alba, Cistus creticus, Lavandula maroccana, Salvia lavandulifolia* and *Olea europaea*. Plant leaves were the most commonly used plant part, and decoction was the most common method of traditional drug preparation. Our study showed that medicinal plants continue to play an important role in the primary healthcare system for the local population of the Tizin'Test region and represents a useful documentation, which can contribute to preserving knowledge on the use of medicinal plants for diabetes treatment and to explore the phytochemical and pharmacological potential of medicinal plant

AishatuShehu (2017) were collected based on an oral interview with the aid of semi structured questionnaire. Only data from willing respondents were obtained and documented. Plant specimens were collected along the line, they were subsequently dried and mounted. It was then taken for identification and authentication in the Herbarium Section of Biological Sciences, Ahmadu Bello University, Zaria where specimen voucher numbers were deposited. Information on sources, safety, methods of preparation and administrations, identity, local and botanical names of medicinal plants used in the management of depression among Hausa tribe of Kaduna State was obtained.

AnamShabir *et al* (2017)collected data by direct comments during field studies, interviews and questionnaires from the local people. The locality, botanical, vernacular names, their family names and uses were elected. During study a total of 98 plants species related to 88 genera and 51 families were recorded. Most abundantly recorded families were Asteraceae, Poaceae, Moraceae, Convolvolaceae, Rosaceae, Fabaceae and Lamiaceae. Ethnobotanical uses classifications showed that major proportion was of medicinal plants species (85 spp, 90.4%) then fodder and forage species were (43 spp, 45.7%) It is followed by other uses such as vegetables (13 spp, 13.8%), fruit (19 spp, 20.2%), Fuel (16 spp, 17%) and timber species (5 spp, 5.3%).

Muhammad Umair, *et al* (2017).This is the first quantitative ethnobotanical study from the area comprising popularity level of medicinal plant species intended by using relative popularity level (RPL) and rank order priority (ROP) indices. Ethnobotanical data were collected by interviewing 166 local informants and 35 traditional health practioners (THPs) from different localities of Hafizabad district. Demographic features of informants; life form, part used, methods of preparation, modes of application and ethnomedicinal uses

were documented. Ethnobotanical data were analyzed using quantitative tools, i.e. Relative frequency citation (RFC), use value(UV), informant consensus factor (ICF) fidelity level (FL), RPL and ROP indices. A total of 85 species belonging to 71 genera and34 families were documented along with ethnomedicinal uses. *Solanum surattense*, *Withania somnifera*, *Cyperus rotundus*, *Solanum nigrum*and *Melia azedarach* were the most utilized medicinal plant species with highest used value. The reported ailments were classified into 11 disease categories based on ICF values and highest number of plant species was reported to treat dermatological and gastrointestinal disorders. *Withania somnifera* and *Ranunculus sceleratus* with maximum FL (100%), were used against gastrointestinal and urinary disorders, respectively. The RPL and ROP values were calculated to recognize the folk medicinal plant wealth; six out of 32 plant species (19%) were found popular, based on citation by more than half of the maximum number of informant viz. 26. Consequently, the ROP value for these species was more than 75. The comparative assessment with reported literature revealed 15% resemblance and 6% variation to previous data;however79% uses of the reported species were recorded for the first time.

ShehlaShinwari, *et al* (2017)Local residents of the study area provided data on 61 medicinal plants belonging to the 34 families and 49 genera. Lamiaceae was the predominant used family consisting of 6 genera (7 species). The highest FL was reported for *Coriandrum sativum*for the treatment of Respiratory disorders (*100%*) and the lowest for *Cedrus deodara*(*78*.57%). The Relative frequency of *Berberis lyceum* (0.39) comes the highest and the lowest for the species of *Oxalis acetosella*(0.15). Leaves were recorded as most used plant part. For the treatment of various diseases, herbs in the area were highly utilized. For

the healing of minor and major illness in northern Pakistan, indigenous medicinal plants are more intended as a form of primary health care.

MayuriTharangaNapagoda*et al.* (2018).The data were collected through semi structured and open-ended interviews from 458 volunteers. Ethno botanical data were analyzed using the Relative Frequency Of Citation (RFC), Family Importance Value (FIV), and Use Value (UV). Out of the total participants, 50.7% claimed the use of medicinal plants for the treatment of inflammatory conditions such as fever, cough, asthma, swellings, and pain in the joints. A total of 43 medicinal plants belonging to 28 plant families were mentioned, out of which *Coriandrum sativum* (RFC = 0.23) was the most cited species. The most cited plant family was fabaceae, and the family importance value was highest in apiaceae. The majority of the nonusers of the herbal remedies mentioned that they would shift to herbal products if scientific information is available on the efficacy of these products.

Mohammad O. *et al* (2018). A total of 159 ethnomedicinal plant species, which were distributed in 132 genera under 62 families, were documented from 174 informants. Of these, 128 plants were native and 31 were exotic. Of a majority of documented species, herbs and leaves were the most utilized plant parts for the preparation of ethnomedicines (45.28%) whereas pastes (63.03%) were the most popular formulations. Among the documented species, the dominant families were the Asteraceae (14 species) and the Lamiaceae (12 species). The highest ICF value was 0.77 for digestive system disorders. Based on UVs, the five most commonly used ethnomedicinal plant species in the study area were *Duabanga grandiflora*(0.43), *Zingiber officinale*(0.41), *Congea tomentosa* (0.40), *Matricaria chamomilla*(0.33) and *Engelhardtia spicata*(0.28). The highest RFC was recorded for *Rauvolfia serpentina*(0.25). The highest RI value was calculated for both *Scoparia dulcis* and

Leucas aspera(0.83). Importantly, 16 species were reported with new therapeutic uses and to our knowledge, 7 species described herein have never been ethnobotanically and pharmacologically studied, viz: *Agastache urticifolia*, *Asarum cordifolium, C. tomentosa, E. spicata, Hypser panitida, Merremia vitifolia* and *Smilax odoratissima.*

Khafsa Malik *et al* (2019). In this study, we recorded 106 plant species belonged to 56 floral families for treatment of skin ailments. The dominant life form reported was herb while the preferred method of utilization was powder, along with leaf as the most used plant part. RFC ranges from 0.07 to 0.25% whereas the highest FIV was recorded for family Pteridaceae. FL values range from 36.8 to 100%. The study reported 88% of new plant reports for the treatment of skin disease.

ElhassanIdm*et al*(2020) collected information by means of open interviews with local people using the questionnaires. The data was analyzed using Use Value (UV), Relative Frequency of Citation (RFC), Fidelity Level (FL) and Informant Consensus Factor (ICF). The analysis of the results allowed us to identify 130 vascular plant species in 57 families with a significant representativeness of Lamiaceae (10%), Asteraceae (9.23%), Fabaceae (8.46%), Apiaceae (6.15%), Poaceae (3.85%), Solanaceae (3.07%) and Amaranthaceae (3.07%). These species are mainly used in the care of the digestive and genito-urinary disorders. The UV ranged from 0.01 (*Aframomum melegueta*) to 0.34 *(Maerua crassifolia*). The RFC ranged from 0.01 (*Aframomum melegueta*) to 0.32 (*Maerua crassifolia*). The highest FL (100%) was found for 38 species, while the highest values of ICF were recorded for gastrointestinal pains (0.972).

HichamOrch, *et al*(2020) were using 480 questionnaire sheets, ethnobotanical field surveys were conducted during two campaigns (2013 to 2015). The determination of the different survey media was carried out using stratified probability sampling techniques. The

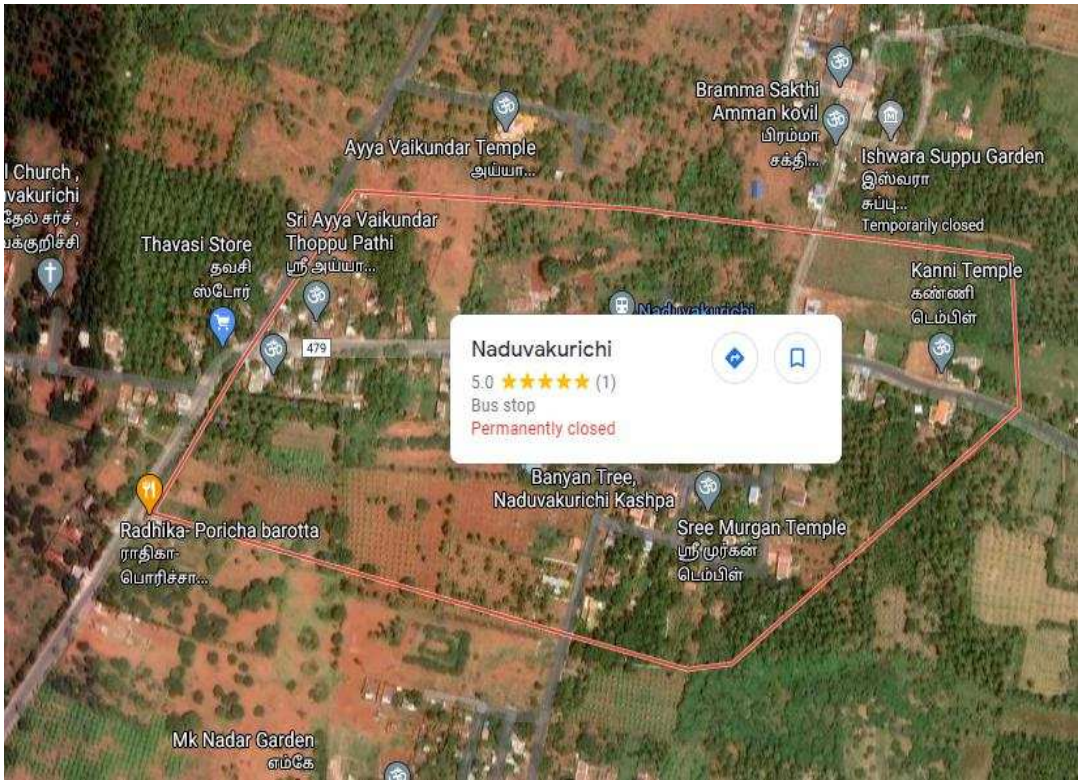
ethnobotanical data were analyzed through the calculation of quantitative indices, such as Relative Frequency of Citation (RFC), Family Importance Value index (FIV), Fidelity Level (FL), Informant Consensus Factor (ICF) and Use Value of the Plant Part (PPV). Analysis of the results revealed 40 plant species, which fall into 19 botanical families. The *Lamiaceae* family was the most represented (14 species, FIV = 0.076). The highest Relative Citation Frequency (RFC) (0.22) was recorded for *Mentha pulegium*. Concerning the diseases treated, asthma had the highest ICF (0.95). The leaf was considered the most used part of the plant (PPV = 0.34), and most of the remedies were prepared as decoctions.

Abhishek Kumar Pandey (2021). The ethno botanical study was carried out by using semi-structured interview in which participatory rural appraisal (PRA) and Rapid rural appraisal methods used to obtain ethnobotanical knowledge of plants of their surrounding form local tribes. The quantitative data were also analyzed by the relative frequency of citation, use value and informant consensus factor. This paper reports 103 medicinal plants belonging to 90 genera representing 40 families. These plants are used by local people to treat different ailments including diabetic, itching, purifying blood, toothache, asthma, fever, low pressure, ulcer and urinary discharge and many other diseases. The highest relative frequency of citation (RFC) was recorded for *Ficus religiosa*(0.92), *Ziziphus marutiana*(0.92) followed by *Ocimum sanctum* (0.90), *Murraya koenigii*(0.89), *Withania somnifera*(0.88) and *Tinospora cordifolia* (0.84). The highest use value recorded for *Moringa oleifera*(1.78) followed by *Ocimum sanctum* (1.75), *Murraya koenigii*(1.62), *Punica granatum*(1.56) and *Mangifera indica*(1.55) Highest Informant Consensus Factor (ICF) was recorded for the gastro-intestinal disorder ailment category.

KodjoviAgbodeka*et al* (2021) prepare a semi-structured questionnaire interviews were used to gather ethnobotanical and socio demographic data from traditional healers of the study area. A total of 61 plants species belonging to 33 families were found to be in use for malaria therapy in the Plateau region. Caesalpiniaceae were the most represented family with 7 species, followed by Euphorbiaceae and Poaceae with 4 species each. According to the relative frequency of citation (RFC), *Newbouldia* laevis(Seem). (RFC =0.52), *Sarcocephalus latifolius*(Sm.) E.A. Bruce (RFC =0.48), *Acanthospermum* hispidum(DC). (RFC =0.43), and *Senna siamea*(Lam). H.S. Irwin and Barne by (RFC =0.40) were the most cited in the treatment of malaria in the traditional medicine in the Plateau region. The parts of plants used could either be the barks, roots, leaves, or whole plants.

PLATE 1

Map showing study area



MATERIALS AND METHODS

Discription of the study area

Naduvakurichi is a small village situated near Sawerpuram town panchayat in Thoothukudi district,Tamilnadu,India. It situated at 8.7408309^{o} N latitude and 77.7952545^{o} E longitude geographically.This village is located 19KM distance from Thoothukudi District. This small village is known for peaceful environment and considerd to be the cleanest small village with less population. It has 0% wind pollution and 0% water pollution. In summer the climate remains warm and on other seasons the climate can be cool and windy. This small village is also known for the cultivation of Bananas (*Musa paradisiaca*)and Rice (*Oryza sativa*) They export the unripened fruit to Kerala. Village is rich source of vegetation and water facilities.

Mode of Survey:

The frequent field survey was conducted from December 2020 to March 2021. Mature and healthy plants were collected. Collected plant samples were identified by using the standard literature such as Floras of Presidency of Madras (Gamble, 1935); Further Illustrations on the Flora of the Tamil Nadu and Carnatic (Mathew, 1983) have been referred for the specimens identified. The medicinal uses of plants were compiled using earlier publications. Maheswerी *et al* (1993),KrishnanMarg(1992);Balasingh *et al* (*2000),* Yoganarasimhan(2000). Dictionary of medicinal plants Balasubramanian(2013). Traditional and local knowledge of medicinal plants and socioeconomic survey was collected through a Questionnaire.

RESULT AND DISCUSSION

SOCIO-DEMOGRAPHIC CHARACTERISTICS OF THE VILLAGE

Naduvakurichi is a small village. There are 553 households with total population of 2488. Out of these 1419 men, 1069 women including adult and children. There are about only 50% of them are literate, remaining 50% of them are illiterates. There are about 53 members are government employers, 53 of them are private, 63 members are self-employers, 53 members are farmers, and nearly 50% ladies are home makers. Majority of them are depend upon agriculture and the remaining families are landless and they eke out their living by daily wages. The details of socio-demographic characteristics were represented in Table 1.

ETHNOBOTANICAL SURVEY

Taxonomically, total of 183 plant species belonging to 149 genera and 61 families were invented from the Naduvakurichi village of Thoothukudi District (Table – 2 & 4).Including one pteridophyte family, and 182angiosperms.Among them 98 species (53%) were herbs, 30 species (16%) were shrubs, 33 (18 %) species were trees, 6 (3 %) species were climber, 15 (8%) were creepers, 1 (0 .5%) were pteridophytes. Majority of the plants recorded in the study area are economically important. Among 150 species recorded 132 species were medicinal. (Table-4) Few species have been used for other purposes such as fodder (*Prosopis juliflora*),fuel wood (*Acacia nilotica*).

The most availability of edible fruit trees are *Annona squamosa, Carica papaya, Psidium gujava, Punica granatum, Terminalia catapa* etc,. The most important vegetable species grown in the study area are *Cocos nucifera, Luff tuberose, Mormordica cybalaria, Moringa oleifera* etc,. The spinach includes *Amaranthus viridis, Pisonia alba, Solanum nigrum*.

The presence of economically important plants in the village helps their basic needs for food, health, energy and housing Tynsong and Tiwari (2010) Mary suba*et al* (2014)

Vegetation around the village are characterized by the mixed type of herbs shrubs trees climbers creepers Pteridophyte

Table: 1 Socio-Demographic Characteristic of Naduvakurichi Village

Background		**Number**	**Percentage%**
Residence		553	
Gender	Male	1419	57%
	Female	1069	43%
Age	>20	72	2.8%
	21 - 30	450	18%
	31 - 40	616	24.8%
	41 - 50	513	20.6%
	51 - 60	553	22.2%
	61 - 70	185	7.4%
	71 - 80	99	0.4%
Occupation	Farmer	53	9.5%
	Home maker	210	3.8%
	Seller	21	3.8%
	Government	53	9.5%
	Private	53	9.5%
	Self employed	68	12.2%
	Others	95	17.1%

Table 2: List of Plant Species of the Study Area

Sl.No	Botanical Name	Family	Habit	Common Name
1.	*Abelmoscus esculentus*(L.)	Malvaceae	Herb	Vendai
2.	*Abutilon indicum*(L.)	Malvaceae	Herb	Thuthi
3.	*Acalypha indica*(L.)	Euphorbiaceae	Herb	Kuppaimeni
4.	*Achras sapota*(L.)	Sapotaceae	Tree	Sapota
5.	*Achyranthes aspera*(L.)	Amaranthaceae	Herb	Nayuruvi
6.	*Adhatoda vasica*(L.)	Acanthaceae	Shrub	Adhathoda
7.	*Aerva lanata*(L.) juss	Amaranthaceae	Herb	Pongal poo
8.	*Allamanda blanchetii* (A.DC)	Apocynaceae	Shrub	Kuduvai poo
9.	*Allamanda cathartica*(L.)	Apocynaceae	Shrub	Golden trumpet
10.	*Alliaria petiolata*(M.Bieb)	Brassicaceae	Shrub	Garlic mustard
11.	*Allium cepa* (L.)	Alliaceae	Herb	Vengayam
12.	*Alocasia macrorrhizos*(L.) G.Don	Araceae	Herb	Yanaikathu
13.	*Aloe vera*(L.) Burm.f	Lilliaceae	Herb	Katralai
14.	*Alternanthera brasiliana*(L.) Kuntze	Amaranthaceae	Herb	Ponnanganni
15.	*Alternanthera paronychioides* A.St.Hil	Amaranthaceae	Herb	Ponnanganni
16.	*Alternanthera sessilis*(L.) R.Br	Amaranthaceae	Herb	Ponnanganni
17.	*Alysicarpus vaginalis*(L.) DC	Fabaceae	Herb	Alyce clover
18.	*Amaranthus blitum*(L.)	Amaranthaceae	Herb	Spreading pig weed
19.	*Amaranthus caudatus*(L.)	Amaranthaceae	Herb	Thandukeerai
20.	*Amaranthus viridis*(L.)	Amaranthaceae	Herb	Kuppaikeerai
21.	*Andrographis paniculata* (Burm) .f	Acanthaceae	Herb	Siriyanangai
22.	*Anisomeles malabarica*(L.) R.Br	Labiatae	Herb	Perumthumbai
23.	*Annona squamosa*(L.)	Annonaceae	Tree	Seetha
24.	*Apium graveolens*(L.)	Apiaceae	Herb	Celery
25.	*Argemone mexicana*(L.)	Papaveraceae	Herb	Brammathandu
26.	*Artocarpus integrifolia* Linn.f	Moraceae	Tree	Pala
27.	*Asparagus setaceus* (Kunth) Jessop	Aspragaceae	Creeper	Asparagus fern

28.	*Asystasia gangetica*(L.)	Acanthaceae	Creeper	Mithikeerai
29.	*Azadirachta indica* A.Juss	Meliaceae	Tree	Vembu
30.	*Bauhinia variegata*(L.)	Caesalpiniaceae	Shrub	Iruvachi
31.	*Boerhavia diffusa*(L.)	Nyctaginaceae	Herb	Punarnava
32.	*Borassus flabellifer*(L.)	Arecaceae	Herb	Panai
33.	*Bougainvillea glabra*	Nyctaginaceae	Tree	Thaal poo
34.	*Bryophyllum pinnatum*(Lam.) Kurz.	Crassulaceae	Herb	Ranakalli
35.	*Caesalpinia pulcherrima*(L.) Sw.	Caesaalpiniaceae	Shrub	Peacock flower
36.	*Caladium bicolour*(Aiton.) Vent.	Araceae	Herb	Heart of jesus
37.	*Calotropis gigantea*(L.) W.T. Aiton	Apocynaceae	Shrub	Erukku
38.	*Canavalia rosea* (Sw.) DC.	Fabaceae	Creeper	Chevalavarai
39.	*Canna indica*(L.)	Cannaceae	Herb	Wild canna
40.	*Capsicum frutescens*(L.)	Solanaceae	Herb	Milagai
41.	*Carica papaya*(L.)	Caricaceae	Tree	Pappali
42.	*Cassia auriculata*(L.)	Caesalpiniaceae	Herb	Aavaram
43.	*Casuarina equisetifolia*(L.)	Casuarinaceae	Tree	Whistling pine
44.	*Catharanthus roseus*(L.) G. Don.	Apocynaceae	Herb	Nithiyakalyani
45.	*Celosia argentea*(L.)	Amaranthaceae	Herb	Koli poo
46.	*Centella asiatica*(L.) Urb.	Apiaceae	Herb	Vallarai
47.	*Cestrum nocturnum*(L.)	Solanaceae	Shrub	Lady of the night
48.	*Chenopodium album*(L.)	Amaranthaceae	Herb	Chakravarthikeerai
49.	*Chrysanthemum indicum*(L.)	Asteraceae	Herb	Sevanthi
50.	*Chrysanthemum morifolium*	Asteraceae	Herb	Sevanthi
51.	*Cissus quadrangularis*(L.)	Vitaceae	Creeper	Pirandai
52.	*Citrullus colocynthis*(L.) Schrad.	Cucurbitaceae	Tree	Bitter cucumber
53.	*Citrus acida*(L.)	Rutaceae	Tree	Narthai
54.	*Citrus aurantium*(L.)	Rutaceae	Tree	Orange
55.	*Citrus limonum*(Linn.) Burm.f	Rutaceae	Tree	Lemon
56.	*Cleome gynandra* (L.)	Cleomaceae	Herb	African spider flower
57.	*Cleome viscose*(L.)	Cleomaceae	Herb	Tick weed

58.	*Clitoria ternatea*(L.)	Fabaceae	Creeper	Sangupoo
59.	*Clivia miniata*(Lindl.) Bosse.	Amaryllidaceae	Herb	Bush lilly
60.	*Coccinia grandis*(L.) Voigt	Cucurbitaceae	Climber	Kovai
61.	*Cocos nucifera*(L.)	Arecaceae	Tree	Thennai
62.	*Codiaeum variegatum*(L.) Rumph.	Euphorbiaceae	Herb	Croton
63.	*Coleus aromaticus* Benth.	Labiatae	Herb	Omavalli
64.	*Commelina benghalensis*(L.)	Commelinaceae	Herb	Tropical spiderwort
65.	*Commelina erecta*(L.)	Commelinaceae	Herb	Creeping day flower
66.	*Convolvulus arvensis*(L.)	Convolvulaceae	Herb	Field bind weed
67.	*Cordia sebestina* (L.)	Boraginaceae	Tree	Scarlet cordial
68.	*Crossandra infundibuliformis* (L.) Nees	Acanthaceae	Herb	Kanagamaram
69.	*Cucumis melo*(L.)	Cucurbitaceae	Creeper	Thumatikkai
70.	*Cucurbita maxima* Duchesne	Cucurbitaceae	Creeper	Poosani
71.	*Cynodon dactylon*(L.) Pers.	Poaceae	Herb	Bermuda grass
72.	*Cymbalaria muralis* G. Gaertn., B.Mey & Scherb.	Plantaginaceae	Herb	Penny wort
73.	*Datura metel*(L.)	Solanaceae	Herb	Oomathai
74.	*Delonix elata* (L.)	Caesalpiniaceae	Tree	White vadachi
75.	*Delonix regia* (Hook.). Raf	Caesalpiniaceae	Tree	Red vadachi
76.	*Desmodium triflorum*(L.) DC.	Fabaceae	Herb	Tropical trefoil
77.	*Digitaria cilliaris* (Retz.) Koeler.	Poaceae	Herb	Wild crab grass
78.	*Dracaena trifasciata* (Prain.) Mabberley	Asparagaceae	Herb	Snake plant
79.	*Eclipta alba* (L.)	Asteraceae	Herb	Karislanganni
80.	*Eichhornia crassipes* (Mart.) Solms	Pontederiaceae	Herb	Vengayathamarai
81.	*Elaeis guineensis* Jacq	Arecaceae	Tree	African oil palm
82.	*Elephantopus scaber* Linn.	Asteraceae	Shrub	Elephants foot
83.	*Eleusine indica* (L.) Gaertn.	Poaceae	Herb	Crow foot grass
84.	*Emblica officinalis* (L.)	Phyllanthaceae	Tree	Nellikai

85.	*Epipremnum aureum* (Linden & Andre) G.S.Bunting,	Araceae	Climber	Money plant
86.	*Euphorbia heterophylla*(L.)	Euphorbiaceae	Herb	Ilai mel roja
87.	*Euphorbia hirta*(L.)	Euphorbiaceae	Herb	Asthma weed
88.	*Euphorbia milii* Des. Moul	Euphorbiaceae	Herb	Crown of thorns
89.	*Euphorbia peplus*(L.)	Euphorbiaceae	Herb	Radium weed
90.	*Euphorbia serpens* Kunth	Euphorbiaceae	Herb	Matted sandmat
91.	*Ficus racemosa*(L.)	Moraceae	Tree	Athi
92.	*Ficus religiosa*(L.)	Moraceae	Tree	Arasamaram
93.	*Glinus lotoides*(L.)	Molluginaceae	Herb	Lotus sweet juice
94.	*Heliotropium indicum*(L.)	Boraginaceae	Herb	Tetkodukki
95.	*Hemidesmus indicus*(L.) R.Br	Apocynaceae	Herb	Nannari
96.	*Hibiscus rosa-sinensis*(L.)	Malvaceae	Herb	Sembaruthi
97.	*Hygrophila auriculata* (K. Schum) Heine	Acanthaceae	Herb	Neermuli
98.	*Indigofera tinctoria*(Linn.)	Fabaceae	Herb	Avuri
99.	*Ipomoea aquatic* Forssk	Convolvulaceae	Creeper	Water morning glory
100.	*Ipomoea triloba*(L.)	Convolvulaceae	Creeper	Little bell
101.	*Ixora coccinia*(L.)	Rubiaceae	Shrub	Idly poo
102.	*Jasminum angustifolium*(L.)	Olaceae	Shrub	Pitchi poo
103.	*Jasminum auriculatum* Vahl	Olaceae	Shrub	Mullai
104.	*Jasminum sambac*(L.) Aiton.	Olaceae	Shrub	Malli
105.	*Jatropha tanjorensis* Ellis&Saroja	Euphorbiaceae	Shrub	Sugar keerai
106.	*Lawsonia innermis*(L.)	Lythraceae	Shrub	Maruthani
107.	*Leucaena leucocephala* (Lam.) de Wit	Mimosaceae	Tree	Wild tamarind
108.	*Leucas aspera* (Willd). Link	Labiatae	Herb	Thumbai
109.	*Ligustrum ovalifolium*Hassk.	Olaceae	Shrub	Oval leaved privet
110.	*Luffa acutangula* (L.) Roxb.	Cucurbitaceae	Herb	Peerkangai
111.	*Lycopersicum esculentum*(L.)	Solanaceae	Herb	Tomato

112.	*Malva sylvestris*(L.)	Malvaceae	Herb	High mallow
113.	*Malvastrum coromandelianum*(L.) Garcke.	Malvaceae	Herb	False mallow
114.	*Mangifera indica* (L.)	Anacardiaceae	Tree	Mango
115.	*Medicago falcata*(L.)	Fabaceae	Herb	Sickle medick
116.	*Medicago sativa*(L.)	Fabaceae	Herb	Alfalfa
117.	*Melia azedarach*(L.)	Meliaceae	Tree	Katuvembu
118.	*Mentha viridis*(L.)	Labiatae	Creeper	Mint
119.	*Mimosa pudica*(L.)	Mimosaceae	Herb	Thottal sinungi
120.	*Mirabilis jalapa*(L.)	Nyctaginaceae	Shrub	5 o clock plant
121.	*Mollgo nudicaulis*(L.)	Molluginaceae	Herb	Carpet weed
122.	*Momordica charantia*	Cucurbitaceae	Climber	Bitter gourd
123.	*Moringa oleifera*(L.)	Moringaceae	Tree	Murungai
124.	*Murraya konigii*(L.) Spreng	Rutaceae	Tree	Karuveppilai
125.	*Musa paradisiaca*(L.)	Musaceae	Shrub	Valai
126.	*Nephrolepis biserrata*(Swartz)	Nephrolepidaceae	Pteridophyte	Fern
127.	*Nerium odorum*(L.)	Apocynaceae	Shrub	Arali
128.	*Ocimum basilicum*(L.)	Labiatae	Herb	Patchailai
129.	*Ocimum sanctum* (L.)	Labiatae	Herb	Thulasi
130.	*Oldenlandia corymbosa*(L.)	Rubiaceae	Herb	Katusayaver
131.	*Oxalis pes-caprae*(L.)	Oxalidaceae	Creeper	Bermuda buttercup
132.	*Parthenium hysterophorus*(L.)	Asteraceae	Herb	Carrot grass
133.	*Passiflora foetida*(L.)	Passifloraceae	Creeper	Love in a mist
134.	*Phyllanthus amarus* Schumach. & Thonn.	Phyllanthaceae	Herb	Keelanelli
135.	*Piper betle*(L.)	Piperaceae	Climber	Vetrilai
136.	*Piper longum*(L.)	Piperaceae	Climber	Thippili
137.	*Pithecellobium dulce* (Roxb.)Benth.	Mimosaceae	Tree	Kodukapuli
138.	*Plantanus orientalis*(L.)	Plantanaceae	Herb	Oriental plane
139.	*Plectranthus scutellarioides* (L.) R.Br	Labiatae	Herb	Coleus
140.	*Poa annua*(L.)	Poaceae	Herb	Annual blue grass
141.	*Portulaca grandiflora* Hook.	Portulacaceae	Creeper	Moss rose
142.	*Portulaca oleracea*(L.)	Portulacaceae	Creeeper	Purslane
143.	*Potentilla reptans*(L.)	Rosaceae	Creeper	Cinquefoil

144.	*Prosopis juliflora* (SW) D.C	Mimosaceae	Shrub	Karuvelam
145.	*Pseuderanthemum carruthersii*(Seem.) Guill	Acanthaceae	Shrub	False eranthemum
146.	*Psidium guajava*(L.)	Myrtaceae	Tree	Koyya
147.	*Punica granatum*(L.)	Lythraceae	Shrub	Mathulai
148.	*Ricinus communis*(L.)	Euphorbiaceae	Shrub	Aamanakku
149.	*Rosa chinensis* F.Spontanea	Rosaceae	Shrub	Pannier rose
150.	*Rosa indica*(L.)	Rosaceae	Shruds	Rose
151.	*Sechium edule*	Cucurbitaceae	Climber	Chow chow
152.	*Setaria pumila* (Poir.) Roem. & Schult.	Poaceae	Herb	Korai pull
153.	*Sida cordifolia*(L.)	Malvaceae	Herb	Heart leaf sida
154.	*Solanum melongena*(L.)	Solanaceae	Herb	Kathari
155.	*Solanum nigrum*(L.)	Solanaceae	Herb	Manathakkali
156.	*Solanum torvum* Sw.	Solanaceae	Herb	Sundaikai
157.	*Solanum trilobatum*(L.)	Solanaceae	Shrub	Thoothuvalai
158.	*Solanum virginianum*(L.)	Solanaceae	Herb	Kandangathiri
159.	*Sphagneticolan trilobata*(L.)	Asteraceae	Herb	Creeping daisy
160.	*Stellaria media*(L.) Vill.	Caryophyllaceae	Herb	Chick weed
161.	*Syngonium podophyllum* Schott.	Araceae	Shrub	Arrow head
162.	*Syzygium arnotianum* (Wight) Walp	Myrtaceae	Tree	Naval
163.	*Tabernaemontana divaricata* (L.) R.Br	Apocynaceae	Shrub	Nandriketan arali
164.	*Talinum paniculatum*(Jacq)	Portulacaceae	Shrub	Pasalai
165.	*Tamarindus indica*(L.)	Caesalpiniaceae	Tree	Pulimaram
166.	*Tecoma stans*(L.) Juss	Bignonaceae	Tree	Manjanathi
167.	*Tectona grandis*(L.) f	Verbenaceae	Tree	Thekku
168.	*Tephrosia purpurea*(L.) Pers	Fabaceae	Herb	Kollukaivelai
169.	*Terminalia catappa*(L.)	Combretaceae	Tree	Vadhumai
170.	*Thespesia populnea*(L.) Soland	Malvaceae	Tree	Poovarasu
171.	*Tithonia diversifolia* (Hemsl)	Asteraceae	Herb	Japanese sunflower
172.	*Tradescantia virginiana*(L.)	Commelinaceae	Herb	Lady's tears
173.	*Trianthema portulacastrum*(L.)	Aizoaceae	Herb	Giant pig weed

174.	*Tribulus terrestris*(L.)	Zygophyllaceae	Herb	Nerunji
175.	*Tridax procumbens*(L.)	Asteraceae	Herb	Thatha poo
176.	*Turnera ulmifolia*(L.)	Passifloraceae	Herb	Yellow buttercup
177.	*Vinca minor*(L.)	Apocynaceae	Herb	Lesser periwinkle
178.	*Vitis vinifera*(L.)	Vitaceae	Herb	Grapes
179.	*Waltheria indica*(L.)	Sterculiaceae	Herb	Sleepy morning
180.	*Xanthium strumarium*(L.)	Asteraceae	Herb	Marul oomathai
181.	*Yucca aloifolia*(L.)	Asparagaceae	Tree	Palm lilly
182.	*Zingiber officinale* Roscoe	Zingiberaceae	Herb	Ginger
183.	*Ziziphus mauritiana*(Lam.)	Rhamnaceae	Shrub	Elanthai

Table 3: Habit of the Vegetation

Sl.No	Habit	No of species	%
1	Tree	33	18
2	Shrub	30	16
3	Herb	98	53
4	Climber	6	3
5	Creeper	15	8
6	Pteridophyte	1	0.5

Table 4: Medicinal flora of the study area

S.No	BOTANICAL NAME	DISEASES															
		Fever, Asthma	Kidney stone	Hair growth and Dandruff	Injury	Skin disease	Dysentery	Eye issue	Intestinal problems	Heat reducer	Tuning	Ulcer	Indigestion	Diabetics	Dental issues	Snake bite	Rheumatism
1.	*Abelmoscus esculentus*						*							*			
2.	*Abutilon indicum*				*		*										
3.	*Acalypha indica*				*		*										
4.	*Achras sapota*								*			*					
5.	*Achyranthes aspera*															*	
6.	*Adhatoda vasica*	*							*								
7.	*Aerva lanata*		*						*								
8.	*Alliaria petiolata*	*							*								
9.	*Allium cepa*			*					*	*							
10.	*Aloe vera*			*					*	*		*	*				
11.	*Alternanthera brasiliana*								*				*				
12.	*Alternanthera paronychoides*								*					*			
13.	*Alternanthera sessilis*							*									
14.	*Alysicarpus vaginalis*						*		*								
15.	*Amaranthus blitum*								*			*					
16.	*Amaranthus caudatus*								*					*			
17.	*Amaranthus viridis*						*							*			
18.	*Andrographis paniculata*	*															

19.	*Anisomeles malabarica*	*															
20.	*Annona squamosa*													*			
21.	*Apium graveolens*								*								
22.	*Argemone mexicana*								*								
23.	*Artocarpus integrifolia*								*								
24.	*Azadirachta indica*	*		*	*				*			*		*	*		
25.	*Boerhavia diffusa*	*				*		*						*			
26.	*Borassus flabellifer*						*		*			*					
27.	*Bryophyllum pinnatum*		*														
28.	*Calotropis gigantea*						*		*			*			*		*
29.	*Canavalia rosea*																*
30.	*Canna indica*								*								
31.	*Capsicum frutescens*																*
32.	*Carica papaya*	*							*			*					
33.	*Cassia auriculata*			*													
34.	*Catheranthus roseus*		*											*			
35.	*Centella asiatica*						*					*					
36.	*Chenopodium album*											*	*				
37.	*Cissus quadrangularis*								*			*					
38.	*Citrulus colocynthis*	*					*						*				
39.	*Citrus acida*												*		*		*
40.	*Citrus aurantium*												*				
41.	*Citrus limonum*								*								
42.	*Cleome gynandra*								*					*			
43.	*Cleome viscose*																*
44.	*Clitoria ternatea*								*								
45.	*Coccinia grandis*								*			*					
46.	*Cocos nucifera*			*	*	*		*		*		*					
47.	*Coleus aromaticus*	*							*				*				
48.	*Commelina benghalensis*					*				*							
49.	*Commelina erecta*				*												

50.	*Convolvulus arvensis*																*
51.	*Cordia sebastina*	*															
52.	*Crossandra infundibuliformis*	*			*												
53.	*Cucumis melo*													*			
54.	*Cucurbita maxima*	*				*			*								
55.	*Cymbalaria muralis*				*												
56.	*Cynodon dactylon*	*	*				*									*	
57.	*Datura metel*																*
58.	*Desmodium triflorum*						*										
59.	*Digitaria ciliaris*								*								
60.	*Dracaena trifasciata*					*											
61.	*Eclipta alba*			*													
62.	*Eichhornia crassipes*	*					*										
63.	*Elephantopus scaber*	*							*								
64.	*Eleusine indica*	*															
65.	*Emblica officinalis*			*											*		
66.	*Euphorbia heterophylla*	*							*								
67.	*Euphorbia hirta*	*															
68.	*Euphorbia peplus*	*							*								
69.	*Euphorbia serpens*								*								
70.	*Ficus racemosa*						*							*			
71.	*Ficus religiosa*					*								*			
72.	*Glinus lotoides*				*												
73.	*Heliotropium indicum*					*											*
74.	*Hemidesmus indicus*				*						*		*				
75.	*Hibiscus rosa-sinensis*			*					*	*			*				
76.	*Hygrophila auriculata*	*															
77.	*Indigofera tinctoria*	*			*				*								
78.	*Ipomoea aquatic*	*							*								
79.	*Ipomoea triloba*	*							*								
80.	*Jasminum angustifolium*					*								*			

81.	*Jasminum auriculatum*								*								
82.	*Jasminum sambac*				*											*	
83.	*Jatropha tanjorensis*												*				
84.	*Lawsonia innermis*			*						*							
85.	*Leucaena leucocephala*												*				
86.	*Leucas aspera*	*															
87.	*Luffa acutangula*	*															*
88.	*Lycopersicum esculentum*					*								*			*
89.	*Malva sylvestris*				*							*					
90.	*Malvastrum coromandelianum*												*				
91.	*Mangifera indica*						*							*			*
92.	*Medicago falcate*					*											
93.	*Medicago sativa*	*							*								
94.	*Melia azedarach*												*				*
95.	*Mentha viridis*						*					*					
96.	*Mimosa pudica*		*										*				
97.	*Mirabilis jalapa*				*	*											
98.	*Mollugo nudicaulis*								*								
99.	*Momordica charantia*												*				
100.	*Moringa oleifera*												*				
101.	*Murraya konigii*			*						*							
102.	*Musa paradisiacal*						*						*		*		
103.	*Nerium odorum*	*				*											
104.	*Ocimum basilicum*	*							*								
105.	*Ocimum sanctum*	*							*								
106.	*Oldenlandia corymbosa*									*							
107.	*Oxalis pescapre*									*							
108.	*Parthenium hysterophorus*					*			*								
109.	*Passiflora foetida*						*										
110.	*Phyllanthus amarus*				*				*			*					
111.	*Piper betle*	*			*												

112.	*Piper longum*	*					*										
113.	*Pithecellobium dulce*														*		
114.	*Plantanus orientalis*																*
115.	*Plectranthus scutellarioides*	*							*								
116.	*Portulaca grandiflora*															*	
117.	*Portulaca oleracea*											*					*
118.	*Potentilla reptans*	*				*							*				
119.	*Prosopis juliflora*									*							
120.	*Pseuderanthemum carruthersii*				*												
121.	*Psidium guajava*								*						*		
122.	*Punica granatum*								*			*			*		
123.	*Ricinus communis*	*										*					
124.	*Rosa chinensis*				*							*					*
125.	*Rosa indica*	*			*	*	*										
126.	*Sechium edule*								*					*			
127.	*Sedum album*					*											
128.	*Setaria pumila*																*
129.	*Sida cordifolia*	*							*								
130.	*Solanum melongena*	*													*		*
131.	*Solanum nigrum*				*				*				*				
132.	*Solanum torvum*								*								
133.	*Solanum trilobatum*	*															
134.	*Solanum virginianum*	*			*	*											
135.	*Sphagneticola trilobata*	*															
136.	*Stellaria media*				*	*											
137.	*Syngonium podophylllum*								*								
138.	*Syzygium arnotianum*	*															
139.	*Tabernaemontana divaricata*							*								*	
140.	*Talinum paniculatum*								*								
141.	*Tamarindus indica*				*												*
142.	*Tecoma stans*								*								

143.	*Tectona grandis*						*		*								
144.	*Tephrosia purpurea*	*										*					
145.	*Terminalia catappa*	*			*												*
146.	*Thespesia populnia*					*											
147.	*Tithonia diversifolia*											*					
148.	*Tradescantia virginiana*		*														
149.	*Trianthema portulacastrum*								*								
150.	*Tribulus terrestris*					*			*								
151.	*Tridax procumbens*				*		*					*					
152.	*Turnera ulmifolia*	*					*										
153.	*Vinca minor*								*			*					
154.	*Vitis vinifera*	*	*			*			*								
155.	*Waltheria indica*	*			*		*										
156.	*Xanthium strumarium*											*					*
157.	*Zingiber officinale*								*								
158.	*Ziziphus mauritiana*			*	*												

SUMMARY AND CONCLUSION

Naduvakurichi is a small village situated near Sawerpuram town panchayat in Thoothukudi district,Tamilnadu,India.Vegetation around the village are characterized by the mixed type of herbs shrubs trees climbers creepers Pteridophyte. Taxonomically, total of 183 plant species belonging to 149 genera and 61 families.

Local population has a rich indigenous knowledge, but is always stay not adequately documented. It should be noted that some listed species are suffering from surex plantation. Which can subjects to the disappearance of the most vulnerable species. It will be urgent and essential to adopt a sustainable management strategy to avoid the degradation of biodiversity of the region.

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Ethnobotanical Survey of Medicinal Plants Used by the Traditional Healers in Sorispuram and Kurangan thattu village of Tuticorin District

A short field project work submitted to

St. Mary's College (Autonomous)

Affiliated to

MANONMANIAM SUNDARANAR UNIVERSITY

In partial fulfilment of the requirement for the Degree of Master of science in Botany

By

Y. ABINAYA - 20APBO01

DEPARTMENT OF BOTANY

ST.MARY'S COLLEGE (AUTONOMOUS)

THOOTHUKUDI-628001

CERTIFICATE

It is certified that this field work entitled "**Ethnobotanical Survey of Medicinal Plants Used by the Traditional Healers in Sorispuram and Kurangan thattu Village of Thoothukudi District**" submitted to St. Mary's college (Autonomous) affiliated to MANONMANIAM SUNDARANARUNIVERSITY in partial fulfilment of the requirements for the degree of Master of science in Botany, and is a record of work done in the Department of Botany, St.Mary's College (Autonomous), Thoothukudi during the year 2020 – 2021 by the following student.

By

Y. ABINAYA **20APBO01**

M. Glory
GUIDE

M. Glory
HEAD OF THE DEPARTMENT

EXAMINER

Lucia Rose
PRINCIPAL
St. Mary's College (Autonomous)
Thoothukudi - 628 001.

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Introduction

India has one of the oldest, richest and most diverse cultural traditions called 'folk tradition" associated with the use of medicinal herbs and it is still a living tradition in India. Traditional folk medicine is the application of indigenous beliefs, knowledge, skills and cultural practices concerned with human health. The sacred 'Vedas' and the other ancient Indian treatises give many references of these medicinal plants.

One of the oldest records in traditional herbal medicine is Vrikshyayurveda compiled by Parashara, which formed the basis of medicinal studies in ancient India. More detailed accouts are in the Atharva Veda (BC 800). Later came the Ayurveda, the practice of which was recorded in Sanskrit. The Vedic and post-Vedic periods roughly from BC 4500 to 5000 AD has celebrated Indian physicians and herbalists. Aterya, Mahabharata, Nagarjuna, Vaghbhata, Sushruta and the Hindu Hippocrates, Charaka were the legendary figures of traditional Indian medicine.

Two memorable treatises, Charaka Samhita and Sushruta Samhita appeared between 400AD and 500AD. The evidence of systematic herbal knowledge, however, was first found in the oldest medicinal book of India, that is, Sushruta-Samhita.

Although traditional medicine flourished in India for quite a long time, yet for a while it was subdued under the impact of modern medicine. But as science revealed the great hazards of synthetic medicines on human health combined with their high cost which is beyond the reach of the common man, the traditional herbal medicine has once again started gaining importance all over the world. India has to revive the traditional herbal medicine and bring it into the mainstream of the national health care programme.

Scope And Various Sub-Disciplines

It has been a century ago that Harashberger (1896) coined the term' ethnobotany'. Basically, ethnobotany deals with aboriginal man and his social, cultural and religious links with plants.

The interests of ethnobotanists include a wide range of subjects like indigenous healing herbal medicines, plants used in religious rituals, cultural activities and musical instruments, foods of plant origin, fossils, ancient trade routes, wild relatives of cultivated plants, new and emergent uses of plants as alternate sources of energy, renewable biomass energy, etc.,

Jain (Retired Director, Botanical Survey of India) now Emeritus Scientist, CSIR, who streamlined the subject and trained and prompted a number of students, and also produced a lot of works may easily be called the ***'Father of Indian Ethnobotany'***.

Vishnu Mitre also contributed a lot through his research on Paleoethnobotany. R.R. Rao, K.S. Manilal, J.K. Maheshwari, K.K. Singh, P.K. Kachroo, S.K. Berthakur, M.M. Bhandari, G.L. Shah, V.D. Vartak and Madhav Gadgil are some noted Indian workers.

Of course, there are many whose names have not been mentioned due to paucity of space, but they too contributed a lot to the branch of science through their excellent research.

Ethnobotany includes general economic botany of the plants used as food, medicine, dyes, fish poisons, arrow head poisons, house building, etc., It also includes social taboos

associated with some plants, for example some plants are considered as 'sacred' or believed to be a 'deity'. Some plants are used in religious ceremonies or an auspicious occasions.

Sources Of Data And Methods Of Study

The source of information about plants in the past and at present and their relationship with human beings are the major tools of study of Ethnobotany.

Sometimes, some fragmentary information from branches like archaeology, literature and field notes, fossils and excavations also form a basis of the study (Schultes, 1962; Jain, 1964).

The importance of ethnobotanical studies as mentioned by Harshberger (1896) is as follows:

- Ethnobotanical studies aid in elucidating the cultural position of the tribes that used the plants for food, shelter, medicine and clothing.
- They throw light upon past distribution of plants.
- They help us to decide as to what were the then existing ancient trade routes.
- Ethnobotany is useful to explore new lines of harnessing the potential of the plant kingdom in developing sustainable alternatives.
- Tribal medicine and participatory conservation strategy to protect and conserve the plant world for human survival in terms of alternate sources of user and his environment-friendly energy resources.

Biodiversity

In developing countries, plants are the main source of medicine. According to the World Health Organisation, as many as 80 percent of the world's people rely for their primary health care on traditional medicine, most types of which use remedies made from plants.

Biological diversity or biodiversity refers to the variety of life forms, the different plants, animals and micro-organisms, the genes they contain, and the ecosystem they form.

Biological diversity is usually considered at three different levels: Genetic Diversity, Species Diversity and Ecosystem Diversity.

- Genetic diversity refers to the variety of genetic information contained in all of the individual plants, animals and micro-organisms. It occurs within and between populations of species as well as between species.
- Species diversity refers to the variety of living species.
- Ecosystem diversity relates to the variety of habitats, biotic communities and ecological processes, as well as the tremendous diversity present within ecosystems in terms of habitat differences and the variety of ecological processes.

Biodiversity is one of the most important capital assets of the country. India encompasses 15 different agro-climatic zones, 10 vegetation zones, 25 biotic provinces and 426 biomes. It has been identified as one of the top 12 mega-diversity countries of the world.

The rich and varied biodiversity of India is the greatest strength and is the bedrock for bio-industrial development. The rich biodiversity of India is also matched with an equally rich cultural diversity and indigenous knowledge systems particularly in food and health care traditions.

Table 1

No	Life Forms in Medicinal Plants	Percentage
1	Herbs	28
2	Herbaceous Climbers	7
3	Lianas	3
4	Shrubs	21
5	Woody Climbers	5
6	Trees	36

Table 2

No	Parts Used Of Medicinal Plants	Percentage
1	Rhizomes	4
2	Roots	30
3	Stems	6
4	Bark	14
5	Wood	3
6	Whole Plant	3
7	Leaves	6
8	Flowers	10
9	Fruits	7
10	Seeds	4

At one time, nearly all medicines were derived from biological resources. Even today, they remain vital because as much as 70-75 per cent of modern medicine is derived from natural products.

In India, almost 95 per cent of the prescriptions are plant-based in the traditional systems of Ayurveda, Unani and Siddha. Many indigenous medicines also utilise animals and their parts/extracts as remedies for various diseases.

Aim of the present study

Ethnobotany deals with the studies among the ethnic group of people for recording their unique knowledge (traditional knowledge) regarding plant wealth.

Traditional knowledge refers to the knowledge, innovations and practices of indigenous and local communities around the world. Developed from experience gained over the centuries and adapted to the local culture and environment, traditional knowledge is transmitted orally from generation to generation.

The present report deals with medicinal use of about thirty plants/ plant parts gathered from two villages namely, Sorispuram and Kuranganthattu in Thoothukudi district. The elders in a family have their own valued and time-tested recipes passed down from one generation to another for treating a wide array of health conditions. Most of the medicines are prepared form commonly available herbs.

Due to rich plant wealth, the inhabitants of this village possess good knowledge about the uses of plants. Much of the knowledge is gradually vanishing because of rapid industrialization and modernization.

Therefore, it was felt that the information about plants should be recorded for proper scientific evaluation, before it is lost forever. The data have been collected either from people of above mentioned villages who are experienced and are actually prescribing these materials to cure different diseases.

Objectives

- Survey, Collection of medicinal plants in Two villages Sorispuram and Kuranganthattu in Thoothukudi District.
- Identification of Medicinal Plants used by the Local people.
- Collecting the relevant information through discussion with the people of two villages.
- Recording audio clips and gathering information about the medicinal plants.
- Enumeration of Medicinal Plants in the order of Botanical name, Vernacular name. Family name, Parts used mode of utilization and plant drug therapy etc.,

Methods and Materials

The plants were collected after regular trips around two villages. For survey, the medicinal plants are identified and collected from inhabitants of two above mentioned villages of Thoothukudi district.

Information regarding the medicinal plants was gathered by meeting village people of two villages. Relevant information regarding medicinal values, local names, audio clips and herbarium were documented.

The plants are identified by Gamble (1957), Santapau and Henry (1973), Henry et al. (1989) and Johan Jothi Prakash (2004).

Enumeration of Medicinal Plants

Based on the investigations of the plants used for remedial purposes against common ailments were documented. About 30 species have been enumerated in alphabetical order along with their vernacular names, families, botanical names, images, audio clips and plant drug therapy.

The enumeration of medicinal plants is as follows:

1. *Acalypha indica*

Botanical Name	*Acalypha indica*
Common Name	Kuppaimeni
Systematic Position:	
Class	Dicotyledons
Order	Malpighiales
Family	Euphorbicaeae
Genus	*Acalypha*
Species	*indica*

Plant drug therapy:

- ♣ Apply Kuppaimeni poultice on wounds and it reduces the inflammation so quickly along with healing the wound.
- ♣ Apply the paste of the plant as a poultice on wounds it helps reduce both the inflammation and pain.
- ♣ Kuppaimeni leaf juice or the decoction of the juice is taken to get rid of intestinal worms.
- ♣ It has both anti-bacterial and anti-fungal properties.
- ♣ We can use Kuppaimeni oil for treating many skin problems like acne, eczema, etc.,

2. *Allium Sativum*

Botanical Name	*Allium Sativum*
Common Name	Vellai Poondu
Systematic Position:	
Class	Monocotyledons
Order	Lilliales
Family	Lilliaceae
Genus	*Allium*
Species	*sativum*

Plant drug therapy:

- ♣ The part is made from log of fresh bulbs along with palm candy is taken to get relief from abdominal pain.
- ♣ Ten grams of the fresh bulbs is boiled with cow's milk and made into paste. The paste is taken orally to treat gastric problems, cough and ulcer.
- ♣ Three cloves of garlic boiled in mile, is used every night to prevent asthma.

3. Aloe Vera

Botanical Name	*Aloe vera*
Common Name	Kattralai
Systematic Position:	
Class	Monoctyledons
Order	Asparagales
Family	Asphodelaceae
Genus	*Aloe*
Species	*vera*

Plant drug therapy:

- ♣ One fresh leaf is taken per day for about 10 days after removing the epidermal peel to cure kidney stones.
- ♣ To reduce hair dandruff, people of this village use this plants fresh leaf.
- ♣ This village people also takes this leaf juice orally to relieve indigestion problems.

4. *Amaranthus dubius*

Botanical Name	*Amaranthus dubius*
Common Name	Araikeerai
Systematic Position:	
Class	Dicotyledons
Order	Caryophyllales
Family	Amaranthaceae
Genus	*Amaranthus*
Species	*dubius*

Plant drug therapy:

- ♣ To increase the blood level in the body, take amaranth with dal for about 20 days.
- ♣ Add 10 fenugreek seeds with spleen amaranth juice and apply it on your scalp, then take head bath after few minutes. It reduces the hair problems like dandruff, lice, grey hair and split ends.
- ♣ Add root of spleen amaranth, turmeric in the boiled water. Filter the ingredients and goggle the water alone for few seconds to make your mouth fresh and free from gum and tooth ache.

5. *Azadirachta indica*

Botanical Name	*Azadirachta indica*
Common Name	Vembu
Systematic Position:	
Class	Dicotyledons
Order	Sapindales
Family	Meliaceae
Genus	*Azadirachta*
Species	*indica*

Plant drug Therapy:

- ♣ Take 30-60gm of neem bark extract twice daily by mouth for 10 weeks helps heal stomach and intestinal ulcers.
- ♣ Applying extract of neem root or leaf to the skin helps repels black flies. Also applying neem oil to the skin to protect against some types of mosquitos.
- ♣ Fresh leaves, bark of neem, dried ginger, garlic, pepper, thippili, betal leaf, kayam and omam are boiled in water and the filtrate is given for relief from head ache, fever and insect bite.

6. *Calotropis gigantea*

Botanical Name	*Calotropis gigantea*
Common Name	Yerukku
Systematic Position:	
Class	Dicotyledons
Order	Gentianales
Family	Apocynaceae
Genus	*Calotropis*
Species	*gigantea*

Plant drug therapy:

- ♣ The leaves are used as a very effective remedy for vatha diseases.
- ♣ The leaf juice along with honey is given internally for intestinal worms.
- ♣ The leaf juice 2-3 drops with equal quantity of honey is given for frequently occurring fever or periodic fever.
- ♣ For poisonous snake bites, 2 to 4 leaves of this plant is chewed well by the patient to reduce the effect of poison.
- ♣ The leaves are dried well and powdered and externally applied for unhealing ulcers.

7. *Carica papaya*

Botanical Name	*Carica papaya*
Common Name	Pappali
Systematic Position:	
Class	Dicotyledons
Order	Violales
Family	Caricaceae
Genus	*Carica*
Species	*papaya*

Plant drug therapy:

- ♣ The unripen fruits are eaten to induce abortion.
- ♣ The latex is applied for skin diseases and ringworms.
- ♣ Mashed papaya is even good for healing skin wounds and has anti-bacterial action.
- ♣ Papaya also helps to lower the blood pressure.

8. *Cassia auriculata*

Botanical Name	*Cassia auriculata*
Common Name	Aavarai
Systematic Position:	
Class	Dicotyledons
Order	Fabales
Family	Fabaceae
Genus	*Cassia*
Species	*auriculata*

Plant drug therapy:

- ♣ Water soaked in flowers are helpful to manage peaking sugar levels. It also reduces thirst, burning sensation of body and removes bad odour of body.
- ♣ The decoction of roots are helpful in curing constipation, fever, diabetes, urinary infections etc.,
- ♣ Flowers can be ground well with turmeric and chick pea powder and used as bath powder to treat burning sensation of body and bad odour.

9. *Catharanthus roseus*

Botanical Name	*Catharanthus roseus*
Common Name	Nithiya Kalyani
Systematic Position:	
Class	Dicotyledons
Order	Gentianales
Family	Apocynaceae
Genus	*Catharanthus*
Species	*roseus*

Plant drug therapy:

- ♣ The leaves are taken along with turmeric and fine paste is made. This is applied on the wounds 2 to 3 times a day. It serves as a wound healer.
- ♣ White periwinkle leaves is pounded well and fresh juice is obtained. 2-3 ml of this juice is taken in the early morning or in late nights. This helps to control the blood pressure.
- ♣ Six to eight fresh leaves of the plant are boiled with 2 cup of water and reduced to half a cup. This is taken regularly for three consecutive menstrual cycles.
- ♣ Fresh juice or fine paste of the leaves if applied to the bite area of the insects and wasps. This reduces the irritation and swelling.

10. Citrus auranttifolia

Botanical Name	*Citrus auranttifolia*
Common Name	Yelumichai
Systematic Position:	
Class	Dicotyledons
Order	Sapindales
Family	Rutaceae
Genus	*Citrus*
Species	*limon*

Plant drug therapy:

- ♣ Lime juice contains healthy acids and helps remove dead cells when applied to the skin. It improves overall texture.
- ♣ Consume 2 glasses of warm water with lime juice a day to reap benefits in a few days to reduce the weight loss.
- ♣ Before take bath, the village people crash the cut fruit on their head to reduce the body temperatures.

11. Carcuma longa

Botanical Name	*Carcuma longa*
Common Name	Manjal
Systematic Position:	
Class	Monocotyledons
Order	Zingiberales
Family	Zingiberaceae
Genus	*Curcuma*
Species	*longa*

Plant drug therapy:

- ♣ If any pain or swell in the joints, turmeric and chunam can be mixed and smeared for gradual recovery.
- ♣ Some people will experience nose block. To clear this they can heat the turmeric and inhale the air that comes from it. While doing this, one should not drink water for 2 hours after done this.
- ♣ For itching skin, turmeric and neem leaves can be applied on the affected area and it cause relief from itching.
- ♣ Turmeric and gooseberry can be powdered in equal measure and the powder should be taken both in the morning and evening to do away with diabetic problem.

12. Cyanodon dactylon

Botanical Name	*Cyanodon dactylon*
Common Name	Arugampul
Systematic Position:	
Class	Monocotyledons
Order	Poales
Family	Poaceae
Genus	*Cyanodon*
Species	*dactylon*

Plant drug therapy:

- ♣ To get instant relief from acidity take 3 teaspoons of arugampul juice mixed in a glass of water on an empty stomach.
- ♣ The juice of this plant is given for urinary tract infection, dysentery and diabetes.
- ♣ Juice of fresh grass is applied to cuts and wounds.

13. Datura metal

Botanical Name	*Datura metal*
Common Name	Oomathai
Systematic Position:	
Class	Dicotyledons
Order	Solanales
Family	Solanaceae
Genus	*Datura*
Species	*metal*

Plant drug therapy:

- ♣ The paste of roasted leaves is applied over the area to relieve pain.
- ♣ The burning leaf smoke of datura is good to treat asthma and bronchitis.
- ♣ Put 3-4 leaves in boiling coconut oil. Filter the medicated oil and apply lukewarm oil on the scalp. It reduces the dandruff.
- ♣ Put the warm leaves on the belly for stomach ache.

14. Eclypta prostrata

Botanical Name	*Eclypta prostrata*
Common Name	Kareesilanganni
Systematic Position:	
Class	Dicotyledons
Order	Asterales
Family	Astraceae
Genus	*Eclypta*
Species	*prostrata*

Plant drug therapy:

- ♣ Plant is rubbed on the gums in toothache and the plant is rubbed with little oil and applied for relieving headache.
- ♣ Kariseelankanni oil can be used for treating various hair and skin problems.
- ♣ The whole plant juice in combination with turmeric taken for jaundice.

15.Hibiscus rosa-sinensis

Botanical Name	*Hibiscus rosa-sinensis*
Common Name	Semabaruthi
Systematic Position:	
Class	Dicotyledons
Order	Malvales
Family	Malvaceae
Genus	*Hibscus*
Species	*rosa-sinensis*

Plant drug therapy:

- ♣ Hibiscus can be used for the blackening and pre-mature greying of hair. It also promote hair growth.
- ♣ If we eat the buds of hibiscus flowers early in the morning on empty stomach, it should cure all the diseases.
- ♣ For increasing the blood level, add flowers in boiled water and blended in a mixer with roasted cumin seeds and salt and consumed after drinking it.

16.Mentha spicata

Botanical Name	*Mentha spicata*
Common Name	Pudhina
Systematic Position:	
Class	Dicotyledons
Order	Lamiales
Family	Lamiaceae
Genus	*Mentha*
Species	*spicata*

Plant drug therapy:

- ♣ Pudhina tea can be used to ease an upset stomach, calming the digestive tract and alleviating in digestion, gas and cramps.
- ♣ Pudhina juice is a perfect drink to quench our thirst in summer.
- ♣ Pudhina can be used for headache.

17.Moringa oleifer

Botanical Name	*Moringa oleifera*
Common Name	Murungai
Systematic Position:	
Class	Dicotyledons
Order	Capparales
Family	Moringaceae
Genus	*Moringa*
Species	*oleifera*

Plant drug therapy:

- ♣ Freshly extracted juice of root is used to relive ear pain by pouring into the ears and also into the hollow of the tooth in the case of dental carries.
- ♣ Fifteen to twenty five of fresh leaves is made into paste along with 2gm of black pepper and 5gm of garlic bulbs. This paste is taken in the early morning hours in empty stomach once in a day for a period of 3-4 days to treat jaundice.

18.Murraya koenigii

Botanical Name	*Murraya koenigii*
Common Name	Curryvepilai
Systematic Position:	
Class	Dicotyledons
Order	Sapindales
Family	Rutaceae
Genus	*Murraya*
Species	*koenigii*

Plant drug therapy:

- ♣ Add curry leaves, fenugreek with coconut oil and heat till it turns into the green colour. Then use this oil regularly for hair growth and prevent hair loss.
- ♣ It also shines the hair and improves the color of the hair.
- ♣ Chewing curry leaves or drinking a cup of curry leaves tea every day can prevent weight gain and reduce body cholesterol.

19. Ocimum sanctum

Botanical Name	*Ocimum sanctum*
Common Name	Tulsi
Systematic Position:	
Class	Dicotyledons
Order	Lamiales
Family	Lamiaceae
Genus	*Ocimum*
Species	*sanctum*

Plant drug therapy:

- ♣ Tulsi tea helps boost your digestive system which is important for losing weight quickly.
- ♣ Tulsi mixed with eggs and mixed can help in tightening skin pores.
- ♣ Krishna tulsi helps to cure infections such as throat infections, respiratory problems, ear aches and skin diseases.
- ♣ Tulsi leaves the best aid to stop smoking.

20. Phyllanthus amarus

Botanical Name	*Phyllanthus amarus*
Common Name	Kilanelli
Systematic Position:	
Class	Dicotyledons
Order	Euphorbiales
Family	Phyllanthaceae
Genus	*Phyllanthus*
Species	*amarus*

Plant drug therapy:

- ♣ Two grams of the fresh and cleaned aerial part is made into juice with 25ml of water. The filter juice is taken orally as such or along with 100ml of cow's milk twice a day for about seven to ten days to treat jaundice.
- ♣ The paste prepared from 10gm of fresh entire plant is taken orally once in a day to treat diabetes.
- ♣ Mix two teaspoon of gingelly oil, roots of kilanelli, cumin seeds and cow milk, then grind them together and drink the filtrate. This will relive headache.

21.Phyllanthus emblica

Botanical Name	*Phyllanthus emblica*
Common Name	Nellikai
Systematic Position:	
Class	Dicotyledons
Order	Euphorbiates
Family	Euphorbiaceae
Genus	*Phyllanthus*
Species	*emblica*

Plant drug therapy:

- ♣ Amla powder mixed with 2 teaspoons of honey. It provides relief from cough and cold when consumed around 3-4 times a day.
- ♣ Amla powder mixed with a tablespoon of honey or jaggery can act as a great natural blood purifier and can increase your blood level when taken regularly.
- ♣ To get relief from ulcers, dilute amla juice in half a cup of water and goggle with it. It helps in improving digestion and may increase immunity level.

22.Piper betle

Botanical Name	*Piper betle*
Common Name	Vetrilai
Systematic Position:	
Class	Dicotyledons
Order	Piperales
Family	Piperaceae
Genus	*Piper*
Species	*betle*

Plant drug therapy:

- ♣ Betal leaves, when applied over a wound and bandaged, can heal a wound and accelerate the healing process.
- ♣ Leaf soaked in mustard oil, warmed and applied to the chest to relieve cough and difficulty in breathing, applied to treat rheumatisms and orchitis.
- ♣ Leaf juice for cure the in digestion of children, its petiole is crushed and its essence is given.
- ♣ Leaf juice is mixed with milk, sweetened and taken for easing urination.

23. Punica granatum

Botanical Name	*Punica granatum*
Common Name	Madhulai
Systematic Position:	
Class	Dicotyledons
Order	Myrtales
Family	Lythraceae
Genus	*Punica*
Species	*granatum*

Plant drug therapy:

- ♣ Pomegranate juice can reduce inflammation in the gut and improve digestion.
- ♣ The peel collected form the fruit is dried and then this dried peel is grind into powder. Everyday morning and evening one teaspoon of this powder mixed with boiled milk to cure diarrhoea and dysentery.

24.Ricinus communis

Botanical Name	*Ricinus communis*
Common Name	Aamanakku
Systematic Position:	
Class	Dicotyledons
Order	Malpighiales
Family	Euphorbiaceae
Genus	*Ricinus*
Species	*communis*

Plant drug therapy:

- ♣ For treatment of Jaundice (initial stage), take 4-5 gm of leaves and grind them. Add this to boiling water and make decoction and drink twice a day.
- ♣ Massage of castor oil cures the cracked heels, rough skin and hyper pigmentation.
- ♣ For arthritis, take few castor leaves and heat them, put in cloth and tie on the affected area at night before sleeping and remove in morning.
- ♣ One teaspoon of oil obtained from the plant seed is taken as vermifuge and purgative.

25. Sesbania grandiflora

Botanical Name	*Sesbania grandiflora*
Common Name	Agathi
Systematic Position:	
Class	Dicotyledons
Order	Fabales
Family	Fabaceae
Genus	*Sesbania*
Species	*grandiflora*

Plant drug therapy:

- ♣ Leaf paste can be applied to treat oral and throat problems.
- ♣ The intake of the leaf of this medicinal plant can remove the intestinal worm.
- ♣ One to two drops of leaf juice is used as nasal drops to cure intermittent fever, sinusitis and headache.
- ♣ The flowers can be used as edible fries. This will cure giddiness, eye-sore, yellowish urination etc.,

26. Solanum nigrum

Botanical Name	*Solanum nigrum*
Common Name	Manathakkali
Systematic Position:	
Class	Dicotyledons
Order	Solanales
Family	Solanaceae
Genus	*Solanum*
Species	*nigrum*

Plant drug therapy:

- ♣ The cooked leaves of this plant can be consumed to get relief from cough.
- ♣ The decoction of this plant can be taken thrice daily for chicken pox and small pox.
- ♣ The juice of this plant is given as an anti-dote to opium poisoning and also to treat rat bit cases.
- ♣ The fresh juice of the leaf is slightly heated and used as ear drops to control earache.

27.Solanum surattense

Botanical Name	*Solanum surattense*
Common Name	Kandankatri
Systematic Position:	
Class	Dicotyledons
Order	Solanales
Family	Solanaceae
Genus	*Solanum*
Species	*surattense*

Plant drug therapy:

- ♣ Take ½ teaspoon of kandankatri powder. Mix it with water or honey and swallow it once or twice a day after taking light food. Continue this till you do not get relief from the symptoms of cough and cold.
- ♣ Cooked fruits are taken to get relief from dry cough.

28. Solanum trilobatum

Botanical Name	*Solanum trilobatum*
Common Name	Thoodhuvalai
Systematic Position:	
Class	Dicotyledons
Order	Solanales
Family	Solanaceae
Genus	*Solanum*
Species	*trilobatum*

Plant drug therapy:

- ♣ Few drops of fresh juice taken form the leaves are applied in the ear to get relief form ear pain.
- ♣ Thoothuvalai soup is very effective for treating chest congestion, cough, blocked nose and it is good even for people who suffer from sinus infections.
- ♣ Powder is mixed with pepper and honey and taken 3 times a day for a period of two days to get relief from cough.

29. Vitex negundo

Botanical Name	*Vitex negundo*
Common Name	Nochi
Systematic Position:	
Class	Dicotyledons
Order	Lamiales
Family	Lamiaceae
Genus	*Vitex*
Species	*negundo*

Plant drug therapy:

- ♣ Boil 10g of nochi leave powder and 1 teaspoon of black pepper in 200ml of water till it is concentrated to about 1/3. Take 50 ml of decoction twice a day. It improves immunity and highly effective in treating many infectious diseases like Dengue fever and relieves body pain.
- ♣ Nochi powder can be used to keep away mosquitoes as well as airborne diseases.
- ♣ Nochi leaf pillow is considered effective against headache and sinusitis.

30. Zingiber officinale

Botanical Name	*Zingiber officinale*
Common Name	Ingi
Systematic Position:	
Class	Monocotyledons
Order	Zingiberales
Family	Zingiberaceae
Genus	*Zingiber*
Species	*officinale*

Plant drug therapy:

- ♣ The juice prepared from the rhizome with lime is taken in a single dose for a period for seven days to treat headache and also arrest vomiting.
- ♣ The juice prepared from 50g of rhizome is taken morning and evening for a period of seven days to reduce blood pressure.
- ♣ Cut ginger into pieces and rub on scalp to prevent baldness and hair growth.
- ♣ To reduce cold, consume 4 teaspoon ginger juice with 4 teaspoon honey and 2 teaspoon lemon juice with water.
- ♣ In addition, ginger with honey cures cough.

Result and Discussion

The present investigation has brought to light some popular and frequently used prescriptions available for minor ailments such as boils, cuts, diarrhoea, headache, jaundice, skin infections, scabies, blood dysentery, killing intestinal worms, giddiness, abortion, toothache, cold, gastric problems, abdominal pain, inflamed wound, kidney stones, bone fraction, pitham, blood sugar, cholera etc.,

The plants enumerated in the text are easily available remedial material which give quick results.

Among thirty plants presently recorded, 25 plants belong to Dicotyledons and 5 plants belong to Monocotyledons. The family wise distribution of enumerated medicinal plants is given in table 3.

Table 3

S.No	Family	Total No. of Genera	Total No.of Species
Dicotyledons			
Polypetalae			
	Caricaceae	1	1
	Fabaceae	2	2
	Lythraceae	1	1
	Malvaceae	1	1
	Meliaceae	1	1
	Moringaceae	1	1
	Rutaceae	2	2
Gamopetalae			
	Apocynaceae	2	2
	Asteraceae	1	1
	Lamiaceae	3	3
	Solanaceae	4	4
Monochlamydeae			
	Amaranthaceae	1	1
	Euphorbiaceae	3	3
	Lilliaceae	1	1
	Piperaceae	1	1
	Phyllanthaceae	1	1
Monocotyledons			
	Asphodelaceae	1	1
	Poaceae	1	1
	Zingiberaceae	2	2

The village people are not running to doctors as and when they encounter any problems, they depend on the herbs available near their habitats.

During the survey, it has been observed that the village people still depend upon herbal plants around them for meeting their needs and possess good knowledge of the medicinal uses of such plants. These medicinal plants are grown by village people in their houses of their own purposes. Each house has several medicinal plants itself for pre-treatment purposes. They have been using these plants from the time immemorial. They have also been using these medicinal plants as a part of their daily food habits. It improves their health and build up natural immunity.

The pandemic disease, COVID-19 which is caused by Novel Coronavirus has been caused many fatalities around the world. In this period, Siddha doctors recommended "Kabasura kudineer" which has the capability to increase our immune power considerably.

Awareness campaigns have to be launched among local people about the medicinal plant usage, conservation and propagation. Nature is a great chemist, starting with water, carbon dioxide and mineral elements, nature has shown her synthetic skill of producing carbohydrates, proteins, fats, vitamins and innumerable secondary metabolites of natural products.

Plants produce these materials as they absorb energy from the sun and convert variety of substances that appear to be infinite. Recent reviews applying IUCN threat criteria found that a significant number of medicinal plant species are threatened or endangered within India. It is the time to save our plants and our planet to get healthy and happy life ever.

FLORA OF BOTANICAL GARDEN OF ST.MARY'S COLLEGE THOOTHUKUDI

A short field project work submitted to

St. Mary's College (Autonomous)

Affiliated to

MANONMANIAM SUNDARANAR UNIVERSITY

In partial fulfilment of the requirement for the Degree of Master of science in Botany

By

S.CHITRA - 20APBO02

DEPARTMENT OF BOTANY

ST.MARY'S COLLEGE (AUTONOMOUS)

THOOTHUKUDI-628001

CERTIFICATE

It is certified that this short term project work entitled "**FLORA OF BOTANICAL GARDEN OF ST.MARY'S COLLEGE THOOTHUKUDI**" submitted to St. Mary's college (Autonomous) affiliated to MANONMANIAM SUNDARANARUNIVERSITY in partial fulfilment of the requirements for the degree of Master of science in Botany, and is a record of work done in the Department of Botany, St.Mary's College (Autonomous), Thoothukudi during the year 2020 – 2021 by the following student.

By

S.CHITRA - 20APBO02

M. Glory
GUIDE

M. Glory
HEAD OF THE DEPARTMENT

M. Glory
EXAMINER

Lucia Rose
PRINCIPAL
St. Mary's College (Autonomous)
Thoothukudi - 628 001.

DECLARATION

Hereby declare that the short term project work entitled " **FLORA OF BOTANICAL GARDEN OF ST.MARY'S COLLEGE THOOTHUKUDI"** is the original work and it has not been submitted for the award of any Degree,Diploma,Felloship or any other similar title and that the short term project work represents independent and original work on the partial fulfillment of the requirements for the degree of **MASTER OF SCIENCE IN BOTANY** during 2020-2021,submitted to St.Mary's College (Autonomous), Thoothukudi-628 001.

S. Chitra

S. CHITRA(20APBO02)

Place: Thoothukudi-628 001

Date:

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CONTENTS

INTRODUCTION

Plants are pivotal part of our planet. Because of the existence of plants, earth is called a green planet. It plays a fundamental role in our life, to understand why we can imagine our life without oxygen which is provided by plants, in addition to the relation of plants to our "food", "medicines" and "furniture", so we can say plants was the basis of life. Plants are divided into smaller groups, according to shared attributes. Plants recognition is rather difficult because Plants are extremely complex. The recognition process of familiar plants was easy by experts, sometimes especially in medicine we need to identify prejudiced or toxic plants, botanists can do that facilely, but he must find a way to categorize the many different species when there are millions of various plant species which composed of similar parts(roots, stems, leaves, etc.).Designing plants recognition system is required in order to save time and decrease cost. Plant recognition or classification can be done based on parts of plant like:

- Leaves of Plant
- Flowers of Plant
- Fruits of Plant

Many research based on leaves in plant recognition, because the leaf was the most important part of plant carrying its characteristics compared with other plant parts, fruits and flowers are not available throughout the year, most plants are seasonal as well as shape, size and color of fruits and flowers are changing during growth. Many studies used leaves to identify plant category based on shape, texture information, venation, and color. The process of identifying leaf was done using various methods chemical methods, instrumental methods, another method was used named optical method which was more advantage than other techniques

Plants Recognition Approaches In this section we will review approaches for plants classification and recognition:

a) Approaches based on leaves

Most of studies based on leaf shape or color to extract features, there are some important leaf features such that:"aspect ratio", "narrow factor", "compactness", "centroid", "eccentricity", "dispersion", "area", "equivalent

diameter", "moments invariant", " etc. presented a system based on leaf image to identify plants, two techniques were used moments-invariant and centroid-radii. Proposed a hybrid approach based on "contrast stretching" and "adaptive thresholding" that at the same time adjusts the intensity level of plant leaf image. The classification approach proposed by K. Gurpreet and K. Gurpinder was based on leaf features: "isoperimetric quotient", "eccentricity", "aspect ratio", "leaf area", "leaf perimeter", "length of the major and minor axes", "solidity" and "upper and lower triangle area of the leaf". Tree identification system that presented by Itheriet detected boundary then described the detected boundary by using the directional fragment histogram, geometric features such that: "rectangularity", "convexity and solidity", "circularity", "Sphericity" and "ellipse variance" are extracted. It was noted that most of the research based on geometric features of leaves as in in which geometric features were extracted with hypersphere classifier to classify more than species of plants.

b) Approaches based on flowers or fruits

Some approaches recognize plants based on flowers, by focusing on the flower region as a whole or on parts of the flower. Tan proposed flower shape descriptors: "rea", "perimeter", "roundness", and "aspect ratio". Some studies extract features like color, texture, and shape. Warisara presented a system for classifying flower, RGB histogram was used as a feature, spices of input images were used which had good quality, for classification random forest algorithm was used. Diah presented a system for recognizing the orchid species based on image of flower, shape and color features were extracted, for segmentation maximal similarity based on region merging was used which was easy to use and more accurate than others, for classification support vector machine method was used. As we know fruit images may have similar color and shape, in plants was Identified by using morphological features of fruits(shape and size). Arivazhagan presented a system based on intensity, color, shape and texture, 15 different types of fruit

were used, minimum distance classifier was used, the good result of classification was obtained when used combined colour and texture features. Saurabh presented plant recognition system based on geometrical features (shape, size and orientation) by defining two classes: fruit and non-fruit, fifty images of fruit plants were collected, for classification support vector machine was used, artificial neural network, fuzzy set rules and image warping technique were used, the results were very satisfactory.

Moreover, some approaches based on two parts when recognize plants, Supapattranon P. and Siriwisesokul N. presented a recognition system could recognize plants by either using leaf or flower image,features like: "height ratio", "area ratio", "width and height ratio", "roundness value"," ripple feature", "half leaf of flower area ratio", "color feature", "boundary feature"were extracted from leaf or flower,they used 30 kinds of flowers and 30 kinds of leaves, euclidean distance was used for recognition.

Features Extraction and Representation Techniques Features extraction is an essential process in any recognition or classification system.Many techniques can be used to extract image attribute, divided into three types:

- Shape

Any object had a special shape, in other words had a contour, a good shape descriptor must beinvariant to scaling, rotation, translation, and reflection.Shape representation divided into two types: "boundary-based" and "region-based"[19,20]. Plants recognition system based on shape of leaf, flower and fruit to identify plant category. Shape features can be represented mathematically

In plants recognition was based on leaf boundary. Elliptic fourier and chain code were computed, the system used to identify plants spices. AbdurrasyidHasim used centroid contour distance to recognize the leaf (boundary-based approach), it calculates the distance between the midpoint and each boundary point, species of tropical plants were used, probabilistic neural network was used as a classifier, we observed that their approach assumed that the leaf shape is always symmetrical, so centroid contour distance was only done on one side of the leaf. Proposed a boundary-based shape descriptor named "multi-scale distance matrix" to extract the geometric features, the approach was invariant to rotation, translation, and scaling. The approach used Euclidean distances it was more efficient compared to other boundary-based approaches. Proposed a region-based shape

descriptor using "multi-scale local binary patterns" which provide a discriminative and robust leaf representation. Some methods cannot be classified as either boundary-based or region-based, it have been applied for plants identification methods for example the morphological shape descriptors which composed of "aspect ratio", "rectangularity measures", "circularity measures", and "the perimeter to area ratio", in "the morphological features" of the leaf's shape.

• Texture

There is no exceptional definition of texture, because it is extremely complex. We can recognize texture by our eyes, but we cannot be able to describe it. A simple definition of image texture is a surface attributes that can use to recognize objects. Texture was described by the number of "primitives" and the spatial layout of these "primitives". Texture is the most important technique used to quantify the patterns in images. It can describe the arrangement of the surface. Texture explains the spatial arrangement of "intensities" or "color" in an image. Mathematically three methods can be used for representation:

a) Structural: It provide effective description of image by using rules,it describes texture as a set ofarranged elements or texels. The extraction process of these texels is difficult.

b) Model based method. it is robust and requires less computation, It uses "fourier descriptor", "wavelet transform" and "gabor descriptor".

c) Statistical: it computes local features at each point in the image, it describes texture using "statistical proprieties" of the gray levels in image, the most common methods are: co-occurrence matrix and moment invariants.

d) In texture features were extracted used gray level co-occurrence matrix, the database was contain 390 images, for testing 65 new image were used, the results showed that the gray level co-occurrence matrix was sensitive for any changes forimages.

• Color

Color is using every day to distinguish among objects. Color plays anessential role for flower analysis than for leaf analysis. Methods that used for color representation were:"color moments" which was simple, "color histograms" which was robust to translation and rotation, "color coherence vector", and "color correlogram", two kinds of histogram can be used "global" and "local". In color histogram, edge histogram and area were used for medicinal plant species identification. as we mentioned, most studies based on leaf in plants identification, we found a specific leaf features namely, leaf venation which was used widelyin plants identification approaches.

• Venation

It means the pattern of veins in the leaf that can be classified as "arcuate", "cross-venulate" and "dichotomous", "longitudinal", "palmate", "parallel", "rotate", „pinnate", "reticulate",

Scope and Objectives

In establishing the Phylogenetic relationship that exists naturally b/w many groups of plants Using nomenclature principles and rules all plants are named. It has a great value in Forestry because all forest trees have been named and classified. It has wide importance in Agriculture, Horticulture, etc To study ecology, the knowledge of taxonomy / systematic botany became essential, plant ecologist must be aware of the names of plants and their relationship to habitat and environment.

Inventory of world's fauna

To provide a method for identification and communication

To produce a coherent and universal system of classification

To demonstrate the evolutionary implications of plant diversity

To provide single Latin "Scientific name" for every group of plants in the world, both living and extinct.

To arrange plants in such a way as to give us an idea about the sequence of their evolution from simpler, earlier and more primitive type to more complexes, more recent, more advanced type in different periods of history.

Systematic Position

Kingdom:	Plantae
Clade:	Tracheophytes
Clade:	Angiosperms
Clade:	Eudicots
Order:	Malpighiales
Family:	Calophyllaceae
Genus:	Calophyllum
Species:	C. inophyllum

1.Taxonomy and Nomenclature

Calophyllum inophyllum L. is a medium sized to large treebelongs to the family Clusiaceae. It is commonly called as Poon.In English, it is known as Alexandrian Laurel. The vernacularnames are Sultanachampa (Hindi), Nagachampa (Sanskrit),Punnai (Tamil), Pouna (Telugu), Punna (Malayalam) and Vuma(Kannada). The generic name comes from the Greek words 'kalos'-beautiful and'phullon'-leaf, meaning beautiful-leafed and thespecific epithet is derived from the Greek words 'in'-fibre and'phullon'-leofi, alluding to the pronounced veins on the undersideof the leaves (Allen, undated).

2.Distribution and Environmental Conditions

World distribution: The species is widely distributedthroughout the tropics. As a native species it is found in Aruba,Cambodia, Cook Islands, Fiji, French Polynesia, India, Indonesia,Japan, Kiribati, Laos, Madagascar, Malaysia, Marshall Islands,Myanmar, New Caledonia, Norfolk Island, Papua New Guinea,Philippines, Reunion, Samoa, Solomon Islands, Sri Lanka,Taiwan, Province of China, Thailand, Tonga, Vanuatu andVietnam. As an exotic, C. inophyllum occrtrs in Djibouti, Eritrea, Ethiopia, Kenya, Nigeria, Somalia, T anzania, Uganda and Unite d States of America (Allen, undated).Distribution in India: It is essentially a littoral tree of the tropics occurring above the high tide mark along the sea coasts of the Indian Peninsula and the Andaman and Nicobar Islands. Onthe West Coast, it is found from Mumbai southwards to Southern Kerala and along the East Coast, from Orissa southwards. It is a characteristic species of the Littoral forest where it occurs in association with Manilkara littoralis, Casuarina equisetifolia, Terminalia catappa, Heritiera littoralis, Pongamia pirtnata, Barringtonia asiatica and Erytltrina uariegata . inophyllum grows in areas with an annual rainfall ranging from about 750 to 5000 mm. The tree grows in a wide variety

of soils, from nearly pure coastal sands to clay, and is capable of growth on degraded and poorly drained sites. It can not withstand indefinite water logging. It is sensitive to frost and fire. Though the tree is a light demander planting in areas with light shade may improve success. It will not grow under dense forest canopies.

3. Botanical Descriptions

It is a medium-sized evergreen, ornamental, submaritime tree with a broad spreading crown of irregular branches. Height is B-20 m sometimes reaching upto 35 m and DBH is 0.5 1.5 m. This tree has stickS, latex clear or opaque and white, cream or vellow; bole usually twisted or leaning, without buttresses. Outer harl often with characteristic diamond to boat-shap ed fis sure s becoming confluent witlr age, smooth, often with :r yellowish or ochre tint, inner bark usually thich, soft, firm, fibror-rs and laminated, pink to red, darkening to brownish on exposure. Though the stem is reported to be short and often crooked, clear boles reaching upto 15 m and a girth of 7 m have been reported from Andaman. Its leaves are dark green and shining, 10 to 18 x 7.5 to 10 cm, broadly elliptic, rounded and often notched at the apex with wavy margins and very close lateral nerves giving a stliate appearance to the blade, base acute; petioies 1 to 1.6 cm long stout, flat. The inflorescence is axillary racemose or paniculate consisting of 4 to 15 flowers. Flower is 1.9 to 2.5 cm in diameter, pure white, fragrant. Sepals 4, ovate-orbicular, concavez reflexed, fringed with fine hairs; Petals 4, oblong, obtuse, spreading- The ball-shaped, light green fruits grow in clusters arc 2.5 to 5.0 cm in diameter. The skin, which turns yellow and then brown and wrinkled when the fruit is ripe, covers the thin pulp, the shell, a corky inner laer , and a single seed kernel.

4. Reproductive Biology and Breeding System

The flowering period is reported to vary depending on the area. In Tamil Nadu, the tree flowers during December-January and in Kerala, March-Apri}. In some areas two flowering seasons have been observed as in the cases of Orissa (May-June and october-November). In Andaman profuse flowering occurs during the rainy season and to a smaller extent at other times of the year. Fruiting season also vary. Tamil Nadu: March; Kerala (May-June), Orissa (July-August and December-January) and Andaman (June-August). The bisexual flowers are pollinated by insects such as bees. It has been suggested that apomixis may occur in calophyllurn, resulting in polyembryony. Hybridization may occur with C. inoph,yllum as one of the parents . The fruit is dispersed by sea currents and by fruit bats.

5. Genetics and Tree Irnprovement

Inophyllum has been identified as a priority species for genetic research by the south Pacific Regional Initiative on Forest Genetic Resources (SPRIG). Studies on distribution, genetic variation, selection and germplasm bank estabrishment can be taken up to intiate genetic improvement of the species.

6. Seed Collection, Processing and Nursery

Techniques seeds can be collected from trees by picking individual fruits or lopping off branches with pruning poles, but it is generally more practical to collect them after the fruits fall to the ground. Ripe fruits (skin is yellow or brown and wrinkled) may be soaked overnight to remove skin. Just prior to planting, it is best to crack shells or shell seeds entirely using a mallet, pliers, or hammer.

No additional treatments are required. Seed storage behaviour is recalcitrant the seeds are very oily, quickly losing their germinative power.

It is found to be desiccation and low temperature sensitive with high seed moisture during maturity. Seeds can be stored in sealed polythene bags within a temperature range of 10 to 20°C. The tree can usually be grown from seed without difficulty, provided the seed is sown soon after ripening. Complete removal of the seed shell is very effective in improving the germination to more than 90 per cent. Sowing seed directly into containers is the most efficient method. Small dibble tubes can be used when the seed is extracted from the shell or use of larger tubes (more than 6 cm diameter) or small pots or sowing in seedbeds followed by transplanting is recommended. Seedlings can be moved safely into full sunlight 1 to 2 months after germination. Seedlings should be hardened in full sunlight for 4 months before outplanting.

7. Silviculture, Plantations and Management

A report from Indonesia revealed that the spacing adopted is usually 2 x 3 m. It is regarded as a slow growing species. Fertilizer can boost its initial growth. While there are no specific fertilizers recommendations for C. inophyllum, seedlings have grown well with 50-170 g of a complete fertilizer of applied per seedling at planting and again after 6 months. Young, actively growing trees benefit from application of fertilizers containing 1-3 kg N per 100 m2 of canopy or planting bed area per year. Fertilizers containing N and K are best

applied in several small applications over the course of the year rather than all at once and best placed in holes dug around the trees. Alternately, or in addition to chemical fertilizers, well composted manures or other organic fertilizers can be added to the planting hole and spread around the base of the tree occasionally. Trees benefit from mulching, but deep mulch should be kept out of direct contact with the trunk . In India it is not used for plantation programme.

8. Agroforestry Practices

It is grown as part of the mixed garden agroforestry systems in many Pacific islands. In the Solomons, C. inophyllum has been traditionally retained or planted along with other trees such as breadfruit, sago palm, Terminalia, Burckella, Pometia, and Canarium in fallow yam and sweet potato fields.

9. Growth and Yield

It is generally described as slow growing. Young trees in Hawai may grow up to 1 m in height per year for the first few years, but after that the growth rate slows. In Malaysia one stand of trees attained a diameter of 50 cm at breast height in 70 years.

10. Important Insect-pests and Diseases

The tree does not have many pest problems. Thrips may attack new leaves, but the trees usually outgrow the infestation and no treatment is needed. Fungus rot may affect adult trees. A fungal p atho gen namely, Lep to gr ap hiu m calo phy lti (Ver ti cilliu m calophylli) causing vascular wilt disease was detected on C. inophyllum in the Seychelles. 11. Wood Properties and Utilization The wood can be used for furniture, beams, heavy packing boxes, tent poles, veneers and plywood. It is a well known material for boat and ship building; logs of long lengths are used as masts and spars. The wood is also a prized timber for carving and cabinet making. The timber is mostly used for constructional purposes (Suited for bridges, poles, ceiling boards, panelling, planking an drafters).

Sapwood and heartwood fairly sharply demarcated in the freshly felled timber, but usually less so on ageing, sapwood pale reddish-white to yellow, heartwood yellowish to reddish-brown with darker streaks on the longitudinal surface. It is a moderately heavy wood and weighs 6bb kgm"3 at 12% moisture content. Its specific gravity varies from 0.47 to 0.84.

The timber is fairly strong . Bending strength in the air-dry condition (about 12% moisture content) is high - comparable to teak. Maximum crushing strength, or compression strength parallel to grain, is high. Some familiar species with high crushing strength parallel to grain include hard maple, teak, and white oak. It is fairiy hard, resisting wear, denting, and marring fairly well. It can be worked to a smooth surface which takes a fine polish .

The timber seasons well but is liable to develop short surface cracks. Drying defects that may occur in this species include end- splits and warp. surface checking may also occur, but it is minimal.

12. Medicinal Uses

The latex or a decoction of the bark is sometimes used medicinally. Bark of the tree is an astringent. Decoction of the leaves was used to treat eye ailments over much of Polynesia and westward into Malaysia. Oil from the seed is used for cosmetic and topical applications for healing of burns and skin diseases. Reported that this species is an under explored source of anticancer drugs. It is also a source of anti- HIV compounds.

13. Other Relevant Information

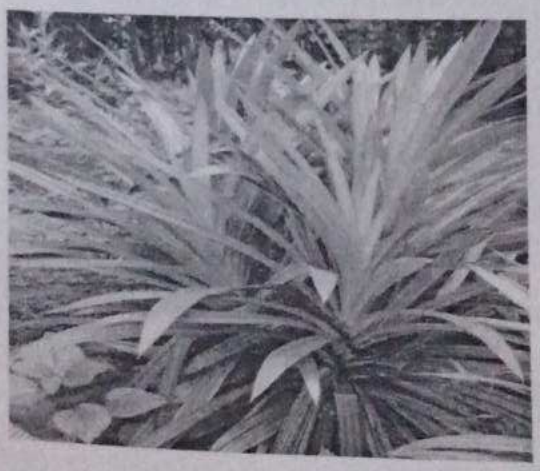
It is a high potential bio-diesel tree. Seed yields 65% oil from its dry weight of 37 44 kg/ha from 400 trees. It can be used in conventional diesel engines in its pure form or as a blend with mineral oil. The thick, dark green oil extracted from the seeds is used in a number of products, including oil for lighting, medicines, soaps and body and hair grease. The seed oil is also used as a wood finish. Oil cake can be used as manure Troup. Oil cake could" be used for the management of disease complex caused by Fusarium solani and Meloidogyrle incognita (root-knot nematode) on brinjal Mittal and Goswami. The mature fruit is burned for mosquito repellent. Latex from the cut bark has been made into a poison to kill rodents and stun fish. The bark of the tree contains tannin (around 72%).In ancient Hawai, a brownish-mauve dye was made from the fruit husks Friday and Okano. The tree is a favourite ornamental in the Pacific' A new prenylated pyranoxanthone, inophyllin A, was isolated from the roots of c. inophyllum and was found to be toxic to the larvae of Aedes aegypti mosquito, the results of which will go a long way in controlling the Dengue Fever. Several antimicrobial and cytotoxic agents have been isolated from the root bark and nuts of this species. Lesari and Yoswita reported that C. inophyllum ts a suitable species for best quality puip. It is an ethnobotanicaliy important species for the Jarawas tribes in the Andaman Group

of islands. The tree is regarded as sacred in some parts of the Pacific and is commonly featured in chants and other folklore of the region.

Systematic Position

Kingdom:	Plantae
Clade:	Tracheophytes
Clade:	Angiosperms
Clade:	Monocots
Order:	Pandanales
Family:	Pandanaceae
Genus:	Pandanus
Species:	P. amaryllifolius

Pandanus amarylifolius



Pandanus amaryllifolius is a true cultigen, and is believed to have been domesticated in ancient times. It is sterile and can only reproduce vegetatively through suckers or cuttings. It was first described from specimens from the Maluku Islands, and the rare presence of male flowers in these specimens may indicate that it is the origin of the species. However, as no other wild specimens have been found, this is still conjecture. The plant is grown widely throughout Southeast Asia. It has also been introduced to South Asia via Malaysia and Indonesia, where they are grown extensively, though South Asian populations have low genetic diversity.

Culinary

Pandan cake, a light, soft and fluffy chiffon cake uses pandan leaf as green colouring and flavouring agent.

In Malaysia, Indonesia and the Philippines, it is commonly called pandan or pandan wangi (fragrant pandan). The green juice acquired from its leaf is used extensively in Malaysian cuisine and Indonesian cuisine as green food colouring and flavouring agents that gave pleasant aroma for kue, a tapioca, flour or glutinous rice-based traditional cakes; including klepon, kue putu, dadar gulung, lapis legit, pandan cake, buko pandan salad, and buko pandan cake. The tied knot of bruised pandan leaf is also added into fragrant coconut rice to enhance the aroma.

In Sri Lanka, it is called rampé and it is grown almost in every household. Most of the Sri Lankan dishes use these leaves for aroma along with curry leaves. In India it is called annapurna leaves; in Bangladesh, it is called pulao pata and in the Maldives, it is called ran'baa along with the other variety of pandan there (Pandanus fascicularis), and is used to enhance the flavor of pulao, biryani, and sweet coconut rice pudding, or payesh if basmati rice is not used. It acts as a cheap substitute for basmati fragrance, as one can use normal, nonfragrant rice and with pandan the dish tastes and smells like basmati is used. The leaves are used either fresh or dried, and are commercially available in frozen form in Asian grocery stores of nations where the plant does not grow. They have a nutty, botanical fragrance that is used as a flavor enhancer in many Asian cuisines, especially in rice dishes, desserts, and cakes

The leaves are sometimes steeped in coconut milk, which is then added to the dish. They may be tied in a bunch and cooked with the food. They may be woven into a basket which is used as a pot for cooking rice. Pandan chicken, , is a dish of chicken parts wrapped in pandan leaves and fried. The leaves are also used as a flavoring for desserts such as pandan cake and sweet beverages. Filipino cuisine uses pandan as a flavoring in some coconut milk-based dishes as well as desserts like buko pandan. It is also used widely in rice-based pastries such as suman and numerous sweet drinks and desserts.

Bottled pandan extract is available in shops, and often contains green food coloring.

Systematic Position

Kingdom:	Plantae
Clade:	Tracheophytes
Clade:	Angiosperms
Clade:	Monocots
Order:	Asparagales
Family:	Asphodelaceae
Subfamily:	Asphodeloideae
Genus:	Aloe
Species:	A. vera

Aloe vera

Distribution

A. vera is considered to be native only to the south-east Arabian Peninsula in the Al-Hajar mountains in north-eastern Oman. However, it has been widely cultivated around the world, and has become naturalized in North Africa, as well as Sudan and neighboring countries, along with the Canary Islands, Cape Verde, and Madeira Islands. It has also naturalized in the Algarve region of Portugal, and in wild areas across southern Spain, especially in the region of Murcia.

The species was introduced to China and various parts of southern Europe in the 17th century. It is widely naturalized elsewhere, occurring in arid, temperate, and tropical regions of temperate continents. The current distribution may be the result of cultivation.

Cultivation

As an ornamental plant

Aloe vera has been widely grown as an ornamental plant. The species is popular with modern gardeners as a putatively medicinal plant and for its interesting flowers, form, and succulence. This succulence enables the species to survive in areas of low natural rainfall, making it ideal for rockeries and other low water-use gardens. The species is hardy in zones 8–11, and is intolerant of heavy frost and snow. The species is relatively resistant to most insect pests, though spider mites, mealy bugs, scale insects, and aphid species may cause a decline in plant health. This plant has gained the Royal Horticultural Society's Award of Garden Merit.

In pots, the species requires well-drained, sandy potting soil and bright, sunny conditions. Aloe plants can burn under too much sun or shrivel when the pot does not drain water. The use of a good-quality commercial propagation mix or packaged "cacti and succulent mix" is recommended, as they allow good drainage. Terra cotta pots are preferable as they are porous. Potted plants should be allowed to completely dry before rewatering. When potted, aloes can become crowded with "pups" growing from the sides of the "mother plant". Plants that have become crowded should be divided and repotted to allow room for further growth and help prevent pest infestations. During winter, Aloe vera may become dormant, during which little moisture is required. In areas that receive frost or snow, the species is best kept indoors or in heated glasshouses.

There is large-scale agricultural production of Aloe vera in Australia, Cuba, the Dominican Republic, China, Mexico, India, Jamaica, Spain, where it grows even well inland, Kenya, Tanzania, and South Africa, along with the USA to supply the cosmetics industry.

Description

Aloe vera is a stemless or very short-stemmed plant growing to 60–100 centimetres (24–39 inches) tall, spreading by offsets. The leaves are thick and fleshy, green to grey-green, with some varieties showing white flecks on their upper and lower stem surfaces. The margin of the leaf is serrated and has small white teeth. The flowers are produced in summer on a spike up to 90 cm (35 in) tall, each flower being pendulous, with a yellow tubular corolla 2–3 cm (3/4–1 1/4 in) long. Like other Aloe species, Aloe vera forms arbuscular mycorrhiza, a symbiosis that allows the plant better access to mineral nutrients in soil.

Aloe vera leaves contain phytochemicals under study for possible bioactivity, such as acetylated mannans, polymannans, anthraquinone C-glycosides, anthrones, and other anthraquinones, such as emodin and various lectins.

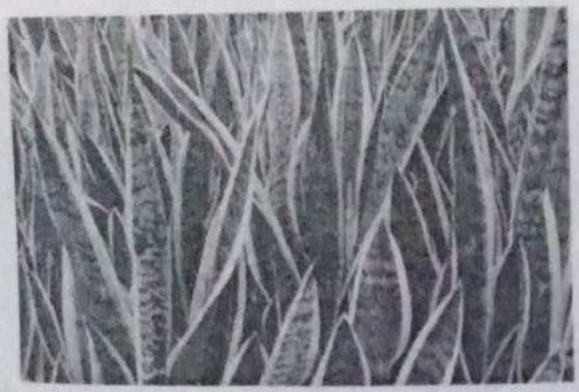
Uses

Two substances from Aloe vera – a clear gel and its yellow latex – are used to manufacture commercial products. Aloe gel typically is used to make topical medications for skin conditions, such as burns, wounds, frostbite, rashes, psoriasis, cold sores, or dry skin. Aloe latex is used individually or manufactured as a product with other ingredients to be ingested for relief of constipation. Aloe latex may be obtained in a dried form called resin or as "aloe dried juice".

Systematic Position

Kingdom:	Plantae
Clade:	Tracheophytes
Clade:	Angiosperms
Clade:	Monocots
Order:	Asparagales
Family:	Asparagaceae
Subfamily:	Nolinoideae
Genus:	Dracaena
Species:	S. trifasciata

Sansevieria trifaciata



Description

It is an evergreen perennial plant forming dense stands, spreading by way of its creeping rhizome, which is sometimes above ground, sometimes underground. Its stiff leaves grow vertically from a basal rosette. Mature leaves are dark green with light gray-green cross-banding and usually range from 70–90 centimetres long and 5–6 centimetre wide, though it can reach heights above 2 m in optimal conditions.The specific epithet trifaciata means "three bundles.

The plant exchanges oxygen and carbon dioxide using the crassulacean acid metabolism process, which allows them to withstand drought. The microscopic pores on the plant's leaves, called the stomata and used to exchange gases, are only opened at night to prevent water from escaping via evaporation in the hot sun. As a result, stored oxygen is released at the opening of the stomata at night, unlike most plants which continuously exchange gases during the day.

Systematic Position

Kingdom:	Plantae
Clade:	Tracheophytes
Clade:	Angiosperms
Clade:	Monocots
Order:	Alismatales
Family:	Araceae
Subfamily:	Aroideae
Tribe:	Aglaonemateae
Genus:	Aglaonema Schott

Aglaonema commutatum schott

Description

These are evergreen perennials with stems growing erect or decumbent and creeping. Stems that grow along the ground may root at the nodes. There is generally a crown of wide leaf blades which in wild species are often variegated with silver and green coloration. The inflorescence bears unisexual flowers in a spadix, with a short zone of female flowers near the base and a wider zone of male flowers nearer the tip. The fruit is a fleshy berry that ripens red. The fruit is a thin layer covering one large seed.Plants of the genus are native to humid ,shady tropical forest habitat.

Cultivation and uses

Aglaonema costatum

Aglaonema have been grown as luck-bringing ornamental plants in Asia for centuries. They were introduced to the West in 1885, when they were first brought to the Royal Botanic Gardens, Kew. They have been cultivated, hybridized, and bred into a wide array of cultivars. They live in low-light conditions and are popular houseplants.

This mainly tropical genus is known for its intolerance of cold temperatures. Chilling injury can begin at 15 °C (59 °F). The injury manifests in dark, greasy-looking patches on the foliage.

Cultivars have been selected for their shape and size, and especially for the color and pattern of the leaves. Many have white or cream-colored stems. Some have also been developed to tolerate colder temperatures. The most common cultivar is 'Silver Queen', which has gained the Royal Horticultural Society's Award of Garden Merit.

Most propagation of Aglaonema is done with cuttings and by dividing the basal shoots. Care of the houseplant involves protecting it from cold temperatures and excessive sunlight and removing any inflorescences that develop, which can prolong the life of the plant. It requires moist soil, and while some cultivars require a small amount of fertilizer, plants are easily injured when oversupplemented. Aglaonema are prone to false mites (Brevipalpus californicus). They may also acquire populations of nematodes, such as root-knot nematodes and Pratylenchus species, which cause root lesions.[8] Pathogens include the fungus Myrothecium roridum and bacteria such as Pseudomonas cichorii, Erwinia chrysanthemi, and Xanthomonas campestris, which can all cause leaf spot. Colletotrichum fungi can cause anthracnose.

The NASA Clean Air Study determined that the species modestum of this plant genus was effective at removing common household air toxins formaldehyde and benzene.

Aglaonema plants are poisonous due to calcium oxalate crystals. If ingested they cause irritation of the mucous membranes, and the juice can cause skin irritation and painful rash.

Systematic Position

Kingdom:	Plantae
Clade:	Tracheophytes
Clade:	Angiosperms
Clade:	Monocots
Order:	Alismatales
Family:	Araceae
Genus:	Dieffenbachia
Species:	D. seguine

Dieffenbachia seguine

Description

The herbaceous perennial grows 3 feet (0.91 m) to 10 feet (3.0 m) in height and 2 feet to 3 feet in width. The plant's leaves are large and green, and often with variegated white patterns. Like other Dieffenbachias, the sap is toxic. It has showy white flowers.

Cultivation

Dieffenbachia seguine is cultivated as an ornamental plant in temperate shade gardens and as a potted house plant. Cultivars emphasize different patterns of variegation

Systematic position

Kingdom:	Plantae
(unranked):	Angiosperms
(unranked):	Eudicots
(unranked):	Asterids
Order:	Gentianales
Family:	Rubiaceae
Genus:	Hamelia
Species:	H. patens

Hamelia patens

Description

Hamelia patns is a large perennial shrub or small tree in the coffee family, Rubiaceae, that is native to the American subtropics and tropics. Its range extends from Florida in the southern United States to as far south as Argentina. Common names include firebush, hummingbird bush, scarlet bush, and redhead. In Belize, this plant's Mayan name is Ix Canaan and is also known as "Guardian of the Forest.

Uses

Hummingbirds are attracted by its flowers and other birds feed on the fruit, both of which will also forage on small insects found in the vicinity, helping to keep down pests. These flowers are also fed on by butterflies, such as the statira sulphur (Aphrissa statira), which are attracted to red flowering plants. The fruits have a refreshing, acidic taste and are also edible by humans; in Mexico, they are made into a fermented drink.

Systematic position

Kingdom:	Plantae
Clade:	Tracheophytes
Clade:	Angiosperms
Clade:	Eudicots
Clade:	Asterids
Order:	Solanales
Family:	Solanaceae
Genus:	*Solanum*
Species:	*S. trilobatum*

Solanum trilobatum

Description

Solanum trilobatum is an herb that can be consumed by mildly frying it in oil or ghee and then grinding it.

The plant is full of thorns, including the leaves. It is important to remove these thorns before cooking as the thorns are considered to be mildly toxic. The herb can be stored in powdered form by drying the leaves under shade and making a powder out of it. It is used to treat fever and common cold. Its native range is India, Sri Lanka and Indochina. Solanum trilobatum is a perennial herb that grows up to a height of 2-3 meters and is fully covered with thorns. One can see thorns even underneath the leaves and sometimes on the stalks of the fruit. The plant produces attractive purple-violet colored flowers. Leaves are triangular and are irregularly lobedSolanum trilobatum Linn (Family: Solanaceae), a thorny creeper with bluish white flower and grows as a climbing under shrub. It is one of the important medicinal plant, more commonly available in Southern India and has been used in herbal medicine to treat various diseases like respiratory problems, bronchial asthma and tuberculosis [4It has been used in Siddha medicine for various home remedies. In India, it's also used to make delicious dish. To get any health benefits of Solanum trilobatum, its thorn has to be cleaned first because it is believed to be mildly toxicSqueeze out the juice of Solanum Trilobatum (Alarka) Leaves. Take one tablespoon twice a day. Take 2 ripe Solanum Trilobatum (Alarka) Fruit. Soak in Honey (Shehad) for 2 hours. Have it once a day. Take one teaspoon juice of Solanum Trilobatum (Alarka) Leaves thrice a day

Systematic position

Kingdom:	Plantae
Clade:	Tracheophytes
Clade:	Angiosperms
Clade:	Eudicots
Clade:	Rosids
Order:	Malpighiales
Family:	Euphorbiaceae
Genus:	*Codiaeum*
Species:	*C. variegatum*

Codiaeum variegatum

Codiaeum variegatum (fire croton, garden croton, or variegated croton; syn. *Croton variegatum* L.) is a species of plant in the genus *Codiaeum*, which is a member of the family Euphorbiaceae. It was described by Carl Linnaeus in 1753. It is native to Indonesia, Malaysia, Australia, and the western Pacific Ocean islands, growing in open forests and scrub.

The garden crotons should not be confused with *Croton*, a cosmopolitan genus also in the Euphorbiaceae, containing more than 700 species of herbs, shrubs and trees.

Description

It is a tropical, evergreen, monoecious shrub growing to 3 m (9.8 ft) tall and has large, thick, leathery, shiny evergreen leaves, alternately arranged, 5–30 cm (2.0–11.8 in) long and 0.5–8 cm (0.20–3.15 in) broad. The leaf blades can, for example, be ruler-lanceolate, oblong, elliptic, lanceolate, ovate inverted, ovate spatulate, or violin-shaped and coloured green, yellow, or purple in various patterns, depending on the variety. The petiole has a length of 0.2 to 2.5 cm. The inflorescences are long racemes, 8–30 cm (3.1–11.8 in) long, with male and female flowers on separate inflorescences; the male flowers are white with five small petals and 20–30 stamens, pollens are oval approximately 52x32 microns in size. The female flowers yellowish, with no petals. The flowering period is usually in early autumn. The fruit is a capsule 9 mm (0.35 in) in diameter, containing three seeds that are 6 mm (0.24 in) in diameter. When cut, stems bleed a milky sap like many of the Euphorbiaceae.

Cultivation

.In tropical climates, crotons make attractive hedges and potted patio specimens, valued for their striking foliage. They only survive outdoors where temperatures do not normally drop below 10° to 13 °C in winter; colder temperatures can cause leaf loss . In colder climates, the plants are grown in greenhouses or as house plants. The cultivated garden crotons are usually smaller than the wild plant, rarely over 1.8 m tall, and come in a wide diversity of leaf shapes and colours. They are sometimes grouped under the name *Codiaeum variegatum* var. *pictum* (Lodd.) Müll. Arg., though this is not botanically distinct from the species and usually treated as a synonym of it.

Cultivars

The several hundred cultivars are selected and bred for their foliage. Depending on the cultivar, the leaves may be ovate to linear, entire to deeply lobed or crinkled, and variegated with green, white, purple, orange, yellow, red, or pink. The colour patterns may follow the veins or the margins, or be in blotches on the leaf. Popular cultivars include 'Spirale', which has spirally twisted red and green leaves; 'Andreanum', which has broadly oval yellow leaves with gold veins and margins; 'Majesticum', which has pendulous branches, with linear leaves up to 25 cm long with midrib veins yellow maturing to red; and 'Aureo-maculatum', which has leaves spotted with yellow.

Toxicity

As with many of the Euphorbiaceae, the sap can cause eczema in some people. The bark, roots, latex, and leaves are poisonous. The toxin is the chemical compound 5-deoxyingenol. The plant contains an oil which is violently purgative and is suspected of being a carcinogen. Consumption of the seeds can be fatal to children.

Systematic position

Kingdom:	Plantae
Clade:	Tracheophytes
Clade:	Angiosperms
Clade:	Monocots
Order:	Asparagales
Family:	Asparagaceae
Subfamily:	Nolinoideae
Genus:	*Ophiopogon*
Species:	*O. planiscapus*

Ophiopogon planiscapus nakai

Description

Ophiopogon planiscapus, black mondo grass, is a species of flowering plant in the family Asparagaceae. It is a small evergreen perennial growing to 20 cm tall by 30 cm (12 in)wide. It grows from short rhizomes, and bears tufts of grasslike leaves, from which purple or white flowers emerge in racemes held on short stems above the leaves. It is native to Japan, where it grows on open and forested slopes.

Garden use

The cultivar 'Nigrescens' (black mondo, black mondo grass or black lilyturf) is grown as groundcover. Its leaves turn from green to dark purple (black) and can grow to 8 in tall and 1/4 inch wide. The flowers are white to pale lilac. This plant is commonly used in rock gardens or raised beds as an ornamental plant; owing to its dwarf qualities it can be lost in borders. Under the cultivar name 'Kokuryu' it has gained the Royal Horticultural Society's Award of Garden Merit.

There are also two variegated forms called 'Little Tabby' and 'Silver Ribbon'. These are green with white borders around the leaves.

Propagation

The plants spread by underground stolons with thick fleshy roots making fair sized colonies which can be separated by division in the spring.

Systematic position

Kingdom:	Plantae
Clade:	Tracheophytes
Clade:	Angiosperms
Clade:	Eudicots
Clade:	Asterids
Order:	Lamiales
Family:	Lamiaceae
Genus:	*Ocimum*
Species:	*O. tenuiflorum*

Ocimum tenuiflorum

Ocimum tenuiflorum (synonym *Ocimum sanctum*), commonly known as holy basil or *tulsi*, is an aromatic perennial plant in the family Lamiaceae. It is native to the Indian subcontinent and widespread as a cultivated plant throughout the Southeast Asian tropics.

Tulsi is cultivated for religious and traditional medicine purposes, and also for its essential oil. It is widely used as a herbal tea, commonly used in Ayurveda, and has a place within the Vaishnava tradition of Hinduism, in which devotees perform worship involving holy basil plants or leaves.

The variety of *Ocimum tenuiflorum* used in Thai cuisine is referred to as Thai holy basil (it is not the same as Thai basil, which is a variety of *Ocimum basilicum*.

Morphology

Holy basil is an erect, many-branched subshrub, 30–60 cm (12–24 in) tall with hairy stems. Leaves are green or purple; they are simple, petioled, with an ovate blade up to 5 cm (2 in) long, which usually has a slightly toothed margin; they are strongly scented and have a decussate phyllotaxy. The purplish flowers are placed in close whorls on elongated racemes.

The three main morphotypes cultivated in India and Nepal are *Ram tulsi* (the most common type, with broad bright green leaves that are slightly sweet), the less common purplish green-leaved (Krishna *tulsi*) and the common wild *vana tulsi*.

Origin and distribution

DNA barcodes of various biogeographical isolates of tulsi from the Indian subcontinent are now available. In a large-scale phylogeographical study of this species conducted using chloroplast genome sequences, a group of researchers from Central University of Punjab, Bathinda, have found that this plant originates from North-Central India.

This basil has now escaped from cultivation and has naturalised into a cosmopolitan distribution.

Chemical composition

Some of the phytochemical constituents of *tulsi* are oleanolic acid, ursolic acid, rosmarinic acid, eugenol, carvacrol, linalool, and β-caryophyllene (about 8%).

Tulsi essential oil consists mostly of eugenol (~70%), β-elemene (~11.0%), β-caryophyllene (~8%), and germacrene (~2%), with the balance being made up of various trace compounds, mostly terpenes.

Genome sequence

The genome of the tulsi plant has been sequenced and reported as a draft, estimated to be 612 mega bases, with results showing genes for biosynthesis of anthocyanins in *Shyama Tulsi*, ursolic acid and eugenol in *Rama Tulsi*.

Uses

Tulsi (Sanskrit-Surasa) has been used in Ayurveda and Siddha practices for its supposed treatment of diseases. For centuries, the dried leaves have been mixed with stored grains to repel insects.

Systematic position

Kingdom:	Plantae
Clade:	Tracheophytes
Clade:	Angiosperms
Clade:	Eudicots
Clade:	Asterids
Order:	Boraginales
Family:	Boraginaceae
Genus:	Cordia
Species:	C. sebestena

Cordia sebestena

Cordia sebestena is a shrubby tree in the borage family, Boraginaceae, native to the American tropics. It ranges from southern Florida in the United States and the Bahamas, southwards throughout Central America and the Greater Antilles. Common names have included siricote or kopté (Mayan) in 19th Century northern Yucatán,scarlet cordia in Jamaica, and Geiger tree in Florida.

Description

Cordia sebestena grows to a maximum height of 25–30 feet at maturity, with a nearly equal spread. The crown is round to vase-shaped. Branches tend to be somewhat drooping, and the tree is naturally multitrunked. When only a single trunk is allowed to develop, it can attain a diameter of 12 inches.

The dense, evergreen foliage consists of dark green, leathery, alternate, ovate leaves, seven inches long, with wavy margins. These leaves are covered with small hairs, lending them a rough, "sandpapery" texture.

Flowers are produced in clusters at branch ends throughout the year, particularly in the spring and summer. Flowers are two inches wide, red-orange in color, tubular, flaring (salverform) with 5-7 lobes, bearing 5-7 stamens of similar height. The species is heterostylous and presumably self-incompatible. Pear-shaped fruits follow the flowers, averaging two inches in length. Fruits are fragrant and edible, but not flavorful.

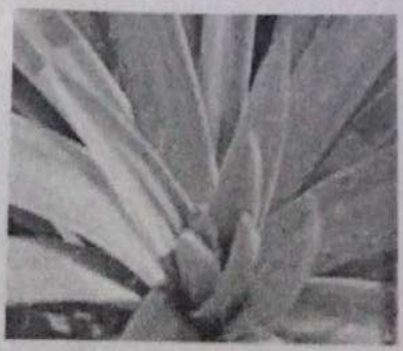
Cultivation

Cordia sebestena is widely planted throughout the tropics as an ornamental plant in gardens for its showy flowers. It is a slow-growing plant, and sheds enough leaves and fruit to require some upkeep. The wood is rather light in density, but branches are not prone to breakage. The tree should be pruned in its youth to establish a structure, as it is prone to low branching. Soil tolerance is fairly broad, provided the soil drains well. *Cordia sebestena* tolerates drought, but not frost. It is not particularly susceptible to pests and diseases, other than the geiger tortoise beetle, which can cause occasional defoliation. This plant tolerates salt spray found near the ocean. It enjoys full sun, and can grow in a part-day sun situation as well. Its uses include: street tree, shade tree, even as a container subject in its youth.

Systematic position

Kingdom:	Plantae
Clade:	Tracheophytes
Clade:	Angiosperms
Clade:	Monocots
Order:	Asparagales
Family:	Asparagaceae
Subfamily:	Agavoideae
Genus:	*Yucca*
Species:	*Y. gloriosa*

Yucca gloriosa



Yucca gloriosa is a species of flowering plant in the family Asparagaceae, native to the southeastern United States. Growing to 2.5 m (8 ft), it is an evergreen shrub. It is widely cultivated as an ornamental for its architectural qualities, and has reportedly become established in warmer climates in the wild in various parts of the world.

Description

Yucca gloriosa is caulescent, usually with several stems arising from the base, the base thickening in adult specimens. The long narrow leaves are straight and very stiff, growing to 30–50 cm long and 2–3.5 cm wide. They are dark green with entire margins, smooth, rarely finely denticulate, acuminate, with a sharp brown terminal spine. The inflorescence is a panicle up to 2.5 long, of bell-shaped white flowers.

Habitat

Yucca gloriosa grows on exposed sand dunes along the coast and barrier islands of the subtropical southeastern USA, often together with *Yucca aloifolia* and a variety formerly called *Yucca recurvifolia* or *Y. gloriosa* var. *recurvifolia*, now *Y. gloriosa* var. *tristis*. In contrast to *Y. gloriosa* var. *tristis*, the leaves of *Y. gloriosa* var. *gloriosa* are hard stiff, erect and narrower. On the other hand, *Y. aloifolia* has leaves with denticulate margins and a sharp-pointed, terminal spine.

Distribution

Yucca gloriosa is native to the coast and barrier islands of southeastern North America, growing on sand dunes. It ranges from extreme southeastern Virginia south to northern Florida in the United States. It is associated with *Yucca filamentosa*, *Yucca aloifolia*, and *Opuntia* species.

Cultivation

The plant is widely cultivated in warm temperate and subtropical climates, and valued as an architectural focal point. It has reportedly escaped from cultivation and naturalised in Italy, Turkey, Mauritius, Réunion, Guam, the Northern Mariana Islands, Puerto Rico, Argentina, Chile and Uruguay In a domestic environment, the plant has average water requirements, and little maintenance is needed other than the removal of dead leaves when the shrub nears its ultimate height. The plant is very hardy, without leaf damage at −20 °C (−4 °F), and can handle occasional snow and freezing temperatures.

Systematic position

Kingdom:	Plantae
Clade:	Tracheophytes
Clade:	Angiosperms
Clade:	Eudicots
Clade:	Rosids
Order:	Sapindales
Family:	Rutaceae
Genus:	*Murraya*
Species:	*M. koenigii*

Murraya koenigii

The curry tree (*Murraya koenigii*) is a tropical to sub-tropical tree in the family Rutaceae (the rue family, which includes rue, citrus, and satinwood), and is native to Asia. The plant is also sometimes called sweet neem, though *M. koenigii* is in a different family to neem, *Azadirachta indica*, which is in the related family Meliaceae.

Its leaves, known as curry leaves, are used in many dishes in the Indian subcontinent, especially curries

Description

It is a small tree, growing 4–6 m (13–20 feet) tall, with a trunk up to 40 cm diameter. The aromatic leaves are pinnate, with 11–21 leaflets, each leaflet 2–4 cm long and 1–2 cm broad. The plant produces small white flowers which can self-pollinate to produce small shiny-black drupes containing a single, large viable seed. The berry pulp is edible, with a sweet flavor.

Distribution and habitat

The tree is native to the Indian subcontinent. Commercial plantations have been established in India, and more recently Australia.

It grows best in well-drained soils in areas with full sun or partial shade, preferably away from the wind. Growth is more robust when temperatures are at least 18°C (65°F).

Uses

The fresh leaves are an indispensable part of Indian cuisine and Indian traditional medicines. They are most widely used in southern and west coast Indian cooking, usually fried along with vegetable oil, mustard seeds and chopped onions in the first stage of the preparation. They are also used to make thoran, vada, rasam and kadhi.

The fresh leaves are valued as seasoning in the cuisines of South and Southeast Asia. In Cambodia, where the leaves are called *sloek kontroap*, the leaves are roasted and used as an ingredient in a soup, *maju krueng*. In Java, the leaves are often stewed to flavor *gulai*. Though available dried, the aroma and flavor is greatly inferior. The oil can be extracted and used to make scented soaps.

The leaves of *Murraya koenigii* are also used as a herb in Ayurvedic and Siddha medicine in which they are believed to possess anti-disease properties, but there is no high-quality clinical evidence for such effects.

Propagation

Seeds must be ripe and fresh to plant; dried or shriveled fruits are not viable. One can plant the whole fruit, but it is best to remove the pulp before planting in potting mix that is kept moist but not wet. Stem cuttings can be also used for propagation.

Chemical constituents

Compounds found in curry tree leaves, stems, bark, and seeds include cinnamaldehyde, and numerous carbazole alkaloids, including mahanimbine, girinimbine, and mahanine.

Systematic position

Kingdom:	Plantae
Clade:	Tracheophytes
Clade:	Angiosperms
Clade:	Monocots
Order:	Dioscoreales
Family:	Dioscoreaceae
Genus:	*Dioscorea*
Species:	*D. communis*

Dioscorea communis

Dioscorea communis or *Tamus communis* is a species of flowering plant in the yam family Dioscoreaceae and is commonly known as black bryony, lady's-seal or black bindweed.

Description

It is a climbing herbaceous plant growing to 2–4 m tall, with stems that twine anticlockwise. The leaves are spirally arranged, heart-shaped, up to 10 cm long and 8 cm broad, with a petiole up to 5 cm long. It is dioecious, with separate male and female plants. The flowers are individually inconspicuous, greenish-yellow, 3–6 mm diameter, with six petals; the male flowers produced in slender 5–10 cm racemes, the female flowers in shorter clusters. The fruit is a bright red berry, 1 cm diameter. Its fairly large tuber is, like the rest of the plant, poisonous.

Distribution

Dioscorea communis is native and widespread throughout southern and central Europe, northwest Africa and western Asia, from Ireland to the Canary Islands, east to Iran and Crimea.

Habitat

Dioscorea communis is a typical plant of the forest understory, from the sea to the mountains, usually in dense woods, but it can also be found in meadows and hedges.

Uses

All components of the black bryony plant, including the tubers, are poisonous due to saponin content, so it is not typically used internally; however, it has been used as a poultice for bruises and inflamed joints. It has been suggested that black bryony be used topically with caution, due to a tendency for the plant to cause painful blisters.

Studies have isolated calcium oxalate deposits and histamines in the berry juice and rhizomes, which may contribute to skin irritation and contact dermatitis associated with black bryony.

Black Bryony is highly poisonous and should not be ingested at all at least when raw. When cooked, young shoots are commonly eaten in southern France, Spain, Portugal, Italy and Croatia.

Chemistry

The rhizome contains phenanthrenes (7-hydroxy-2,3,4,8-tetramethoxyphenanthrene, 2,3,4-trimethoxy-7,8-methylenedioxyphenanthrene, 3-hydroxy-2,4,-dimethoxy-7,8-methylenedioxyphenanthrene, 2-hydroxy-3,5,7-trimethoxyphenanthrene and 2-hydroxy-3,5,7-trimethoxy-9,10-dihydrophenanthrene).

Result and discussion

- In this field project, to study the vegetative character and floral character which is essentially used for identification and description of plants. Therefore the plants which are classified by benthem and hooker classification.The living genera and species of angiosperms which are usually studied on medicinal purposes and pharcological activity.The floral discussion can be substitute for tha taxonomy study.If such generation can be matched to data derived from biological plants ,then it may be possible to model the process by which real ,observd plants have been produced which in could be of greate interest to plant science not leaves for thesis identification . Both grasses and legumes are easy to identify when they are flowering which seldom occurs within intensity graze pastures .
- Therefore,the challenge is to identify grasses and legumes w hen they are in a vegetative state ,which only leaf and supporting cluster present.
- The plant identification practice is an internationally recognized practice that can either stand alone are be incorporated into any programe can concrete solution to an overarching throughout the industry.
- The methods of identification include expert determination ,recognition ,comparisonand theuse of keys and similar devices ,for a through and technical discussion of specimen identification see sneath and sokal.
- In terms of reliability or accuracy the best method of identification is expert determination.

Conclusion

- To study the plant identification approaches focus on leaves.
- Most researches avoid segmentation ,images were used with plain background.
- Shape is the preeminent feature for plant identification ,most studies based on leaf shape analysis.

A field survey on edible oil industries with reference to manufacturing potential and marketing trends

A short field project work submitted to

St. Mary's College (Autonomous)

Affiliated to

MANONMANIAM SUNDARANAR UNIVERSITY

In partial fulfilment of the requirement for the Degree of Master of science in Botany

By

C.ERUTHAYA ABISHA - 20APBO03

DEPARTMENT OF BOTANY

ST.MARY'S COLLEGE (AUTONOMOUS)

THOOTHUKUDI-628001

CERTIFICATE

It is certified that this short field project work entitled "**A field survey on edible oil industries with reference to manufacturing potential and marketing trends** "submitted to St. Mary's college (Autonomous) affiliated to MANONMANIAM SUNDARANAR UNIVERSITY in partial fulfilment of the requirements for the degree of Master of science in Botany, and is a record of work done in the Department of Botany, St.Mary's College (Autonomous), Thoothukudi during the year 2020 – 2021 by the following student.

By

C.ERUTHAYA ABISHA - 20APBO03

M. Glory
GUIDE

M. Glory
HEAD OF THE DEPARTMENT

EXAMINER

Lucia Rose
PRINCIPAL
St. Mary's College (Autonomous)
Thoothukudi - 628 001.

DECLARATION

Hereby declare that the short field project entitled " **A field survey on edible oil industries with reference to manufacturing potential and marketing trends"** is a original work and it has not been submitted for the award of any Degree , Diploma , fellowship or any other similar title and that the short field project represents independent and original work on the partial fulfilment of the requirements for the degree of **MASTER OF SCIENCE IN BOTANY** during 2020-2021, submitted to St.Mary's College (Autonomous),Thoothukudi.

Place :Thoothukudi-628 001

Date :

C.ERUTHAYA ABISHA(20APBO03)

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We offer our praise and sincere thanks to the Almighty God, his for avalanche of graces and abundant blessings, enabling to complete this project.

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CONTENTS

Introduction

Edible oil is a fatty liquid that is physically extracted from several vegetables. In the earlier days oil was prepared in traditional method using chekku. Bullocks were used to move around the marachekk(wooden pestle)and oil seeds in the chekku were crushed without generating any heat. The same system is replicated by replacing Bullocks with a Motor and Gearbox.The wooden pestle revolves at a speed of 14 rotations per minute. This is to ensure that oil is not heated and no nutrients is lost.

Traditional chekku consist of a fixed stone bottom and the pestle is made up of a wood called "vagai". In traditional wood chekku, the seeds are crushed with a There are other types of chekku where the bottom and the pillar is made of steel. Marachekku oil is the oil which is manufactured by the traditional method by using the wooden Chekku (also known as Marachekku in Tamil)**.** The oil is not heated during the Manufacturing Process, hence it contains the original Nutrition and Flavors. The oil prepared by this process was the one that gave strength, stamina and long life to our Ancestors. Pure Marachekku oil is not refined. It is also rich in antioxidants, which boosts immunity.

They retain healthy anti-oxidants that are otherwise destroyed by heat. These anti-oxidants fight harmful free radicals and prevent the growth of tumors. At low temperatures, the good fatty acid bonds in the groundnut don't get destroyed, keeping its heart-protecting abilities intact, along with vitamins and minerals. Groundnuts, with their high Niacin(Vitamin B family) content, helps stabilize blood sugar.

The advantages of wood chekku over steel chekku is that the oil does not heated. Hence the vitamins and minerals are retained.

It's Famous because of its Benefits..

- The main advantage of cold pressed oil or mara chekku oil is that it has better nutritional benefits as the nutrients do not get destroyed by heat.
- Cold pressed groundnut oil retains natural anti-oxidants like phytosterol and tocopherol.

- Cold pressed sesame oil retains liganens, anti-oxidants and polyphenols which act as cholesterol lowering agents and prevents cholesterol.
- Cold pressed coconut oil has medium fatty acids that are easily digestible and for people with liver problems, help in getting quick energy

The pure oil is extracted and we do not add any chemicals or preservatives.

Types of chekku

- ❖ Wooden chekku (Big)
- ❖ Wooden chekku (Medium)
- ❖ Wooden chekku (domastic single phase)
- ❖ Stone chekku (Big)
- ❖ Stone chekku (Medium)
- ❖ Stone chekku (domestic single phase)
- ❖ Piller types stone chekku
- ❖ Piller type wooden chekku
- ❖ Rotary chekku

Types of oils

- ❖ Seasame oil
- ❖ Ground nut oil
- ❖ Coconut oil
- ❖ Mustard oil
- ❖ Neem oil
- ❖ Castor oil
- ❖ Iluppai oil
- ❖ Punga oil
- ❖ Sunflower oil

The study was discussed based on the manufacture process, raw material procurement, oil production and sales statement.

S.NO	Chekku Industry (small scale)	Chekku	Oil production	Annual oil sales (per oil)
1	Shri Madhuram mark	Wooden chekku	Coconut oil, Groundnut oil, Mustard oil, Gingelly oil	6,000 lier
2	Jevajothi chekku	Steel chekku	Coconut oil, Gingelly oil	4,800 liter
3	Jeeva chekku	Wooden chekku	Coconut oil, Groundnut oil, Gingelly oil	5,400 liter
4	Raja Chekku	Steel chekku	Coconut oil, Groundnut oil, Gingelly oil	3,600 liter

In all the industries mentioned here, oil is produced in the following manner.The only difference here is the chekku size but in all the 4 industries only 15 kg chekku is used.

Shri madhuram mark marachekku oil

Shri madhuram mark marachekku oil is a small scale industry located at M.Kumaran nagar , thoothukudi.

Four types of oils are produced here. The oil is traditionally made from wooden chekku. the wooden chekku oil production industry is ideal for self-employment. Good returns are obtained by being made with a small investment. The chekku is available in three variants of 15 kg,20 kg, 25 kg but 15 kg is used in shri madhuram mark marachekku. The price of a 15 kg steel chekku is two lakh ten thousand. The price of a 15 kg wooden chekku is two lakh twenty five thousand.Shri madhuram mark marachekku , chekku was procurement from the hardware industries of Coimbatore.

Oil is made in the same way in all ckekku shops.

Labour

10 people work here.

Transport

They use the road to carry goods.

Product procurement

Drying and storage

Coconut

Sources

Copra Coconut are procured from pollachi.

The production of copra

- Removing the shell
- Breaking it up
- Drying

Drying

- Drying is usually done where the coconut palms grow.

Types of Drying method

- Sun drying
- Smoke drying
- Solar drying
- Kiln drying

Sun drying

In madhuram mark marachekku , sun drying method can be used.

Sun drying requires little more than racks and sufficient sunlight. halved nuts are drained of water, and lift with the meat facing the sky; they can be washed to remove mold- creating contaminants.

After two days the meat can be removed from the shell with ease, and the drying process is complete after three to five more days .

Other types of drying

Kiln drying

Sun drying is often combined with kiln drying , eight hours of exposure to sunlight means the time spent in a kiln can be redused by a day and the hot air the shells are exposed to in the kiln is more easily able to remove the remaining moisture.

The drying time can be reduced to 3-4 days if proper solar dryers are used. The batch type solar cabinet dryer of takes only 3 days for drying.

- A solar copra dryer with a drying time of three days, a small holders dryer having 400 nuts per batch capacity with a drying period of 36 hours, Medium holders dryer with a capacity of 3500 to 4000 nuts capacity, an electrically operated dryer with forced hot air circulation to dry 1000 nuts per batch in 28 hours and a smoke free copra dryer of 1000-1500 coconuts per batch with a drying period of 24 hours have been devepled.

Husking

- Manual dehusking with knife is a common practice.
- In madhuram mark only this can be used.
- A power operated dehusker which can dehusk about 600 nuts per hour is also available.

Advantage of storage

- Increase in thickness of copra
- Increase in oil content
- Greater meat resistance to bacterial sliming while sun drying, easier husking, cleaner
- Easier shelling

Uniform quality of copra.

Copra moisture meter

For safe storage of copra, the optimum level of moisture content recommended is 6%.

10 Benefits of Coconut Oil

- A Boost in Good Cholesterol
- Good for Blood Sugar and Diabetes
- Helps Fight Back Against Alzheimer's Disease
- Helps Stop Heart Disease and High Blood Pressure
- Aids in Liver Health
- Boosts Energy
- Aids with Digestion
- Acts as a Salve for Wounds and Burns.

Major Benefits of Marachekku Coconut Oil

- The cold-pressing process retains the essential antioxidants and vitamins.
- Lauric acid present in coconut oil helps to kill harmful pathogens like bacteria and viruses. This essential component is lost in the commercial oil extraction process and retained during cold pressing.
- **Marachekku Coconut oil** contains a type of fat known as medium-chain triglycerides (MCTs). These fats are converted to ketones in the liver.
- Ketones have a powerful effect on the brain; therefore their long-term use can help treat conditions like epilepsy.
- When the body goes into ketone metabolism, weight loss becomes easier because appetite is reduced on a ketone diet.

- Several studies have shown that coconut oil consumption is good for heart health since it helps raise the HDL (good cholesterol) levels in the body.
- **Marachekku Coconut oil** is very stable when heated to high temperatures due to the presence of short and medium-chain fatty acids. Hence it is recommended for deep frying.
- Cold-pressed virgin coconut oil retains all the essential nutrients and is healthy for the skin, hair, and teeth.

The cold-pressing process retains the antioxidants and essential vitamins in the oil.

Storage of sesame seeds

- In madhuram mark sesame seeds are stored in a dry place.
- Sesame seeds should be stored in an airtight container.
- Unrefrigerated seeds can be kept in a cool, dry place for up to three months.
- If you refrigerate the seeds, they will last up to six months; frozen ones will be good for up to one year.
- Sesame oil, on the other hand, is remarkably stable and will keep for years without turning rancid, even in hot climates.

Benefit

- Sesame seeds are rich in minerals; they are an excellent source of copper and a very good source of calcium, iron, magnesium, manganese, and phosphorus.

- They have two types of beneficial lignan fiber that are not found in other plants. Three tablespoons of sesame seeds provide 3.5 grams of fiber (12 percent of the reference daily intake).
- Not only is this fiber good for digestive health, but it may also have effects in lowering bad cholesterol.
- While sesame seeds have a high-fat content, it is primarily polyunsaturated and monounsaturated fat.
- These seeds can be a good source of protein, with 5 grams per 3 tablespoons.

composition

- Sesame oil is composed of the following fatty acids: linoleic acid(41% of total), oleic acid (39%), palmitic acid (8%), stearic acid (5%) and others in small amounts.

Manufacturing process

- Sesame seeds are protected by a capsule which only bursts when the seeds are completely ripe. This is called dehiscence.
- The dehiscence time tends to vary, so farmers cut plants by hand and place them together in an upright position to continue ripening until all the capsules have opened.
- The steady growth in demand being observed here is in line with rising household income figures and urbanization, as well as an increase in the use of sesame oil for food products and Asian dishes.
- sesame oil derived from quality seeds already possesses a pleasant taste and does not require further purification before it can be consumed.
- Many consumers prefer unrefined sesame oil due to their belief that the refining process removes important nutrients. Flavour, which was traditionally an important attribute, was best in oils produced from mild crushing.
- Sesame oil is one of the more stable natural oils, but can still benefit from refrigeration and limited exposure to light and high temperatures during extraction,

processing and storage in order to minimize nutrient loss through oxidation and rancidity. Storage in amber-colored bottles can help to minimize light exposure.

- Sesame oil is a polyunsaturated (PUFA) semi-drying oil. Commercial sesame oil varies in colour from light to deep reddish-yellow depending on the colour of the seed processed and the method of milling. Provided the oil is milled from well-cleaned seed, it can be refined and bleached easily to yield a light-coloured limpid oil.
- Sesame oil is rich in oleic and linoleic acids, which together account for 85% of the total fatty acids.
- Sesame oil has a relatively high percentage of unsaponifiable matter (1.5-2.3%). In India and in some other European countries it is obligatory to add sesame oil (5-10%) to margarine and generally to hydrogenated vegetable fats which are commonly used as adulterants for butter or ghee.

Varieties

- There are many variations in the colour of sesame oil: cold-pressed sesame oil is pale yellow, while Indian sesame oil (gingelly or til oil) is golden.
- This dark colour and flavour are derived from roasted/toasted sesame seeds.
- Cold-pressed sesame oil has a different flavour than the toasted oil, since it is produced directly from raw, rather than toasted, seeds.
- Sesame oil is traded in any of the forms described above: Cold-pressed sesame oil is available in shri madhuram mark industry.

nutrient

- The only essential nutrient having significant content in sesame oil is vitamin K, providing 17% of the Daily Value per 100 grams (ml) consumed supplying 884 calories (table).
- For fats, sesame oil is approximately equal in monounsaturated fat (oleic acid, 40% of total) and polyunsaturated fat (linoleic acid, 42% of total), together accounting for 80% of the total fat content (table). The remaining oil content is primarily the saturated fat, palmitic acid .

Uses

Cooking

- One type of sesame oil, a pale yellow liquid with a pleasant grain-like odor and somewhat nutty taste, is used as frying oil.
- A second type of oil, amber-colored and aromatic, is made from pressed and toasted sesame seeds and is used as a flavoring agent in the final stages of cooking.
- Despite sesame oil's high proportion (41%) of polyunsaturated (omega-6)fatty acids, it is least prone, among cooking oils with high smoke points, to turn rancid when kept in the open.This is due to the natural antioxidants, such as sesamol, present in the oil.
- Light sesame oil has a high smoke point and is suitable for deep-frying, while dark sesame oil (from roasted sesame seeds) has a slightly lower smoke point and is unsuitable for deep-frying. Instead it can be used for the stir frying of meats or vegetables, sautéing, or for the making of an omelette.
- Sesame oil is most popular in Asia, especially in Korea, China, and the South Indian states of Karnataka, Andhra Pradesh, and Tamil Nadu, where its widespread use is similar to that of olive oil in the Mediterranean.

- East Asian cuisines often use roasted sesame oil for seasoning.
- The Chinese use sesame oil in the preparation of meals.
- In Japan, rāyu is a paste made of chili-sesame oil seasoning and used as a spicy topping on various foods, or mixed with vinegar and soy sauce and used as a dip.
- In South India, before the advent of modern refined oils produced on a large scale, sesame oil was used traditionally for curries and gravies. It continues to be used, particularly in Tamil Nadu and Andhra Pradesh, mixed with foods that are hot and spicy as it neutralizes the heat. It is often mixed in with a special spice powder that accompanies idli and dosa as well as rice mixed with spice powders .

Groundnut

Drying and Cleaning of Groundnut

- The pods have to be cleaned and dried to bring down the **moisture to 10%** or less for safe **storage** or marketing.
- Fungi attack groundnut pods after lifting during drying and storage whenever environmental conditions are favourable for their growth and development.
- **Drying** has to be done rapidly to prevent molding or other forms of deterioration but not very rapidly as to lower the quality. This will help in maintaining the desirable flavour, texture, germination and overall quality.

Storage

- After cleaning and drying of groundnut pods to a safe moisture level, they have to be stored to keep them dry and protected against insects and rodents to prevent loss of natural colour and flavour and absorption of off flavours and development of rancidity.
- Hence proper storage of groundnut pods is an important step whether the pods are used for oil extraction or seed or for edible purpose.
- In madhuram mark ground nut only used for oil production.

Storage Structures

- Farmers usually dispose off their groundnut pods (apart from the quantities retained by them for seed and edible use) within 3-4 weeks or sometimes 3-4 months depending on their economic conditions.
- Pods used for seed purpose are stored for 7-8 months and those intended for edible purposes are stored till the commencement of next harvesting season.
- Storage is invariably in the form of unshelled pods. Pods for seed purposes are generally stored in earthen pods, mud bins, bamboo baskets or wicker baskets which are often plastered with mud or cowdung.
- Gunny bags are also sometimes used for storage. Pods kept in gunny bags are more liable to be damaged by dampness, rodents and other storage pests.
- In the regulated markets, decorticating factories and oil mills, the pods are generally stored loose in godowns or in gunny bags. Pods are also heaped loose or stacked in gunny bags in the open if rains are not expected. The stacks are covered with tarpaulin or gunny cloth.
- As the kernels are more liable to damage faster than pods, they are shelled only a week or two before they are required.
- At the terminal markets and ports storage is in the form of kernels which are most often packed in gunny bags. The pile of bags in godowns is kept 4-5 feet below the roof to allow free circulation of air. The floor is sometimes covered with matting to prevent dampness.
- The problems of storage in groundnut are of greatest significance in our country on the account of the high temperature in summer when bulk storage is required.
- Kernels deteriorate faster than pods as the shells afford protection for the kernels against pests and protect the desirable qualities in them. However, the extra space required for unshelled pods makes it impractical to store large quantities. Huge losses are sustained to store annually by the growers, traders, millers and processors.

  The following aspects are to be considered for better storage of groundnut

- All produce intended for storage should be well dried to have not more than 5% moisture.
- As far as possible groundnut should be stored as pods rather than as kernels.
- If storage is to be done as kernels, pods should be decorticated carefully to avoid splits and broken in kernels. The period of storage as kernels should be reduced to the minimum possible.
- Storing of kernels on hard floor or hard bedding material and piling of bags to great height should be avoided to minimize caking up of kernels and damage to gunnies.

- Produce from the summer crop should not be stored for a long period as it deteriorates more rapidly than that from the winter crop. The summer produce is best utilized for local crushing.
- For storing small quantities of kernels, bins appear to be most suitable.
- Deterioration of groundnut can be considerably reduced by storing them in properly built stores.
- The most satisfactory stores are those which have good cement floors, well-plastered walls, proper and adequate windows and ventilators and tight fitting doors. A tin strip fixed along the bottom 10 to15 cm of the doors passing its lower end below the door to the other side will make the doors rat proof.
- Windows should be at last 3 feet above ground level and half-an-inch wire mesh should be fixed on them to prevent damage by rodents.
- The stack base should be on raised stands not touching the hard flooring. Sand should be spread on the floor to about a foot high and covered with a gunny cloth. Stacks should be in not more than 10 bags piled one over the other depending upon the height of the godown and the space available. The pile of bags has to be kept 4-5 feet below the roof to allow free circulation of oil.
- About 20% of the total space should be allowed between the top layer of the stack and ceiling. Each stack has to be separated from the wall and its neighbour by about 2 feet for ventilation and proper inspection. Ventilators should be kept open only on dry days.
- Gunny bags should be cleaned before use to get rid of dirt and insect pests. Spoiled seeds should be removed daily and the torn bags containing pods should be replaced immediately.
- The store should be cleaned thoroughly each season. Split kernels, groundnut shells, cobwebs and other debris should be completely removed.

Storage insect pests

- Several insects attack groundnut and its products in storage. Approximately 6-10% of the groundnut kernels stored in bags are damaged by insects.
- Insect infestation causes loss in dry mass of the kernels, increased levels of free fatty acids in the oil (thereby lowering the quality) and if the seeds are heavily damaged, reduction in germination potential. The heat and moisture generated by large insect population within heaps or stacks of groundnuts may also increase the risk of mold growth.
- The major pests of stored groundnuts in India are groundnut bruchids, red-flour beetles, rice moth, saw-toothed beetle and almond moth.

Management of storage insect pests

- Groundnuts must be dried properly after harvest to reduce the moisture content of the kernels to below 7%, the upper limit for safe storage. At high moisture levels, insect populations develop more rapidly and there is an increased risk of invasion by toxigenic fungi, with a consequent danger of aflatoxin contamination.
- The empty stores may be disinfected with dichlorvos as a space treatment before storing the produce.
- The stacks should be constructed on wooden pallets to reduce the possibility of ground water seeping into the bottom sacks.
- For large scale storage, the most suitable fumigants are methyl bromide and phosphine.
- Admixture of abrasive materials such as fine sand, kaolin or wood ash to protect grain in farmer-level storage.
- Use of certain plant materials such as crushed neem seed, neem leaves or neem oil, which have an antifeedant or repellant effect on storage pests.
- In madhuram mark only crushed neem seeds are used. they do not keep for long as they keep the peanuts for oil production. So they are not given these strategies to repel insects.

Decortication

- Dry the pods to 16 percent moisture content and decorticate manually using hand operated decorticator wih proper adjustment.
- Dry the kernels to 7 to 8 percent moisture.

Uses of Ground nut oil

Groundnut oil, especially if it is organic, is considered fairly healthy. The following are the health benefits of groundnut oil:

- Source of unsaturated fats

Groundnuts contain 40 to 50 percent fat, and a tablespoonful of the oil made from them contains 13.5 grams of fat. This fat occurs in the form of different types of fatty acids such as oleic acid, linoleic acid, stearic acid, etc. Most of this is unsaturated fat (both poly and mono) which is believed to be a good, healthy source of fat. This reduces the risk of heart disease and lowers bad cholesterol levels.

- Maintains heart health

Groundnut oil contains absolutely no cholesterol and therefore does not add unnecessary dietary cholesterol to our daily consumption. Atherosclerosis, a condition in which plaque forms around the arteries and leads to thickening, can thus be avoided. It also has a substance called resveratrol that decreases blood pressure and reduces stress on the cardiovascular system.

- Contains antioxidants

Antioxidants protect the body from toxins and free radicals. Groundnut oil contains phytochemicals and vitamin E, both of which are natural antioxidants. It also reduces inflammation if consumed regularly. It is said to keep many diseases like cancer at bay.

- Skin care

The vitamin E helps maintain good skin health, making it look young and healthy. It prevents premature aging, wrinkles, and marks caused by free radical.

- Slows cognitive disorders

Resveratrol in groundnuts, in addition to decreasing blood pressure, also slows down cognitive disorders like dementia and Alzheimer's disease. Being an antioxidant, it prevents free radicals from breaking down neural pathways in the brain, thus stopping the progress of the disease.

The antioxidants present in groundnut oil are its greatest assets. If extracted with the use of heat, these antioxidants may be damaged. Cold-pressed oils, on the other hand, retain them.

Mustard

Storage of mustard seeds

- Mustard is considered to be dry at 9.5 per cent seed moisture. For safe, long-term storage the seed should be at nine per cent moisture.

- If the moisture of the grain is between 9.5 and 15 per cent, drying is recommended to ensure safe, long-term seed storage. When moisture is greater than 15 per cent, the drying should occur in two stages. The grain should be first dried down to 13 per cent moisture and allowed to cool to the outside temperatures. The grain can then be dried down to nine per cent and allowed to cool before being placed in the bin

- Drying should not exceed 65˚C (150˚F) air temperature or 45˚C seed temperature. It is important to remember that mustard seed is denser than cereal seed and that it will require two to three times more static pressure to force the air through the crop.

- . Mustard should be stored below 18˚C and, for safe storage, should be kept as cool as possible.

- For safe storage, it is important to not only monitor stored mustard frequently but also to check different areas of the bin for temperature and moisture conditions. If possible, turn at least 1/3 of the bulk in the bin to minimize risk of spoilage from initiating.

Storage insects

- As an oilseed, mustard does not have frequent problems with insect pests in storage. Most commonly, insects observed in stored mustard are related to poor storage conditions and fungal growth associated with moist conditions.

- book lice are an example of fungus-feeding insects. Although not damaging to the commodity itself, they are an indication of fungal growth.

- Cooling and bringing down the moisture level in the storage bin will stop the fungal growth and in turn eliminate the fungal-feeding insects.

Uses of Mustard oil

- Mustard oil, which is produced from the seeds of the mustard plant, is a common ingredient in Indian cuisine.

- Known for its strong flavor, pungent aroma, and high smoke point, it's often used for sautéing and stir-frying vegetables in many parts of the world, including India, Bangladesh, and Pakistan.

- Although pure mustard oil is banned for use as a vegetable oil in the United States, Canada, and Europe, it's often applied topically and used as a massage oil, skin serum, and hair treatment .

- Mustard essential oil, a type of essential oil produced from mustard seeds using a steam distillation process, is also available and approved for use as a flavoring agent .

Here are 8 benefits of mustard oil and mustard essential oil, along with some simple ways to use them.

May support heart health

- Mustard oil is high in monounsaturated fatty acids, a type of unsaturated fat found in foods like nuts, seeds, and plant-based oils .

- Monounsaturated fatty acids have been linked to a variety of benefits, especially when it comes to heart health.

- .For example, one small study in 137 people in North India found that those who consumed a higher amount of mustard oil were more likely to have a history of heart disease .

May block microbial growth

- Some studies have found that mustard essential oil possesses powerful antimicrobial properties and may help block the growth of certain types of harmful bacteria.

- According to one test-tube study, white mustard essential oil decreased the growth of several strains of bacteria, including *Escherichia coli*, *Staphylococcus aureus*, and *Bacillus cereus* .

May promote skin and hair health

- Pure mustard oil is often applied topically to help optimize hair and skin health.

- As well as adding it to homemade face masks and hair treatments, it's sometimes mixed with wax and applied to the feet to help heal cracked heels.

- However, although many report improvements in fine lines, wrinkles, and hair growth, most available evidence on the topical benefits of pure mustard oil is purely anecdotal.

- If you decide to use mustard oil on your skin or scalp, be sure to perform a patch test first and use only a small amount to prevent irritation.

May alleviate pain

- Mustard oil contains allyl isothiocyanate, a chemical compound that has been well studied for its effect on pain receptors in the body .

- Although research in humans is lacking, one animal study found that administering mustard oil to the drinking water of mice desensitized certain pain receptors and helped treat widespread pain .

- Mustard oil is also rich in alpha-linolenic acid (ALA), a type of omega-3 fatty acid that may help decrease inflammation and relieve pain caused by conditions like rheumatoid arthritis .

- However, keep in mind that prolonged topical exposure to pure mustard oil has been shown to cause serious skin burns.

- More research in humans is needed to evaluate the safety and effectiveness of using mustard oil for pain relief.

May slow cancer cell growth

- Promising research suggests that mustard oil may help slow the growth and spread of certain types of cancer cells.

- In one older study, feeding pure mustard oil to rats blocked the growth of colon cancer cells more effectively than feeding them corn oil or fish oil .Another animal study showed that mustard seed powder rich in allyl isothiocyanate inhibited bladder cancer growth by nearly 35%, as well as helped prevent it from spreading into the muscle wall of the bladder .

- A test-tube study observed similar findings, reporting that administering allyl isothiocyanate extracted from mustard essential oil decreased the spread of bladder cancer cells .

Reduces inflammation

- Traditionally, mustard oil has been used topically to relieve symptoms of arthritis, soothe pain and discomfort, and decrease inflammation caused by conditions like pneumonia or bronchitis.

- Mustard oil is also rich in omega-3 fatty acids, including alpha-linolenic acid (.

- Studies show that omega-3 fatty acids are involved in regulating inflammatory processes in the body and may help decrease oxidative stress and inflammation .

- Still, more research is needed to determine how using mustard oil may affect inflammation in humans.

May help treat cold symptoms

- Pure mustard oil is often used as a natural remedy to treat cold symptoms, such as coughing and congestion.

- It can be mixed with camphor, a compound often found in creams and ointments, and applied directly to the chest.

- Alternatively, you can try a mustard oil steam treatment, which involves adding a few drops of pure mustard oil to boiling water and inhaling the steam.

- However, there's currently no evidence to support the use of mustard oil for respiratory issues, nor any research to show that it offers any benefits.

High smoke point

- A smoke point is the temperature at which an oil or fat begins to break down and produce smoke.

- This can not only negatively affect the flavor of your final product but also cause fats to oxidize, producing harmful and highly reactive compounds known as free radicals .

- Pure mustard oil has a high smoke point of around 480°F (250°C), putting it on par with other fats like butter.

- This makes it a common choice for methods like frying, roasting, baking, and grilling in areas like India.

- Plus, it's comprised mostly of monounsaturated fats, which are more resistant to heat-induced degradation than polyunsaturated fatty acids.

OIL PRODUCTION

Coconut oil Production

- Chekku : wooden chekku
- Copra Coconut:15 kg
- Hours :2
- Yield :8 liter
- By product: 6 ½ kg punnaku

Cold pressed coconut oil is manufactured by the old traditional mehod by using chekku.

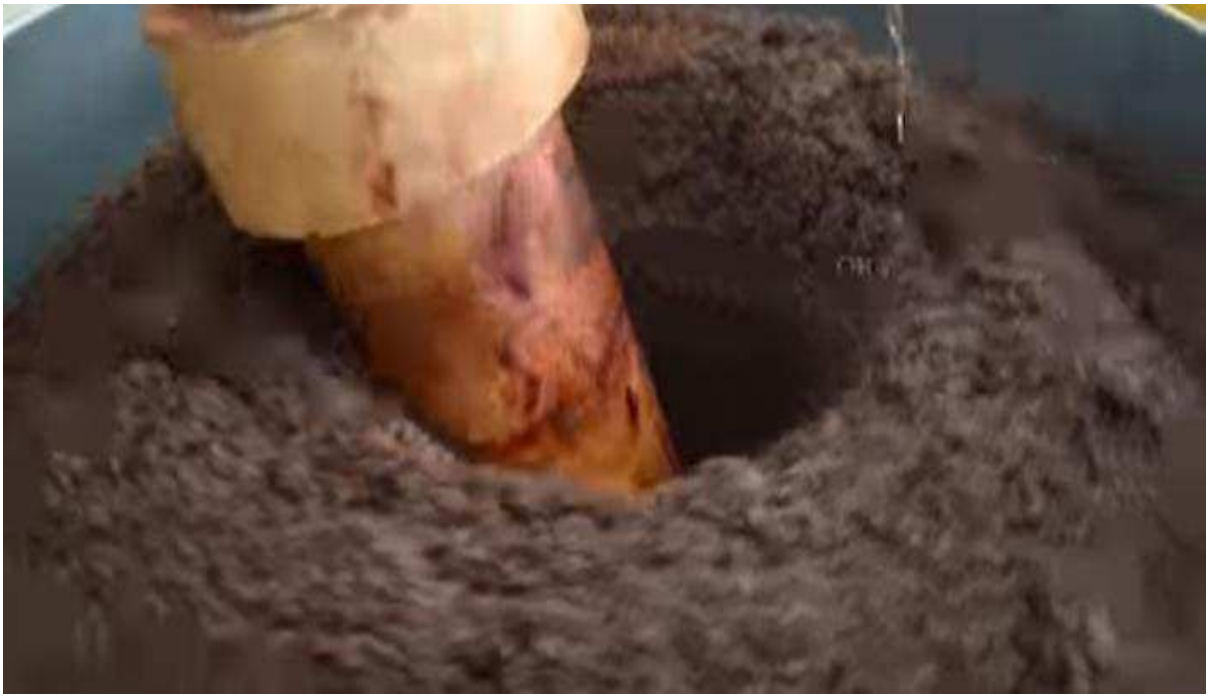
These oils contain all the original nutrition and flavour . also called as chekku coconut oil.

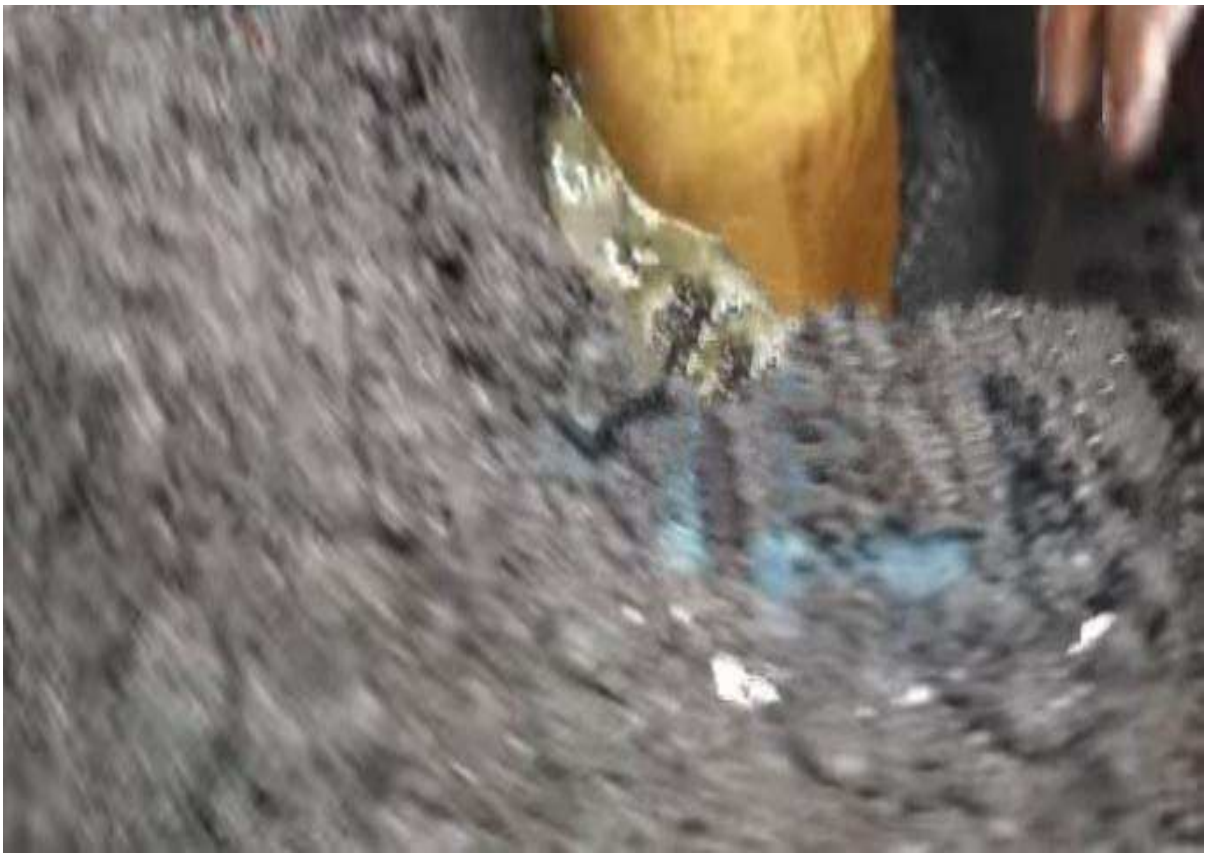
Gingelly oil

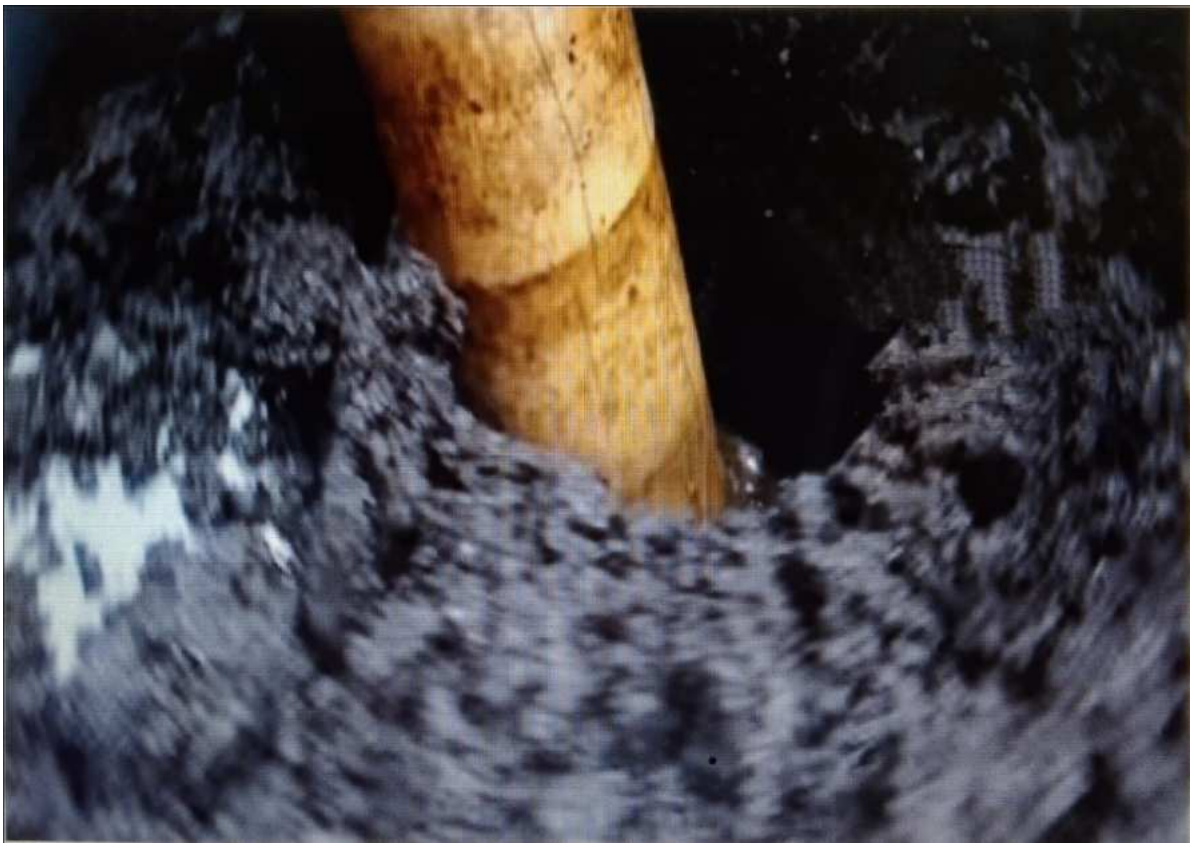
- Chekku : marachekku
- Sesame seeds: 15 kg
- Jaggary :2 kg
- Hours : 2
- Yield :8 liter
- By product: 8 kg punnaku

Cold pressed gingelly oil is manufactured by the old traditional method by using wooden chekku (marachekku in tamil)

These oils contain all the original nutrition and flavor.also called as marachekku gingelly oil.







Ground nut oil production

- Chekku : Marachekku
- Ground nut :15 kg
- Hours :2
- Yield: 6 1/2 liter
- By product: 6 kg punnaku

Cold pressed ground nut oil is manufactured by the old traditional method by using wooden chekku (marachekku in tamil)

These oils contain all the original nutrition and flavour ,also called as marachekku groundnut oil.

Mustard oil production

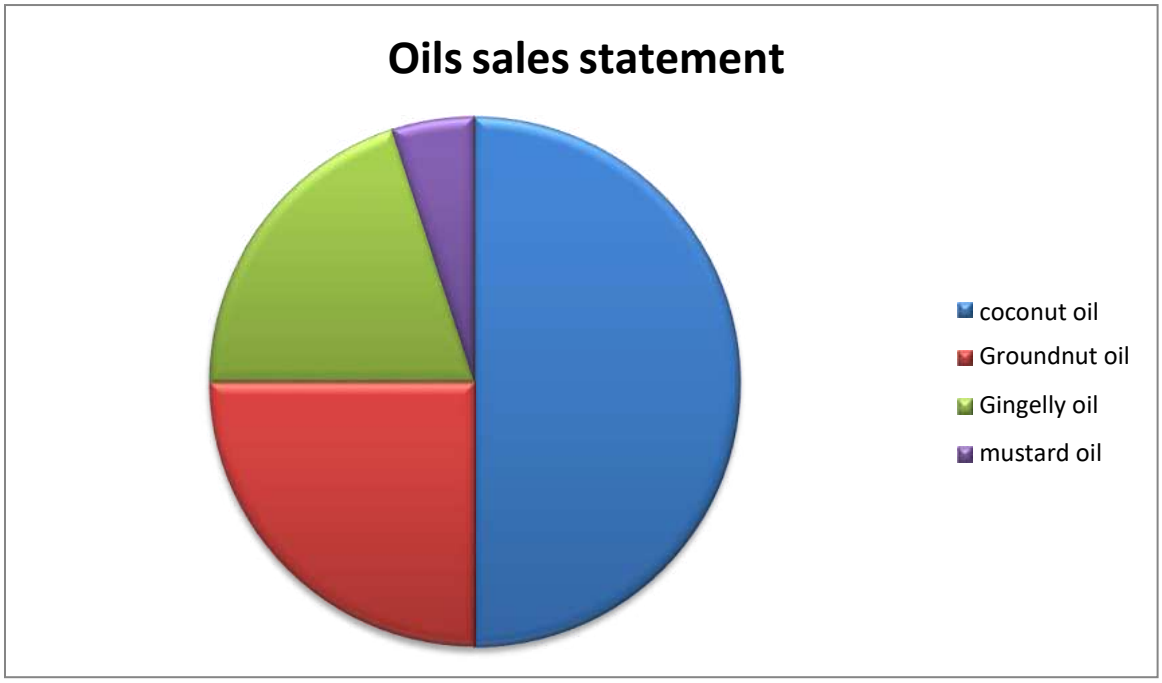
Chekku : marachekku

Mustard seeds : 15 kg

Hours : 2

Yield : 8 liter

Sales statement



Shri madhuram mark produces 6,000 liters per year. 6,000 liters are sold per year. The amount of oil they produce is equal to the amount of oil they sell.

Conclution

Now the idea of health prevails among the people. People love to get it naturally. So now people's mind is turned on oil made of wooden chekku. It is rich in all kinds of nutrients and is suitable for the body. It has become a highly profitable self- employment with little investment. This industry is improving their economy. The main advantage of cold pressed oil or mara chekku oil is that it has better nutritional benefits as the nutrients do not get destroyed by heat.

- Cold pressed groundnut oil retains natural anti-oxidants like phytosterol and tocopherol.
- Cold pressed sesame oil retains liganens, anti-oxidants and polyphenols which act as cholesterol lowering agents and prevents cholesterol.
- Cold pressed coconut oil has medium fatty acids that are easily digestible and for people with liver problems, help in getting quick energy
- The pure oil is extracted and we do not add any chemicals or preservatives. The oil is not heated during the Manufacturing Process, hence it contains the original Nutrition and Flavors. The oil prepared by this process was the one that gave strength, stamina and long life to our Ancestors. Pure Marachekku oil is not refined. It is also rich in antioxidants, which boosts immunity.
- Sesame seeds are rich in minerals; they are an excellent source of copper and a very good source of calcium, iron, magnesium, manganese, and phosphores.
- The antioxidants present in groundnut oil are its greatest assets. If extracted with the use of heat, these antioxidants may be damaged. Cold-pressed oils, on the other hand, retain them.

Pollachi is popularly called the coconut city as tender and ripe coconuts are send from here to many cities and towns across india. It's geographical closeness to the UNESCO world heritage site of western ghats ensures that it has amazing climate throughout the year.

As a botany student, I told shri madhuram mark wooden chekku company that you can not only produce oil but also sell other by products and make a profit .example They have not yet used coconut shells. Home decor can be made in coconut shells.

Crafts men can sell coconut shells and make a profit from it.

In addition , large scale production of charcoal is currently underway. the dealer buys the shells and sells it to the wholesaler. He will be in the business of charcoal burning.

The charcoal industry is to keep the charcoal burning for 24 hours and then pour water over it to get bored Charcoal is used in gold smelting, ironing, carbon sheet and water filer.

A PRELIMINARY STUDY ON SOIL QUALITY ANALYSIS AND CULTIVATION METHODS OF SELECTED GREENS IN FARMLANDS OF PUDUKOTTAI, THOOTHUKUDI DISTRICT

A short field project work submitted to

St. Mary's College (Autonomous)

Affiliated to

MANONMANIAM SUNDARANAR UNIVERSITY

In partial fulfillment of the requirement for the Degree of Master of science in Botany

By

P.ESAKKIAMMAL - 20APBO04

DEPARTMENT OF BOTANY

ST.MARY'S COLLEGE (AUTONOMOUS)

THOOTHUKUDI-628 001

CERTIFICATE

It is certified that this short field project work entitled "**A PRELIMINARY STUDY ON SOIL QUALITY ANALYSIS AND CULTIVATION METHODS OF SELECTED GREENS IN FARMLANDS OF PUDUKOTTAI, THOOTHUKUDI DISTRICT**" submitted to St. Mary's college (Autonomous) affiliated to MANONMANIAM SUNDARANARUNIVERSITY in partial fulfilment of the requirements for the degree of Master of science in Botany, and is a record of work done in the Department of Botany, St. Mary's College (Autonomous), Thoothukudi during the year 2020 – 2021 by the following student.

By

P.ESAKKIAMMAL - 20APBO04

M. Glory
GUIDE

M. Glory
HEAD OF THE DEPARTMENT

M. Glory
EXAMINER

Lucia Rose
PRINCIPAL
St. Mary's College (Autonomous)
Thoothukudi - 628 001.

DECLARATION

Hereby declare that the short field project entitled **" A PRELIMINARY STUDY ON SOIL QUALITY ANALYSIS AND CULTIVATION METHODS OF SELECTED GREENS IN FARMLANDS OF PUDUKOTTAI, THOOTHUKUDI DISTRICT"** is the original work and it has not been submitted for the award of any Degree, Diploma, Fellowship or any other similar title and that the short field project represents independent and original work on the partial fulfilment of the requirements for the degree of **MASTER OF SCIENCE IN BOTANY** during 2020-2021, submitted to St. Mary's College (Autonomous), Thoothukudi – 628 001.

Place: Thoothukudi – 628 001

Date: **P. ESAKKIAMMAL (20APBO04)**

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CONTENTS

INTRODUCTION

"Leafy greens" or "greens" are broad terms used for a number of vegetable crops with edible leaves. Greens help build your internal rainforest and strengthen the blood and respiratory system. Leafy greens are also high-alkaline foods which may be beneficial to people exposed to higher amounts of pollution in urban areas.

Nutritionally, greens are high in calcium, magnesium, iron, potassium, phosphorous, zinc, and vitamins A, C, E and K. They are crammed with fiber, folic acid, chlorophyll and many other micronutrients and phyto chemicals.

Some of the benefits gained from eating dark leafy greens are:

- Blood purification
- Cancer prevention
- Improved circulation
- Immune strengthening
- Increased energy
- Improve organs function
- Clearing of congestion in lungs

The diversity of production and processing methods in the lettuce/leafy greens industry makes a single, universally applicable approach to food safety planning complicated. It is important that each firm assess its operations and implement methods that meet its individual needs.

A quantity of 100 g of greens contains over four times the recommended daily intake of **vitamin K.** For this reason, individuals taking the anticoagulant warfarin – which acts by inhibiting vitamin K – are instructed to minimize consumption of spinach (as well as other dark green leafy vegetables) to avoid blunting the effect of warfarin.

Leafy greens are an important part of a healthy diet. They're packed with **vitamins**, **minerals** and **fiber** but low in **calories**. Eating a diet rich in leafy greens can offer numerous health benefits including reduced risk of obesity, heart disease, high blood pressure and mental decline.

To boost your **daily** nutrition, aim to **eat** about 2 cups of dark, leafy **greens** every day. Two cups of raw **greens** is equal to 1 cup of **vegetables**, and 2.5 cups is recommended **daily** for a 2000-calorie diet.

The low caloric value of leaves makes them ideal for weight management. Greens are a rich source of nutrients, high in dietary fiber, low in lipids, and rich in folate, ascorbic acid, vitamin K, Mg, and K. They also carry plenty of phytochemicals such as β-carotene flavonoids.

The good nutrition profile of greens is beneficial in lowering the risk of cardiovascular diseases and cancer. Greens are also valued for individuals with type 2 diabetes due to their high Mg content, high fiber content, and low glycemic index.

These contain a good blend of polyphenols and antioxidants, which render them unique for therapeutic values. They also possess antimicrobial activity and can be used in different food products to extend storage life. The burden over synthetic chemicals can be reduced by encouraging the use of greens in food and food products.

In addition, dark **green leafy vegetables** act as antioxidants in the body.

Green leafy vegetables are vital for growth and good health as they contain all important nutrients.

In India, a wide range of greens are consumed.

The recommended dietary allowance of leafy greens for an adult women is 100g/day, adult man 40g/day, preschool children (4-6 yrs) and for boys and girls beyond 10 yrs of age it is 50g/day.

SOIL PHYSICAL CHARACTERS:

All soils contain mineral particles, organic matter, water and air. The combinations of these determine the soil's properties – its texture, structure, porosity and colour.

Soil texture:

Soil is made up of different-sized particles. Soil texture refers to the size of the particles that make up the soil and depends on the proportion of sand, silt and clay-sized particles and organic matter in the soil.

Soils are made up of different combinations of sand, silt and clay particles. Soils that are a mixture of sand, silt and clay are called loams. The name of the soil often identifies the dominant particle.

Soil texture can influence whether soils are free draining, whether they hold water and how easy it is for plant roots to grow.

Soil structure:

Soil structure is important for plant growth, regulating the movement of air and water, influencing root development and affecting nutrient availability.

Soil porosity:

Soil porosity refers to the pores within the soil. Porosity influences the movement of air and water. Healthy soils have many pores between and within the aggregates. Poor quality soils have few visible pores, cracks or holes. The way in which a soil is managed can affect its porosity.

Soil colour:

Soil colour gives an indication of the various processes going-on in the soil as well as the type of minerals in the soil.

Soil **colour** is influenced primarily by soil mineralogy – telling us what is in a specific soil. Soils high in iron are deep orange-brown to yellowish-brown. Those soils that are high in organic matter are dark brown or black.

Soils rich in humus tend to be dark because decomposed organic matter is black or brown. Soils with high humus content are usually very fertile, so dark brown or black soils are often referred to as 'rich'. [Some dark soils may be dark because of other soil forming factors and may have little or no humus]

Red or yellow soils typically indicate the presence of iron.

Bulk density:

It is the proportion of the weight of a soil relative to its volume. It is expressed as a unit of weight per volume, and is commonly measured in units of grams per cubic centimeters (g/cc).

The difference in bulk density relates to a difference in "particle density" of mineral soil material versus organic soil material. The average particle density of mineral soil material is 2.65 g/cc, which approximates the density of quartz. Conversely, the average particle density of organic soil material is 1.25 g/cc. Organic soil material weighs less than mineral soil material, so it will lower the bulk density of a mineral soil when added, as it reduces the overall weight of the soil.

Specific gravity:

The **specific gravity** (G_S) of a **soil** refers to the ratio of the solid particles' unit **weight** to the unit **weight** of water. G_S should not be confused with the **soil** density since it is a dimensionless unit and expresses the ratio of two particular densities. The bulk density of soil depends greatly on the mineral make up of soil and the degree of compaction.

About half of most soils are **inorganic** materials, such as the products of weathered rock, including pebbles, sand, silt, and clay particles.

About half of all soils are organic materials, formed from the partial breakdown and decomposition of plants and animals. The organic materials are necessary for a soil to be fertile. The organic portion provides the nutrients, such as nitrogen, needed for strong plant growth.

In between the solid pieces, there are tiny spaces filled with air and water.

SOIL CHEMICAL CHARACTERS:

Chemical properties of soils include the following aspects: inorganic matters of soil, organic matters in soil, colloidal properties of soil particles and soil reactions and buffering action in acidic soils and basic soils. The chemical side of a soil is extremely important of course and is about the correct balance of the available nutrients in the soil. This is largely determined by the organic-matter content and its humus percentage; this is the 'store house' of nutrients on any farm. The extent to which minerals have a dominant presence or not, affects the release of

specific nutrients. Supplementing shortages is important, but the right balance is even more important. The soil only produces nutrients if you have the right balance. Chemical and physical properties impact biological properties. Optimal chemical and physical properties will lead to optimal biological properties and soil functions

Clays and organic matter in the soil carry negative charges. Water in the soil dissolves nutrients and other chemicals. Nutrients like potassium and ammonium have positive charges. They are attracted to the negatively charged organic and mineral matter, and this prevents them from being lost through leaching as water moves through the soil. Nitrate has a negative charge so it is not protected from leaching in most soils.

SOIL TEST:

Soil testing begins with soil sampling. A soil analysis can be only as good as the sample sent to the laboratory. It's important to realize only a tiny portion of a field is analyzed in the laboratory.

Samples are collected from randomly selected locations in the field.

Soil analyses can provide information that's important for maximizing nutrient use efficiency and agricultural productivity. Furthermore, a historical record of soil properties provided by long-term soil testing is useful for determining the effectiveness of fertilizer management strategies.

Soil sampling is the critical first step in a soil testing program. The second is selection of a laboratory that will use analysis procedures appropriate for regional soils and conditions. Third, an understanding of the accuracy and limitations of individual procedures and of the meaning of soil test results is essential. The last steps, interpreting soil analysis values and developing a fertilizer management program, are crop specific and sometimes dependent on additional soil and climatic properties.

a soil test determines the soil's nutrient supplying capacity by mixing soil for only a few minutes with a strong extracting solution

Every greens soil analysis will include information about the physical attributes of the soil.

Traditionally, the greens soil analysis report has been something to glance at once a year and then file away, which is good to some degree, because it is a very useful reference tool for monitoring trends in your green over the long term.

Importance of soil test:

A soil test is important for several reasons: to optimize crop production, to protect the environment from contamination by runoff and leaching of excess fertilizers, to aid in the diagnosis of plant culture problems, to improve the nutritional balance of the growing media and to save money and conserve energy by applying only the amount of fertilizer needed. Pre- plant media analyses provide an indication of potential nutrient deficiencies, pH imbalance or excess soluble salts. This is particularly important for growers who mix their own media. Media testing during the growing season is an important tool for managing crop nutrition and soluble salts levels. To use this tool effectively, you must know how to take a media sample to send for analysis or for in-house testing, and be able to interpret media test results.

Advantages of soil test:

Soil fertility is determined by the soil's biological, chemical, and physical properties. Properties such as structure, soil texture, and colour are visible to the eye. However, it is hard to see the chemical composition of soil. Therefore, there is need for soil diagnosis and that's why soil sampling is critical.

Soil tests are used to determine the soil's nutrient level and pH content. Armed with this information, farmers can define the quantity of fertilizer and exact type that is needed for application to improve the soil on your farm. This is essential because fertile soils are necessary to grow healthy crops.

So the present was aimed to take a survey on cultivation of greens in Pudukottai, town panchayath. The survey involve the following objectives:

- Soil quality of fields under cultivation greens.
- Methods and types of greens cultivation.
- Estimate the yield potential marketing.
- To assent the liveshood people greens cultivation.

STUDY AREA:

Village is located near the Thoothukudi, (13KM)

Farm area : Pudukottai

Land under cultivation : 1 acre

Acre of land : 1 acre

No. of farmers hold : 120

Selected greens (Alternanthera sessilis, Amaranthus caudatus, Amaranthus,Moringa olerifera, Sesbania grandiflora, Solanum nigrum, Spinacea oleracea) normally cultivated in study area.

SOIL CHARACTERS OF STUDY AREA:

Soil behavior:

Soil pH:

pH is the measure of acidity or alkalinity of a soil.

pH is an important criterion for the evaluation of soil quality measurement

Soil pH is important because it influences several soil factors affecting plant growth, such as soil bacteria, nutrient availability, toxic elements and soil structure.

The results eight in pH of Study area is given in the tabular column .

pH of the study area was analysed as :

Table: 1

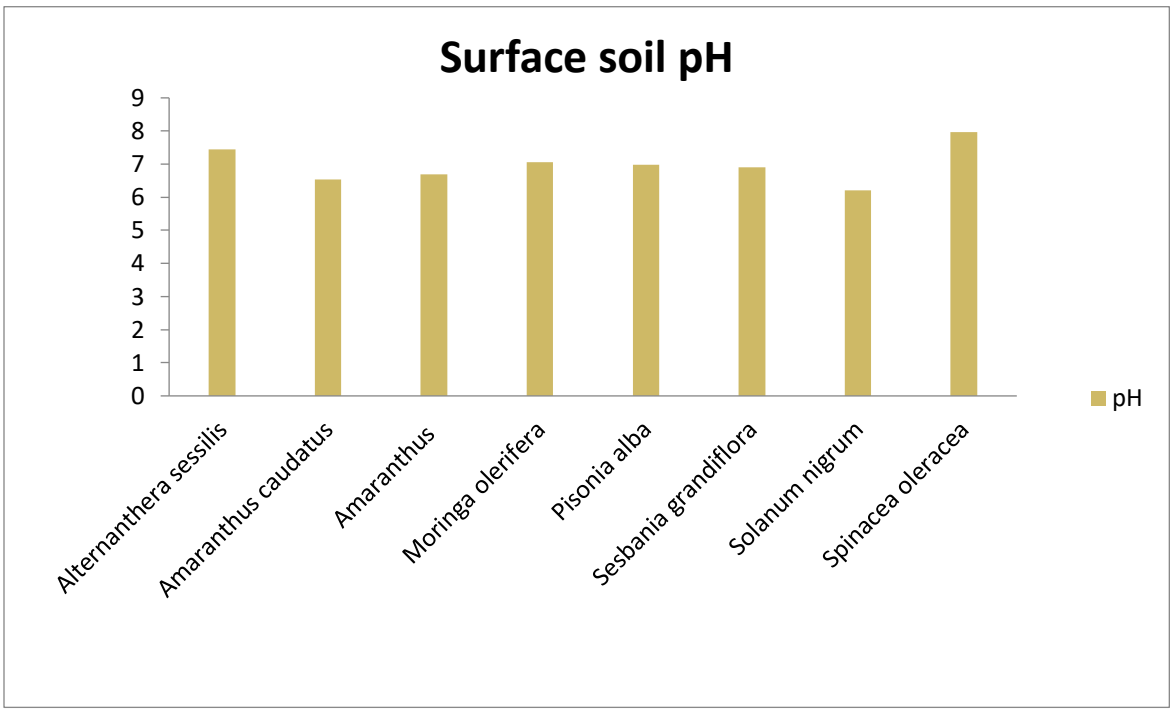
Farm No:	Name of the greens in the farmland	Soil pH
1	Field cultivated with ***Alternanthera sessilis***	Surface soil:7.45 Depth soil :6.90
2	Field cultivated with ***Amaranthus caudatus***	Surface soil:6.54 Depth soil :6.57
3	Field cultivated with ***Amaranthus***	Surface soil:6.69 Depth soil :7.27
4	Field cultivated with ***Moringa olerifera***	Surface soil:7.05 Depth soil :7.69
5	Field cultivated with ***Pisonia alba***	Surface soil:6.98 Depth soil :7.49
6	Field cultivated with ***Sesbania grandiflora***	Surface soil:6.91 Depth soil :7.08
7	Field cultivated with ***Solanum nigrum***	Surface soil:6.20 Depth soil :6.43
8	Field cultivated with ***Spinacea oleracea***	Surface soil:7.96 Depth soil :7.97

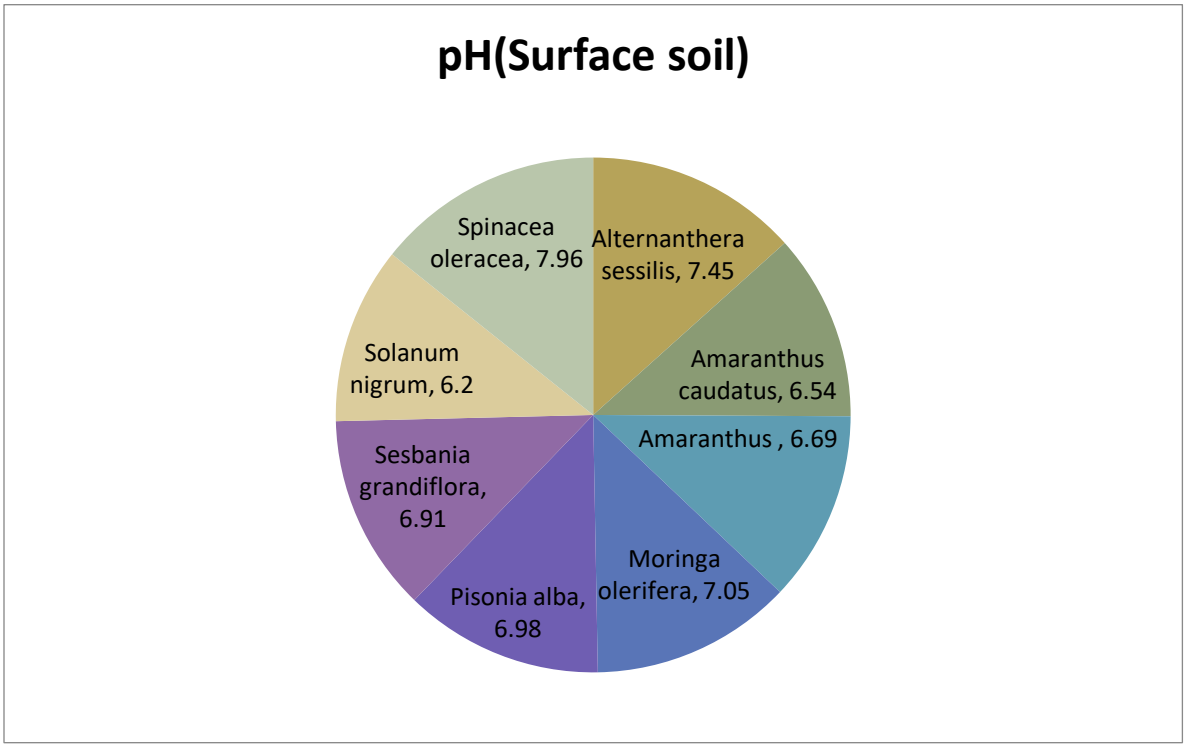
Average pH of study area:

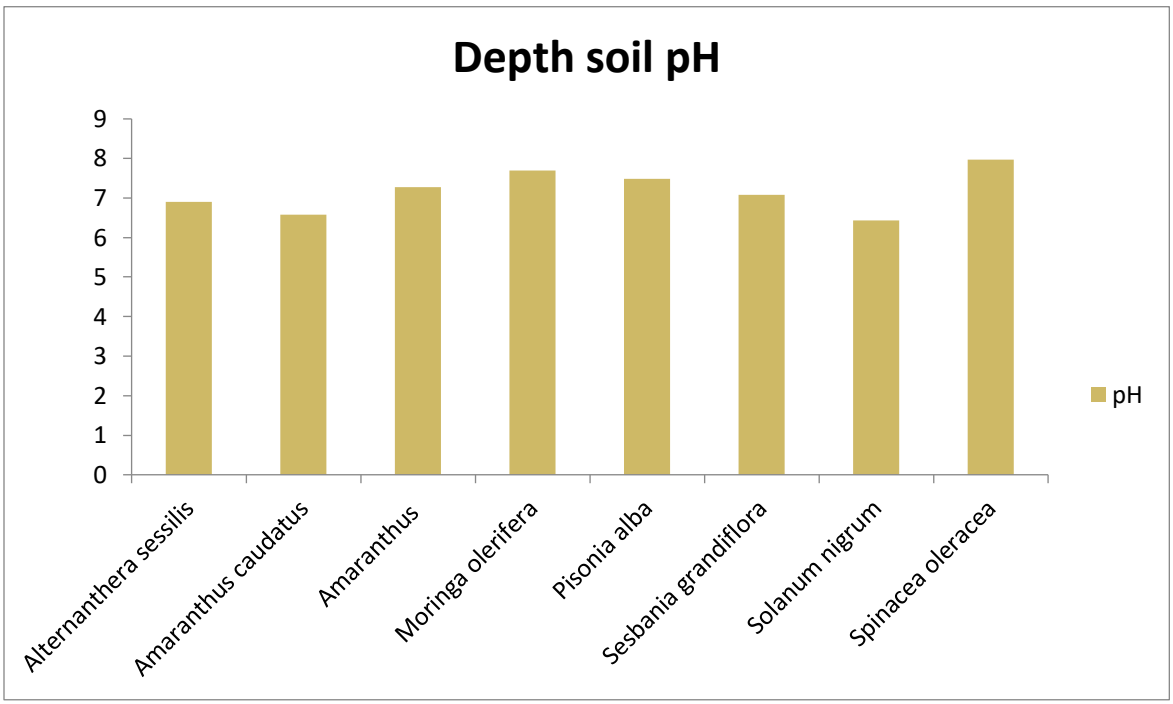
The Study area shows ,

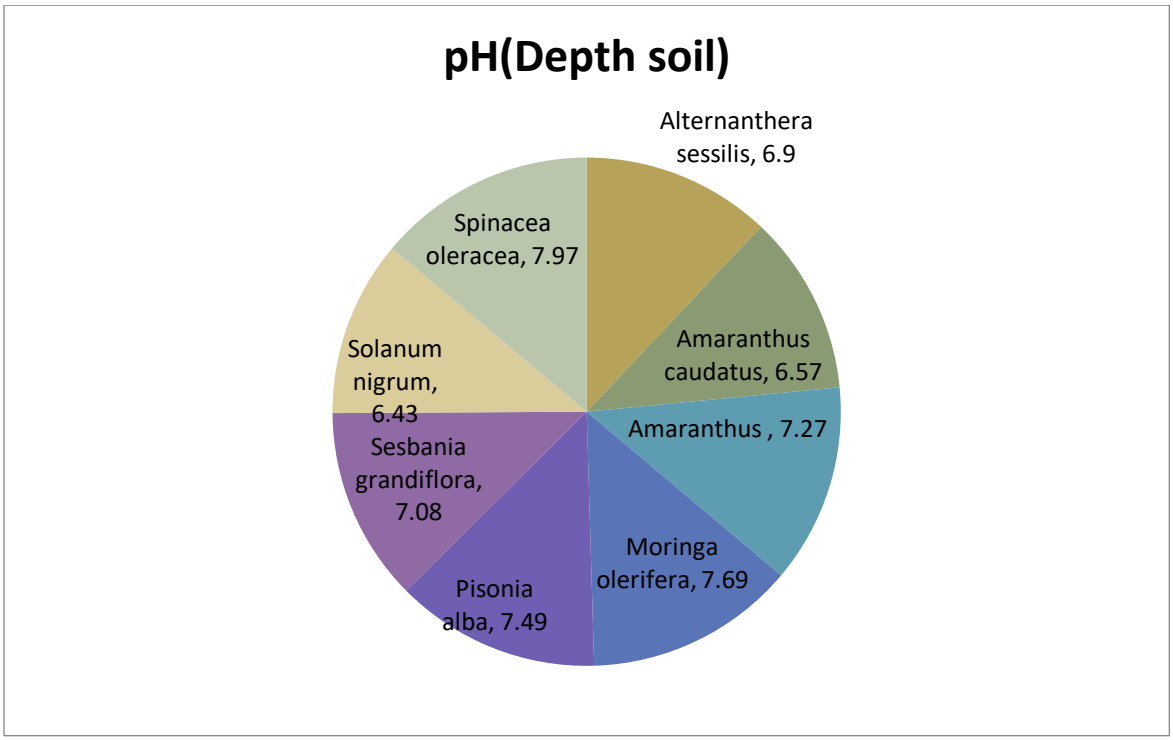
Farm land of the study area is slightly alkaline. (7.97)

In some farm land under the cultivation of greens soil are acidic. (6.2)


Surface soil pH
9
8
7
6
5
4
3
2
1
0
Alternanthera sessilis
Amaranthus caudatus
Amaranthus
Moringa olerifera
Pisonia alba
Sesbania grandiflora
Solanum nigrum
Spinacea oleracea
pH


pH(Surface soil)
Spinacea oleracea, 7.96
Alternanthera sessilis, 7.45
Solanum nigrum, 6.2
Amaranthus caudatus, 6.54
Amaranthus , 6.69
Sesbania grandiflora, 6.91
Moringa olerifera, 7.05
Pisonia alba, 6.98


Depth soil pH
9
8
7
6
5
4
3
2
1
0
Alternanthera sessilis
Amaranthus caudatus
Amaranthus
Moringa olerifera
Pisonia alba
Sesbania grandiflora
Solanum nigrum
Spinacea oleracea
pH


pH(Depth soil)
Alternanthera sessilis, 6.9
Amaranthus caudatus, 6.57
Amaranthus , 7.27
Moringa olerifera, 7.69
Pisonia alba, 7.49
Sesbania grandiflora, 7.08
Solanum nigrum, 6.43
Spinacea oleracea, 7.97

Bulk density:

It is the proportion of the weight of a soil relative to its volume. It is expressed as a unit of weight per volume, and is commonly measured in units of grams per cubic centimeters (g/cc).

The difference in bulk density relates to a difference in "particle density" of mineral soil material versus organic soil material.

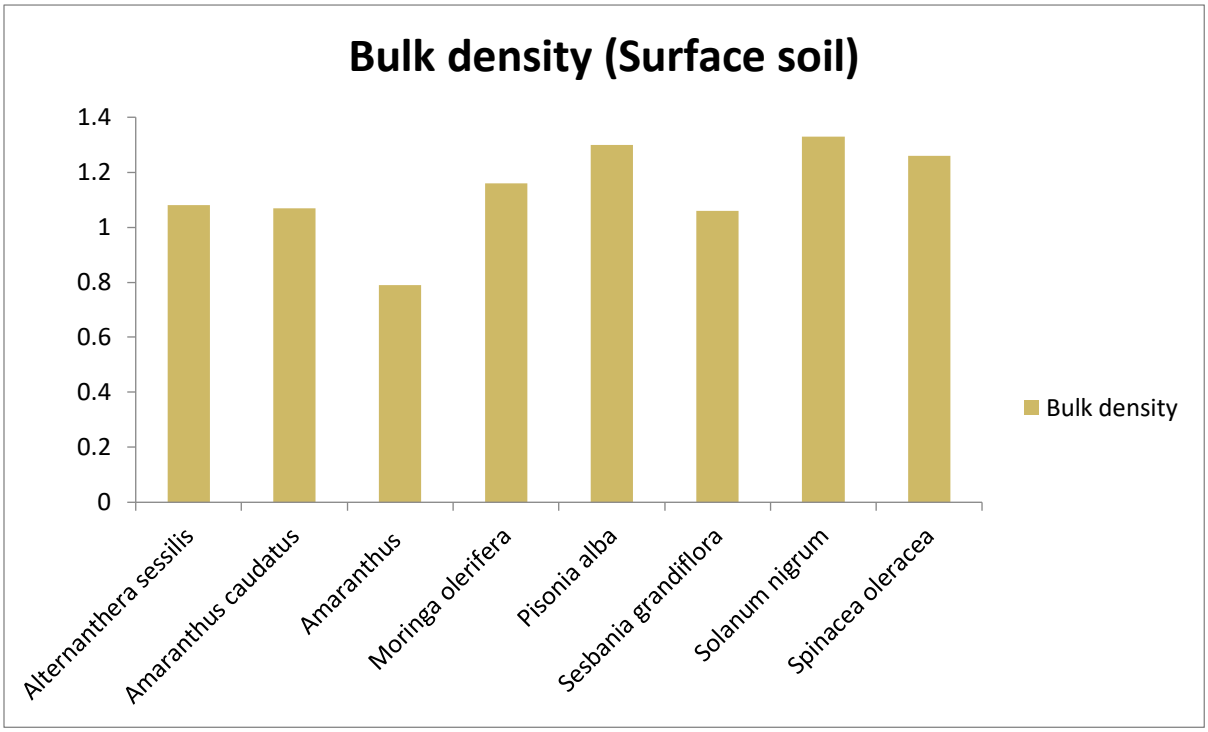
The results eight in Bulk Density of Study area is given in the tabular column .

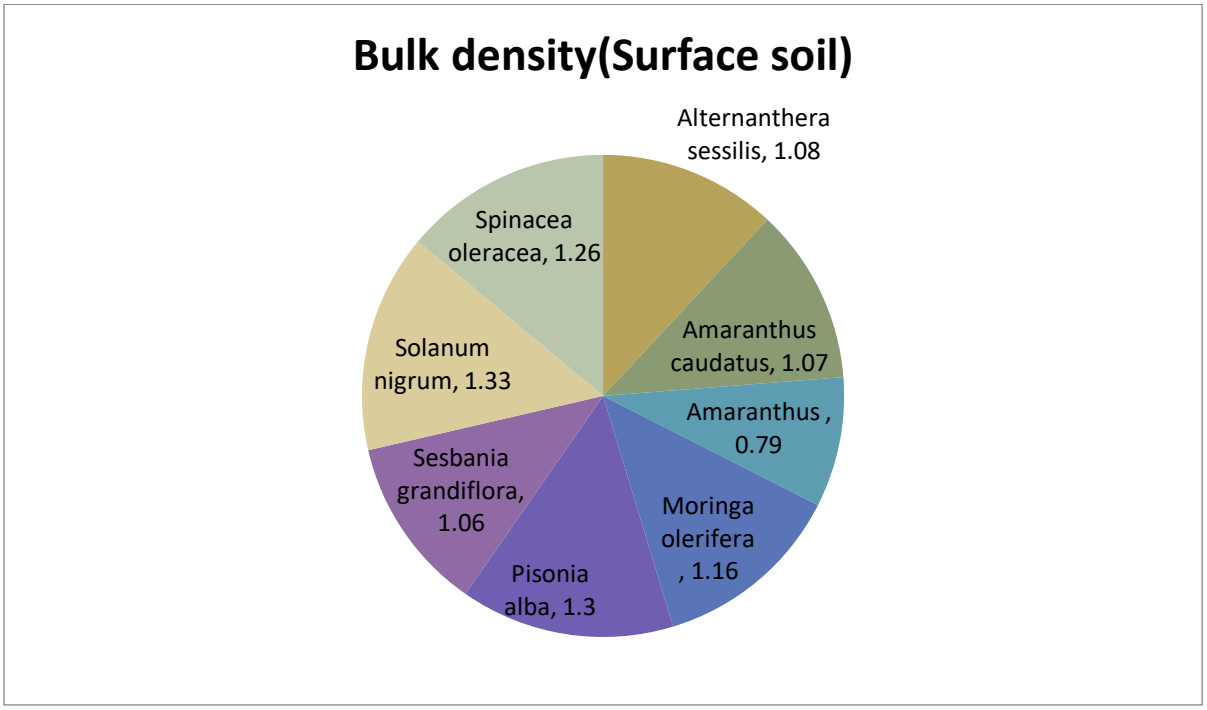
Table: 2

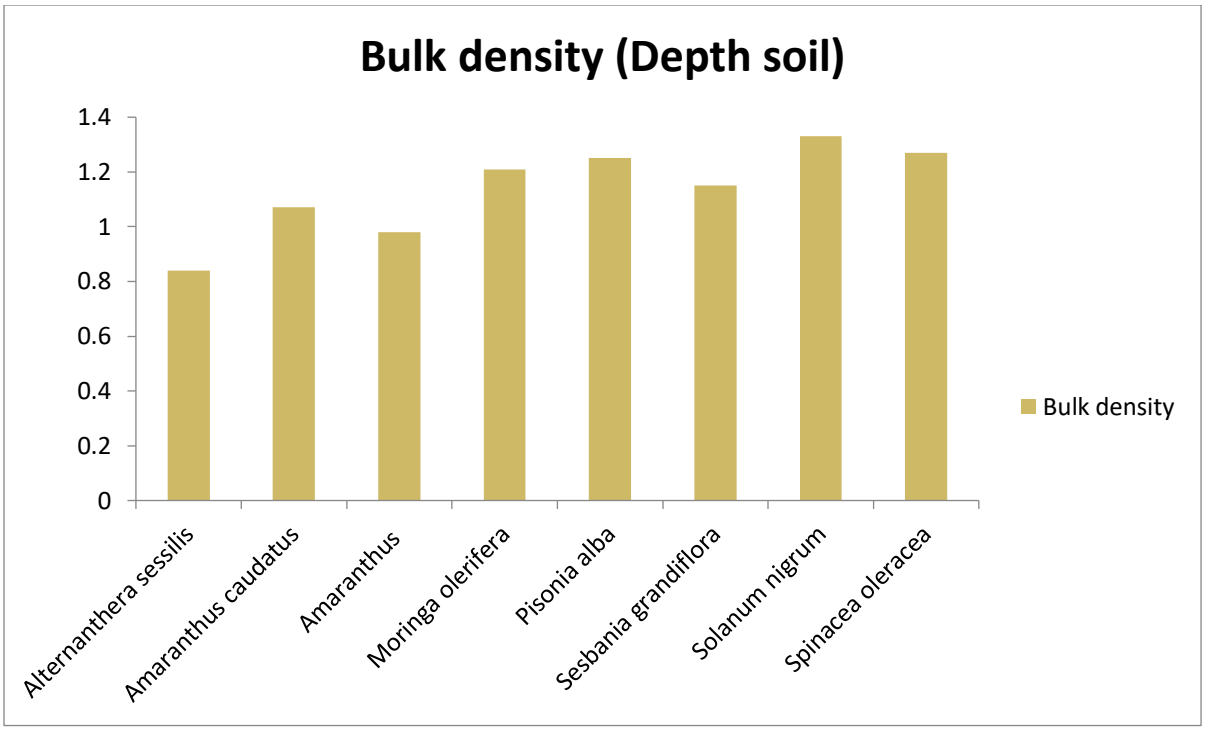
Farm No:	Name of the greens in the field	Soil sample	Bulk Density (g/cm3)
1.	***Alternanthera sessilis***	Surface soil Depth soil	1.08 0.84
2.	***Amaranthus caudatus***	Surface soil Depth soil	1.07 1.07
3.	***Amaranthus***	Surface soil Depth soil	0.79 0.98
4.	***Moringa olerifera***	Surface soil Depth soil	1.16 1.21
5.	***Pisonia alba***	Surface soil Depth soil	1.30 1.25
6.	***Sesbania grandiflora***	Surface soil Depth soil	1.06 1.15
7.	***Solanum nigrum***	Surface soil Depth soil	1.33 1.33
8.	***Spinacea oleracea***	Surface soil Depth soil	1.26 1.27

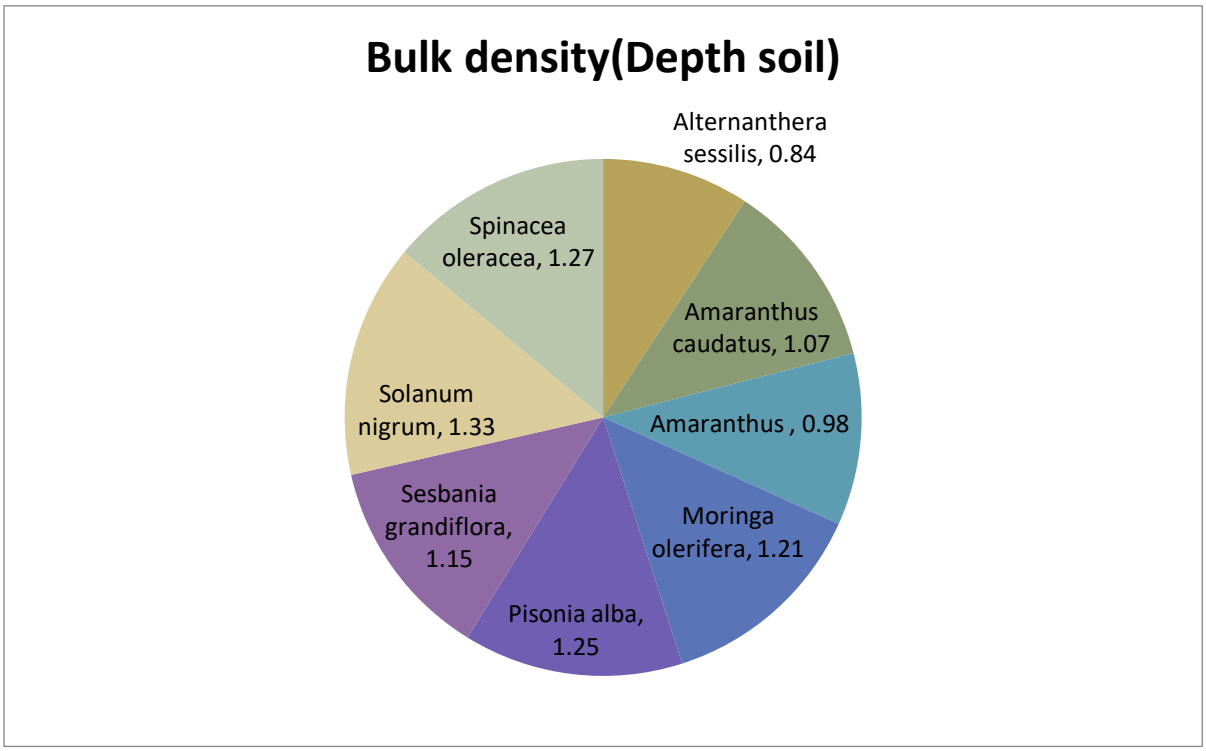
The study area shows,

Average Bulk Density of the farm land ranged from 0.79 to 1.33


Bulk density (Surface soil)
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Alternanthera sessilis
Amaranthus caudatus
Amaranthus
Moringa olerifera
Pisonia alba
Sesbania grandiflora
Solanum nigrum
Spinacea oleracea
Bulk density


Bulk density(Surface soil)
Alternanthera sessilis, 1.08
Amaranthus caudatus, 1.07
Amaranthus , 0.79
Moringa olerifera , 1.16
Pisonia alba, 1.3
Sesbania grandiflora, 1.06
Solanum nigrum, 1.33
Spinacea oleracea, 1.26


Bulk density (Depth soil)
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Alternanthera sessilis
Amaranthus caudatus
Amaranthus
Moringa olerifera
Pisonia alba
Sesbania grandiflora
Solanum nigrum
Spinacea oleracea
Bulk density


Bulk density(Depth soil)
Alternanthera sessilis, 0.84
Amaranthus caudatus, 1.07
Amaranthus , 0.98
Moringa olerifera, 1.21
Pisonia alba, 1.25
Sesbania grandiflora, 1.15
Solanum nigrum, 1.33
Spinacea oleracea, 1.27

Moisture content of soil:

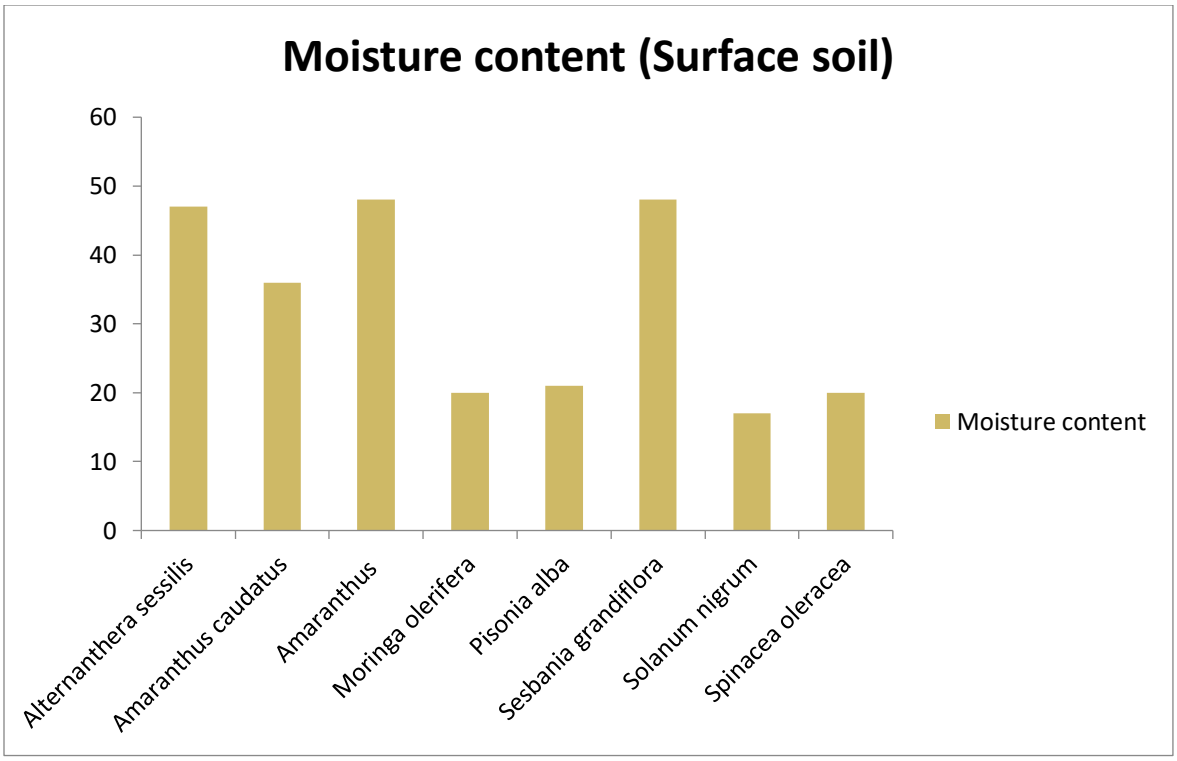
Moisture content of the soil is its water content. Presence of water in soil determines its texture and compactness which ultimately reflects its suitability to support life. Source of moisture in the soil is infiltration and irrigation. Persistence of moisture in soil depends on several factors such as water holding capacity, evaporation and plant uptake.

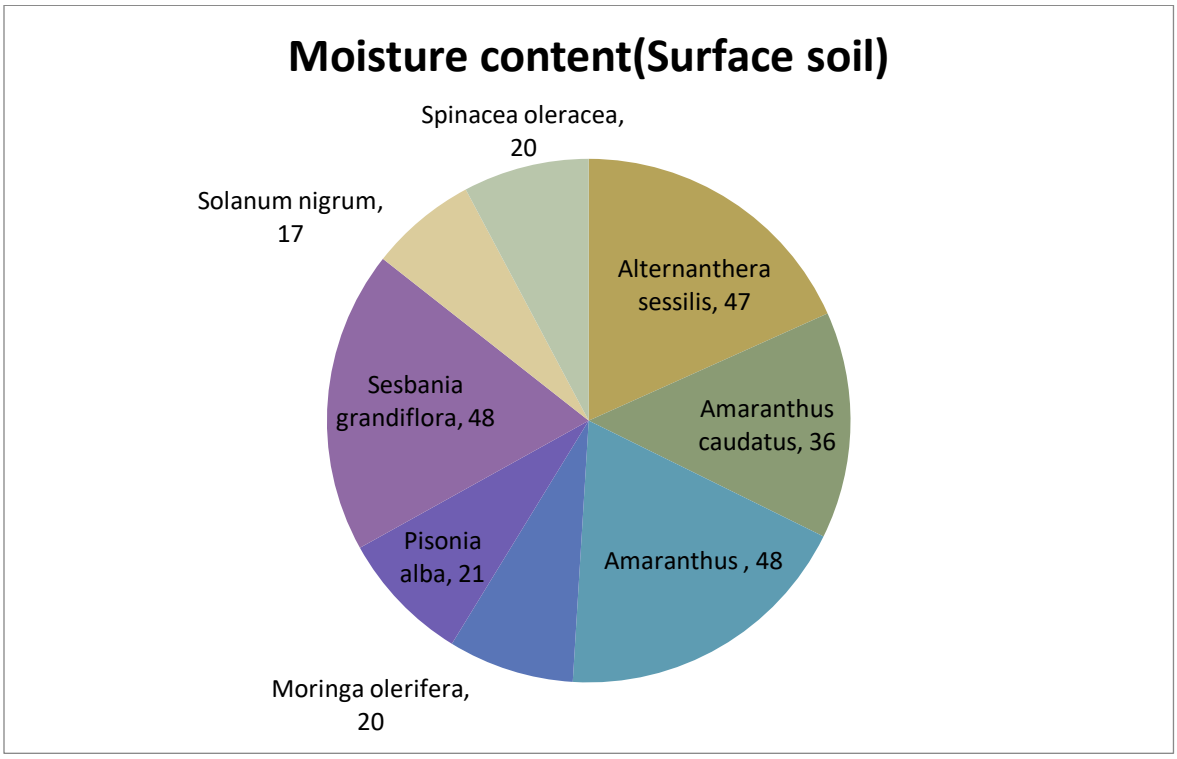
Table :3

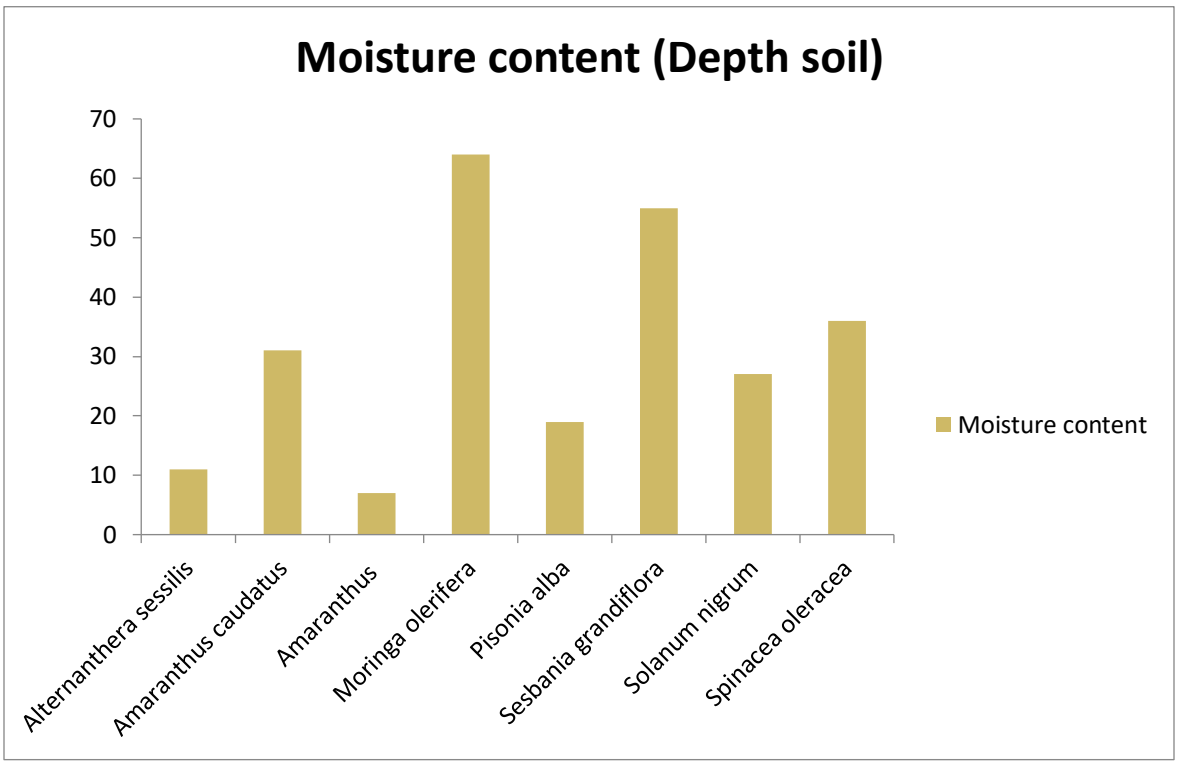
Farm No.	Name of the greens in the field	Soil sample	Moisture content %
1	*Alternanthera sessilis*	Surface soil	47
		Depth soil	11
2	*Amaranthus caudatus*	Surface soil	36
		Depth soil	31
3	*Amaranthus*	Surface soil	48
		Depth soil	7
4	*Moringa olerifera*	Surface soil	20
		Depth soil	64
5	*Pisonia alba*	Surface soil	21
		Depth soil	19
6	*Sesbania grandiflora*	Surface soil	48
		Depth soil	55
7.	*Solanum nigrum*	Surface soil	17
		Depth soil	27
8.	*Spinacea oleracea*	Surface soil	20
		Depth soil	36

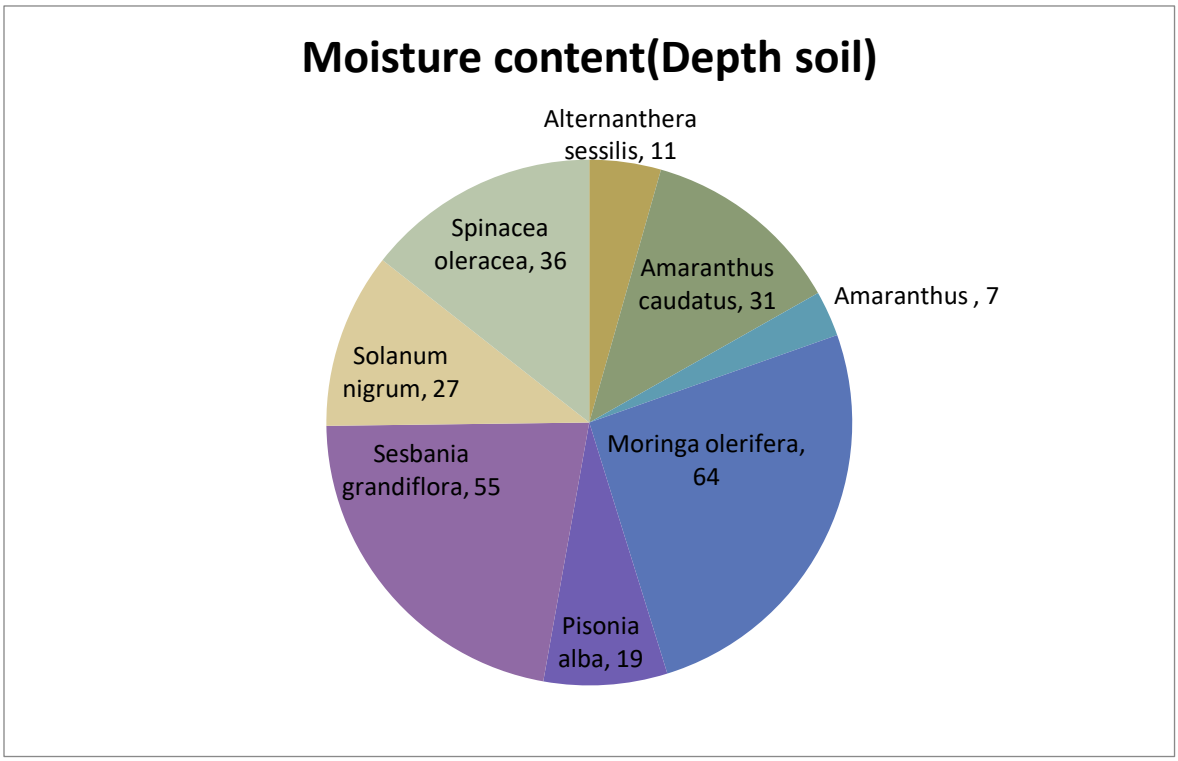
The study area shows,

Average Moisture content of the farm land ranged from 7 to 64


Moisture content (Surface soil)
60
50
40
30
20
10
0
Alternanthera sessilis
Amaranthus caudatus
Amaranthus
Moringa olerifera
Pisonia alba
Sesbania grandiflora
Solanum nigrum
Spinacea oleracea
Moisture content


Moisture content(Surface soil)
Spinacea oleracea, 20
Solanum nigrum, 17
Alternanthera sessilis, 47
Sesbania grandiflora, 48
Amaranthus caudatus, 36
Pisonia alba, 21
Amaranthus , 48
Moringa olerifera, 20


Moisture content (Depth soil)
70
60
50
40
30
20
10
0
Alternanthera sessilis
Amaranthus caudatus
Amaranthus
Moringa olerifera
Pisonia alba
Sesbania grandiflora
Solanum nigrum
Spinacea oleracea
Moisture content


Moisture content(Depth soil)
Alternanthera sessilis, 11
Amaranthus caudatus, 31
Amaranthus , 7
Moringa olerifera, 64
Pisonia alba, 19
Sesbania grandiflora, 55
Solanum nigrum, 27
Spinacea oleracea, 36

CULTIVATION OF GREENS IN STUDY AREA

Common greens are cultivated in Study area.

Crop :

Greens

Season :

Winter

The common greens are cultivated in the study area is given below:

Alternanthera sessilis

Amaranthus caudatus

Amaranthus

Sesbania grandiflora

Solanum nigrum

Spinacia oleracea

Moringa olerifera

Plots of farm land

Water source of farm land

Pesticides used for control pest in the farmland

Alternanthera sessilis:

Sowing method:

Stick

Cultivation area:

0.2 acre

Cultivation method:

- Plough the land using tractor till the sand become very loose condition.
- After 10 days to make plots for ploughed land.
- To make plot, apply the water for minimum amount.
- Next day, take stick of green and planted in the plot of field.
- Apply the water in the plot.
- After 7 days of planting the stick, leaves emerge out of stick.
- Watered regularly.
- After 25 days greens are completely grown.
- To pick the grown greens using handling method.

Manure:

Urea-46-0-0

Seedlings start from:

7 days

Pest:

White fly

Pesticide:

Hunter Bio-pesticide (Alkaloid, salt of fatty acid with mixture of wild plant oil)

Harvested bundles:

100 bundles / day

Yield:

Rs. 500 / day

Amaranthus caudatus:

Sowing method:

Broadcasting

Cultivation area:

0.1 acre

Cultivation method:

- Plough the land using tractor. Till the sand become very loose condition.
- After 10 days to divide the land to make plots for the ploughed land.
- To make plot, apply the water for minimum amount.
- Next day, to sow the seeds and the seeds placed in randomly distance using hand.
- Apply the water in the plot.
- After 3 days seeds are germinated. A very small leaves emerge out from the seeds.
- Watering the seedling regularly.
- After 25 days greens are completely grown.
- To pick the grown greens using handling method.

Manure:

Urea

Seedlings start from:

3 days

Pest:

White fly, beetle

Pesticide:

Bang insecticide, Hunter bio pesticide

Harvested bundles:

100 bundles / day

Yield:

Rs. 500 / day

Amaranthus:

Sowing method:

Broadcasting

Cultivation area:

0.25 acre

Cultivation method:

- Plough the land using tractor, till the sand become very loose condition.
- After 10 days to divide the land to make plots for the ploughed land.
- To make plot, apply the water for minimum amount.
- Next day, to sow the seeds and the seeds placed in randomly using hand.
- Apply the water in the plot.
- After 3 days seeds are germinated, leaves emerge from the seeds.
- Seedlings are watered regularly.
- After 25 days greens are completely grown.
- To pick the grown greens using handling method.

Manure:

Urea

Seedlings start from:

3 days

Pest:

White fly, beetle, Green worm

Pesticide:

Bang insecticide (Alkaloid, Growth promoting enzyme)

Harvested bundles:

2500 bundles / day

Yield:

Rs. 12500 / day

Moringa olerifera:

Sowing method:

Cutting

Cultivation area:

0.12 acre

Cultivation method:

- Plough the land using tractor, till the sand become very loose condition.
- In *Moringa* need minimum amount of water.
- Take the plant cutting and planted in the land.
- The cutting start planting after 7 days.
- To pick the well grown *Moringa* leaves after 60 days.

Manure:

Urea

Pest:

Hairy caterpillar

Pesticide:

Carbaryl 50wp

Harvested bundles:

240 bundles / day

Yield:

Rs. 1200 / day

Sesbania grandiflora:

Sowing method:

Hill dropping

Cultivation area:

0.15 acre

Cultivation method:

- Plough the land using the tractor, till the plough the soil become very loose condition.
- In this green is grown in a tree.
- So seeds are sowing between the another plants. So this green placed in irregular position.
- After 5 days seeds are germinated.
- After the completely grown to cut the greens using sickle.

Manure:

Urea

Seedlings start from:

5 days

Pest:

Beetle

Pesticide:

Hunter bio pesticide (Alkaloid, salt of fatty acid with mixture of wild plant oil)

Harvested bundles:

300 bundles / day

Yield:

Rs. 1500 / day

Solanum nigrum:

Sowing method:

Transplanting

Cultivation area:

0.8 acre

Cultivation method:

- Plough the land using tractor, till the sand become very loose condition.
- After 10 days to divide the land to make plots for the ploughed land.
- To make plot, apply the water for minimum amount.
- To sow the seeds and the seeds are germinated.
- Now the seedlings are transplanted into main field.
- To pick the grown greens using handling method.

Manure:

Urea

Seedlings start from:

3 days

Pest:

Aphid, black ant, white fly

Pesticide:

Hunter bio pesticide, Bang insecticide

Harvested bundles:

80 bundles / day

Yield:

Rs. 400 / day

Spinacea oleracea:

Sowing method:

Broadcasting

Cultivation area:

0.1 acre

Cultivation method:

- Plough the land using tractor, till the sand become very loose condition.
- After 10 days to divide the land to male plots for the ploughed land.
- To make the plot, apply the water for minimum amount.
- Next to sow the seeds by broadcasting method.
- After 5 days seeds are germinated. Leaves emerge from the seeds.
- After 25 days greens are completely grown.
- To pick the grown greens using handling method.

Manure:

Urea

Seedlings start from:

5 days

Pest:

Flea beetles, green worms

Pesticide:

Hunter bio pesticide, Bang insecticide

Harvested bundles:

100 bundles / day

Yield:

Rs. 500 / day

Result of my field work:

Soil analysis:

The eight soil samples collected randomly from the study area were analysed for physical characteristics such as pH, Bulk density, Moisture content.

The results were presented in the Table (1, 2, 3)

pH:

The Study area shows ,

Farm land of the study area is slightly alkaline. (7.97)

In some farm land under the cultivation of greens soil are acidic. (6.2)

Bulk Density:

The study area shows,

Average Bulk Density of the farm land ranged from 0.79 to 1.33

Moisture content:

The study area shows,

Average Moisture content of the farm land ranged from 7 to 64

CONCLUSION:

In this field work clearly explains the variety of greens and explain soil physical properties of that farm area.

Physical characters of soil reported suited for these greens.

So this area soil best suitable for the greens.

Greens cultivation gives a best income for the workers.

Many families to believe that work. Because the cultivation gives best income for daily.

Some reasons of best income for daily;

1. The rate of one bundle is very cheap. So most of the people to buy it.
2. All greens contain high nutritional value, low calories. It is very healthy product.
3. Easy to grow and harvest time is short period.

The cultivation work is heredity of the family by family.

Workers are very experts of this cultivation. Irrigation should be suited for plants growth.

The people well known of the initiation of the greens to till harvest method.

This cultivation is enough to maintain the life time and earn best money of each family.

Greens are cultivated in all season except rainy season, best grown in winter season.

So maximum it give yield for throughout the year. Hence, no loss this work.

These reasons are favourable for the peoples. So most of my village people believe that greens cultivation.

I am suggested that farmer you give a best advantages related in health benefits of greens like good for eyes, reducing high blood pressure, preventing heart disease, maintaining healthy blood vessels and preventing bone loss with aging., during the selling time to customers, nutritional value of greens are recommended.

Phenology of *Abelmoschus esculentus*

A short field project work submitted to

St. Mary's College (Autonomous)

Affiliated to

MANONMANIAM SUNDARANAR UNIVERSITY

In partial fulfillment of the requirement for the Degree of Master of science in Botany

By

J. JEYARANCHINI - 20APBO05

DEPARTMENT OF BOTANY

ST.MARY'S COLLEGE (AUTONOMOUS)

THOOTHUKUDI- 628001

CERTIFICATE

It is certified that this short field project work entitled "**PHENOLOGY OF *Abelmoschus esculentus***" submitted to St. Mary's college (Autonomous) affiliated to MANONMANIAM SUNDARANAR UNIVERSITY in partial fulfillment of the requirements for the degree of Master of science in Botany, and is a record of work done in the Department of Botany, St. Mary's College (Autonomous), Thoothukudi during the year 2020 – 2021 by the following student.

By

J. JEYARANCHINI - 20APBO05

M. Glory
GUIDE

M. Glory
HEAD OF THE DEPARTMENT

EXAMINAR

Lucia Rose
PRINCIPAL
St. Mary's College (Autonomous)
Thoothukudi - 628 001.

ACKNOWLEDGEMENT

We offer our praise and sincere thanks to the Almighty God, his for avalanche of graces and abundant blessings, enabling to complete this project.

We consider is a privilege to express our gratitude to Rev. **Dr. Sr. A.S.J. Lucia rose M.Sc., PGDCA, M. Phil., Ph.D., Principal,** St. Mary's College (Autonomous), Thoothukudi For giving permission for doing research and to utilize the facilities in the Department of Botany and her constant encouragement.

We proudly express our indebtedness to **Dr. M. Glory M.Sc., M. Phil., Ph.D.,** Head of the department of Botany, for her constant encouragement and support. We sincerely thank all of our department for this constant encourage.

We also thank the non-teaching staff members of Department of Botany St. Mary's College (Autonomous) for help in various stages of my work.

DECLARATION

Hereby declare that the short field project entitled **"PHENOLOGY OF *ABELMOSCHUS ESCULENTUS*"** is the original work and it has not been submitted for the award of any Degree, Diploma, Fellowship or any other similar title and that the short field project represents independent and original work on the partial fulfillment of the requirements for the degree of **MASTER OF SCIENCE IN BOTANY** during 2020-2021, submitted to St. Mary's College (Autonomous), Thoothukudi-628 001.

Place: Thoothukudi-628 001

Date: 16.04.2021

J. Jeya Ranchini.

J. JEYA RANCHINI (20APBO05)

CONTENTS

Phenology of *Abelmoschus esculentus*

Introduction

Phenology is the study of periodic events in biological life cycles and how these are influenced by seasonal and inter annual variations in climate, as well as habitat factors. Examples, include the date of emergence of leaves and flowers, the first flight of butterflies, the first appearance of migratory birds, the date of leaf colouring and fall in deciduous trees, the dates of egg-laying of birds and amphibia, or the timing of the developmental cycles of temperate -zone honey bee colonies.

In the scientific literature on ecology, the term is used more generally to indicate the time frame for any seasonal biological phenomena, including the dates of last appearance (e.g., the seasonal phenology of a species may be from April through September).

Because many such phenomena are very sensitive to small variations in climate, especially to temperature, phenological records can be a useful proxy for temperature in historical climatology, especially in the study of climate change and global warming.

Observations of phenological events have provided indications of the progress of the natural calendar since ancient agricultural times. Many cultures have traditional phenological proverbs and sayings which indicate a time for action.

Since the ancient times, humans have been taking phonological observations for various agricultural purposes. From the 18th and 20th centuries, very organized networks for systematic phonological observations have been developed in china, Europe, United States and Russia. These observations are very useful for the development of the science of phenology.

Plant phenology – the timing of plant life-cycle events, such as leaf bud burst, flowering, and fruiting – has cascading effects on multiple levels of biological organization, from individuals to ecosystems.

Phenology not only affects the fitness of individual plants, it also affects the fitness of organisms that depend on them, which, in terrestrial ecosystems, includes virtually all animals. Despite the importance of understanding phenology for managing biodiversity and ecosystem services, studying plant phenology at transcontinental or global scales remains very challenging.

Thus, changes in plant phenology can negatively impact demography, cause rapid evolutionary shifts, and result in agricultural losses. Furthermore, the phenological responses of plants are known to be highly responsive to environmental drivers and thus strongly influenced by climate change.

Advancing our understanding of the drivers of phenological response can provide insight into future states of species distributions, biogeochemistry, and ecosystem services such as pollination. Therefore, increasing scientific understanding of relationships between phenology and the structure and function of ecosystems can help inform adaptive management of natural resources.

phenological data, and it also provides a rigorous description logic-based foundation for these terms so that phenology data can be used directly with knowledge representation systems that support automated reasoning. The

phenology of legumes Van Assche, J A , Debucquoy, K I A & Rommens W A I (2003). Stagno, L , Abbate, C , Intrigliolo, F , Abbate, V & Gennari, M (2008)

Survey of literature showed that vast studies have been done on phenology of legumes Osman, A. E., Pagnotta, A. M., Russi, L., Cocks, P. S. & Falcinelli, M (1990). Yet, & no such comprehensive studies have been carried out in *Abelmoschus esculentus*. By knowing this gap, I have intented to have a similar knowledge on *Abelmoschus esculentus*. This plant has good nutrition & pharmaceutical value. So, knowledge of this plant is imperative. The pertinent study have the following objectives.

Characteristic features of lady's finger plant:

1. **Root:**

   Lady's finger plant has a deep taproot system. There is only one main root or primary root which arises from the radicle of embryo. It persists throughout the life of plant. It gives rise to lateral secondary roots which in turn bear branches of tertiary order. Tertiary roots bear root hairs.

2. **Stem:**

   The stem is semi woody and green in colour. It is erect, robust,variable in branching, with many short branches that are attached to thick semi woody stem. It is 0.5m – 4m in height.

3. **Leaves:**

   In the first week 2 leaves began to appear out. Two days later, two leaves began to grow. The length of leaves is 3cm to 10cm. Each week the leaves continued to grow like this. Lady's fingers are averaging 7-15 centimeters in length.

The leaves are heart-shaped, medium in size. Bright green leaves are covered in small bristles or spines. Leaves have serrated edges, and tapers to a point on the non – stem end.

The leaves grow alternately with 5-7 lobes per stem. Young lady's finger leaves are petite, tender, and mildly grassy while mature leaves will become tougher. The edge of the leaf blade has lobes. It has teeth edges.

4. **Flower:**

The flower shape is funnel – shaped. Flowers are about 2 inches in diameter size. Flower is yellow in color. Life span is 1 day. The flower is solitary.

5. **Fruit:**

The fruit is capsule. The fruit is 18cm in size. It is lantern in shape. The fruit is green in color. The fruit is an elongated, conical or cylindrical capsule. The fruit is actually long pod and generally ribbed, developing in the leaf axil.

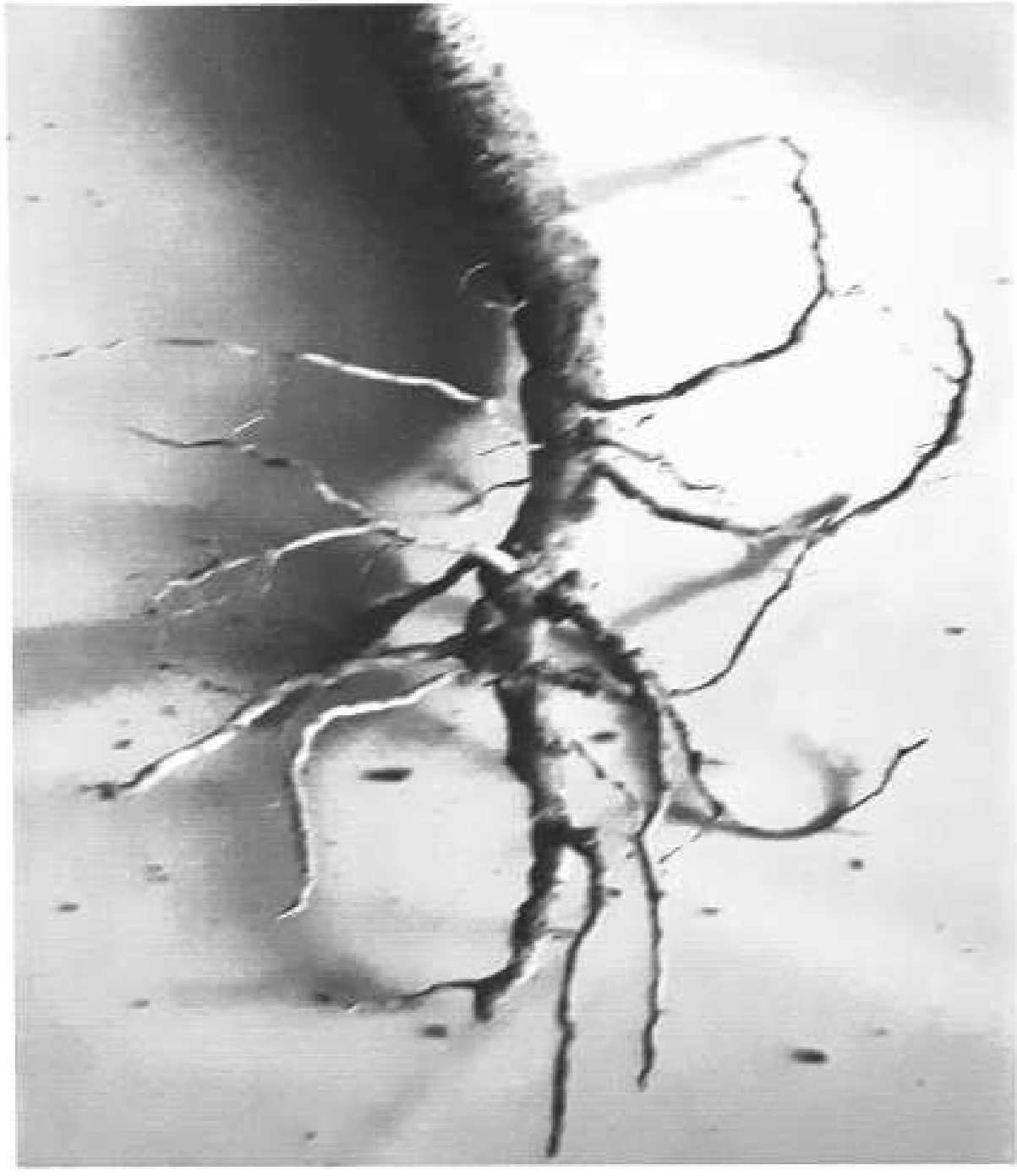
The fruit is normally yellowish green. Lady's finger colour is green when young and colour is dark green when older. It has a smooth texture. It has a horizontal shape with pointed tips.

The plants bears first flower one two month after sowing the fruit is capsule and grows quickly after flowering. The greatest increase in fruit length height and diameter occurs during 4th and 6th day after pollination.

6. **Seed:**

Seed is black in colour. Texture is Smooth, dark green to dark brown seeds.

Root:



Morphology of lady's finger:

Stem

Leaves

Flower

Fruit

Phenology of *Abelmoschus esculentus*:

1. Soaking the seeds:

   Lady's finger is easy to grow but the seeds have a hard coat that can slow germination. So I speed up the process, soak the seeds overnight in warm water before planting. I wrapped the seeds in moist paper towels.

2. Selection of soil & soil bed preparation:

   Lady's fingers requires full sun light (at least 8 hours a day) and prefer soil that is loose, fertile and slightly alkaline. The basic rule is to keep plants separated from each other by about 15 inches plants in beds that are four feet wide by about 20 feet long. Make three rows, and plant seeds about 18 inches apart within each row.

In the presenting & the selected soil PH was estimated. The results are presented (Table 1).

Sample	Soil PH
Abelmoschus esculentus	7. 98

The report shows PH is slightly alkaline. In the present investigation selected soil PH was analyzed & presented. The study showed, the soil is slightly alkaline.

3. Fertilizing:

The garden soil from our backyard is mixed with cow dung, goat dung, ash and vermicompost. The soil ensure fertility of fertilizer. Before planting, use 2-3 pounds of fertilizer such as 4x5 = 20 Square feet of area. Spread the fertilizer evenly over the area, and then mix it well into the 4 inches of soil. Finally, add a half cup of slow release 5- 10 fertilizers for 20 square feet of the soil.

4. Watering:

Irregation was done regularly at morning and evening. Watering should be morning and evening twice in a day. Lady's finger will do fairly well under dry conditions. However, if you water the plants every days, the yield will be higher.

Light watering was done to encourage shallow rooting of the plants. Watering in such a way that moisten the soil to a depth of 6 inches. The soil was kept by regular sprinkling of water.

5. Planting of the soaked Seed :

The first growth stage of lady's finger is the seed phase. Planting the seeds was done at about half an inch deep, about one seed at each spot. Covered it with soil and then watered it. After 3 -7 days seeds started germination.

Lady's finger needs warm weather to grow well. This takes few days in order to produce some roots and eventually some small leaves. One need to take care for it to grow it well. Temperature is 65o F.

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6. Germinating:

This growth stage is start to germinate. Two cotyledonary leaves were exposed become to greener. The cotyledonary seeds seeds were produce some roots and small green leafy.

I have a quality seeds so, Germinating lady's finger seeds were have high percentage of germination .The quality seeds are the mature seeds which are capable of growing. Temperature is 75o F.

7. Young seedling :

This growth stage is seedling phase. This means that the lady's finger is in the form of small plant that show of its sprouted roots and few leaves appear. The seedlings required more care as there stem are very soft and It is very easy to break. It should be protected from wind.

Young seedling lady's finger needs regular watering to ensure healthy germination. It also needs regular water to grow better further. Knowing this, regular water was done periodically. Temperature ranges above 65o F (18o C).

8. Older lady's finger :

This growth stage is in the form of mature plant. The lady's finger started to produce more green leafy hard leaves and produce more stronger stem.

When it gets enough water, sunlight and fertilizer, the best okra was appear. I applied enough fertilizer and continuous watering as it was produce a healthier and stronger plant. I was more effort. So it was surely make to help the plants produced bears more flowers.

9. Flowering :

This growth stage is it start blooming flowers. This means that the lady's finger is in 4 – 5 weeks 1- 2 months old. During this time the beautiful yellow smooth flower began to show off. The flowers are large, pale yellow and fairly ornamental.

Flower opening was initiated between the hours of 6.00am – 6.30am and closes between 11.30am – 12.00pm. Each flower blooms for only one day and eventually forms one lady's finger pod.

The anthesis takes place at the end of the night the flower is open at down, remained open all morning and close in the middle of the afternoon Flower bud appears in the axial of each leaf above 6th to 8th leaves depending the cultivar. Lady's finger flowers can be very attractive.

This stage needs more fertilizer and water. I was applied water every day as it very important. I was putted fertilizer in order for it was to grow healthy. I was looked for some pests that may attack my plants. Temperature is 35o C – 40o C.

10.Lady's finger Bearing :

Lady's finger was began to produced pods for the first time. From the flowers it was formed into a young lady's finger pods.

When the lady's finger started to produced pods, it was already 1-2 months old. I was more care as pest can attacked anytime. I was take some adjustments and improvements. It was help to create a best lady's finger bearing.

11.Harvesting :

This growth stage lady's finger is ready for harvesting. This mean that the lady's finger is mature enough. This is perfect to harvest.

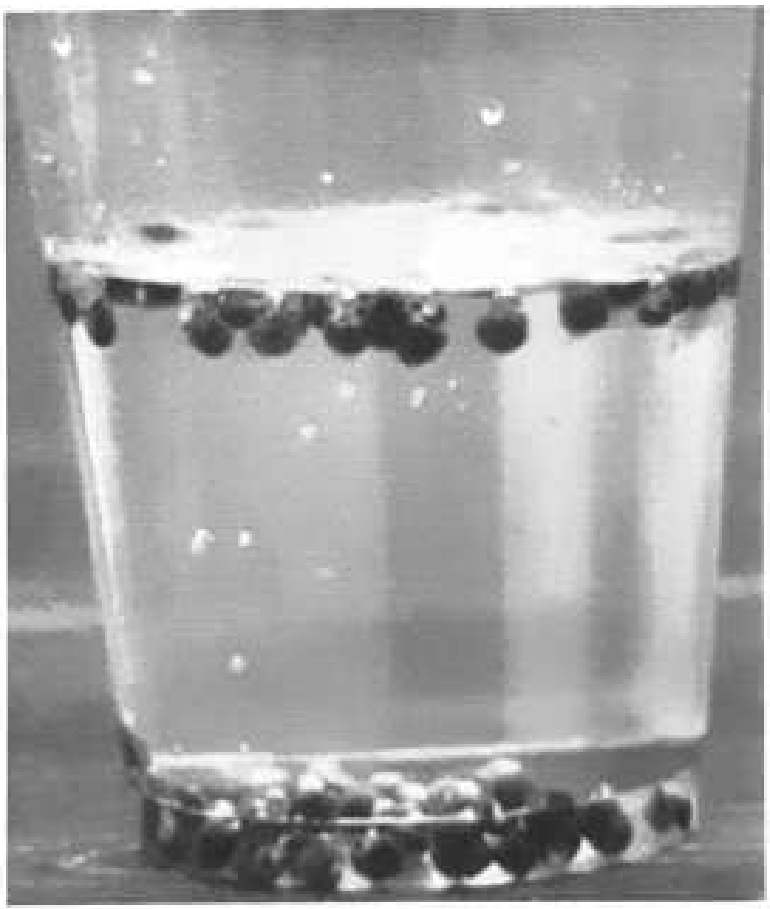
About 50 to 60 days after planting edible pods was started to appear they are tough when mature, so harvest daily with a sharp knife when they are no more than finger sized and when stems are still tender and easy to cut.

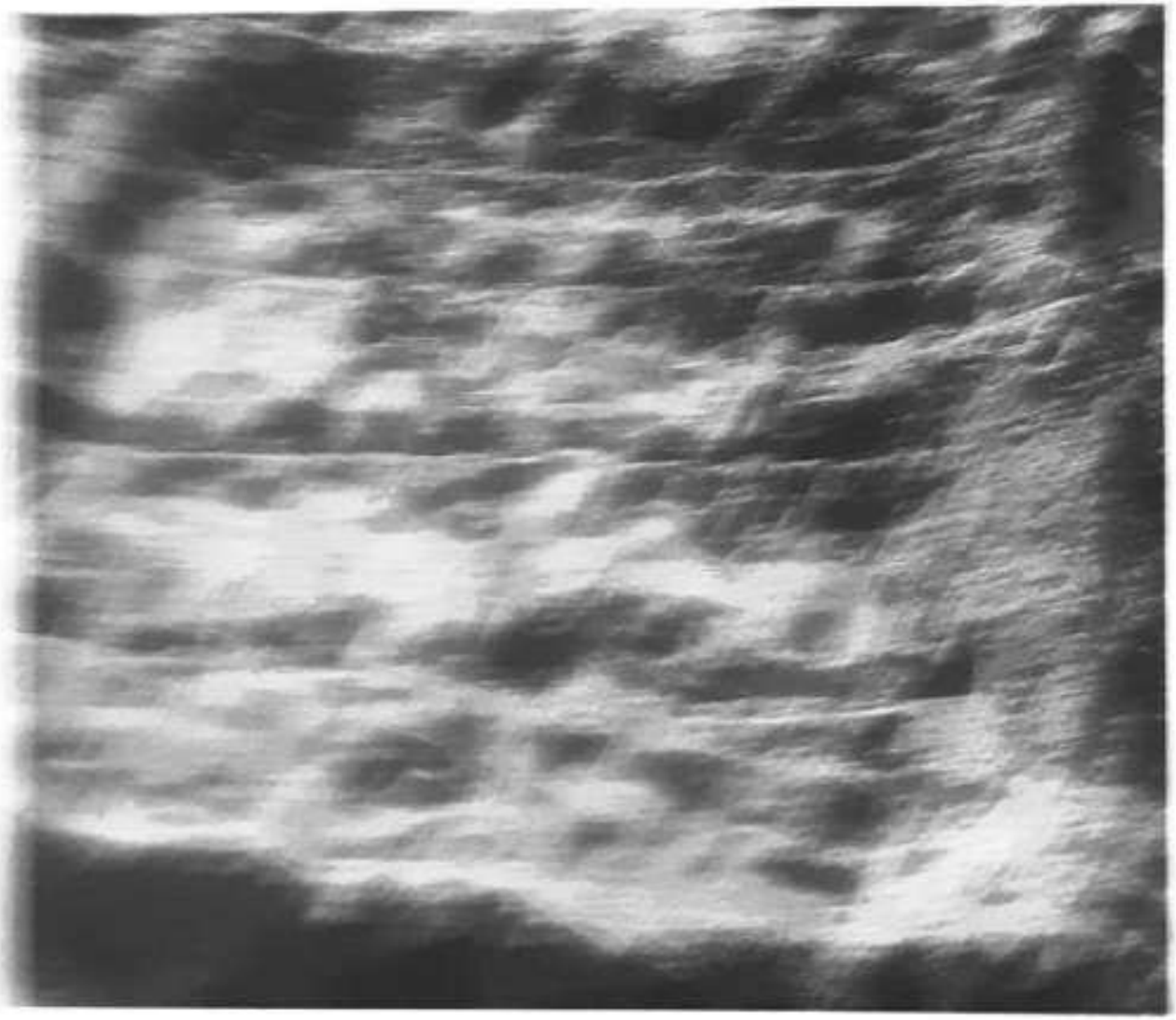
When the lady's finger pod has a hard pod I was now get it. It is not so hard, the pods are just mature enough but so smooth. I saw the colour of lady's finger.

A mature lady's finger pod should have a yellow – green to green in color which indicates it is maturity. It is not good to eat super hard lady's finger pods I was get it when young lady's finger. It was enough length and look differ compared to the previous weeks.

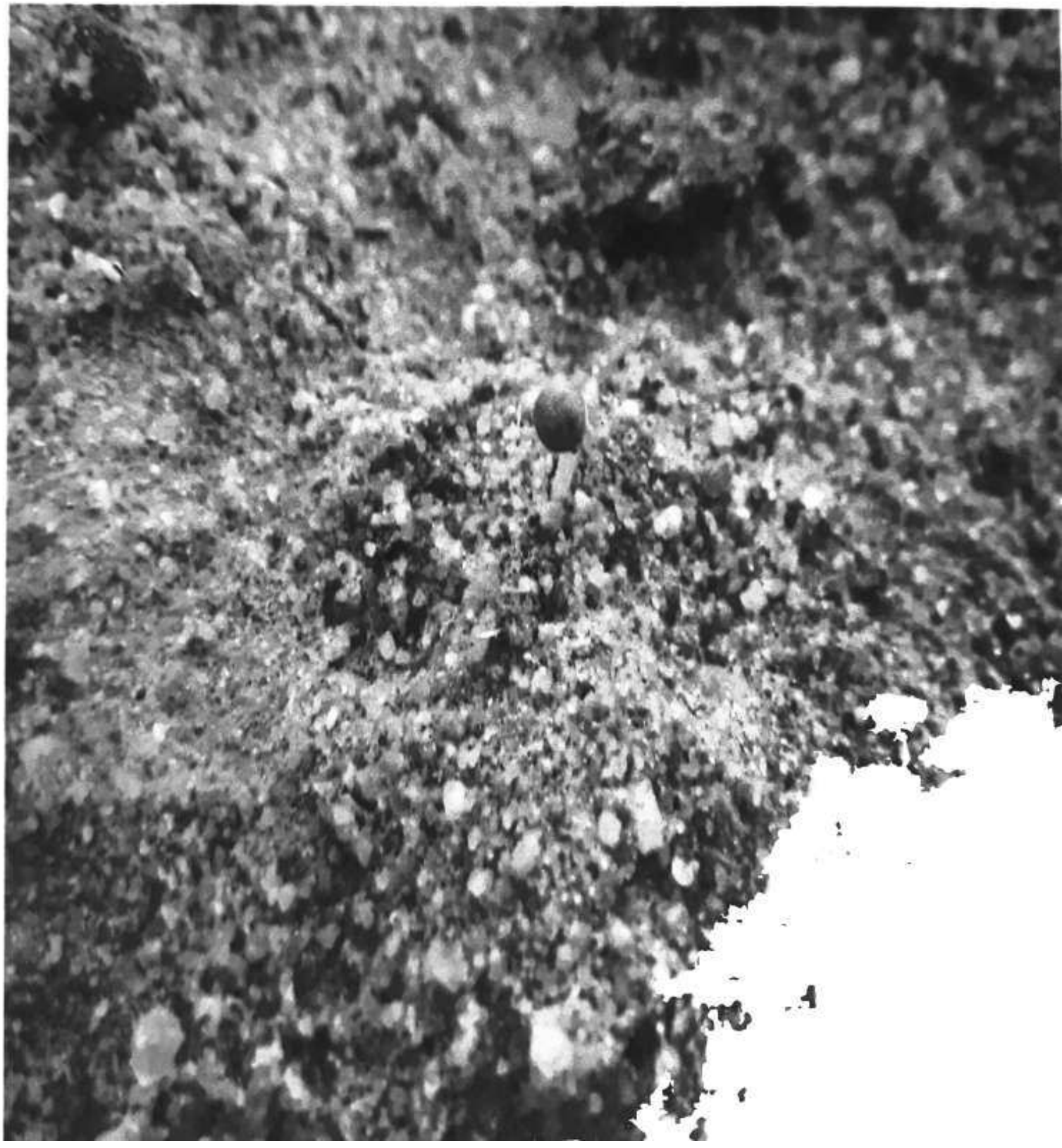
Growth stages ok lady's finger:

Soaking the seeds





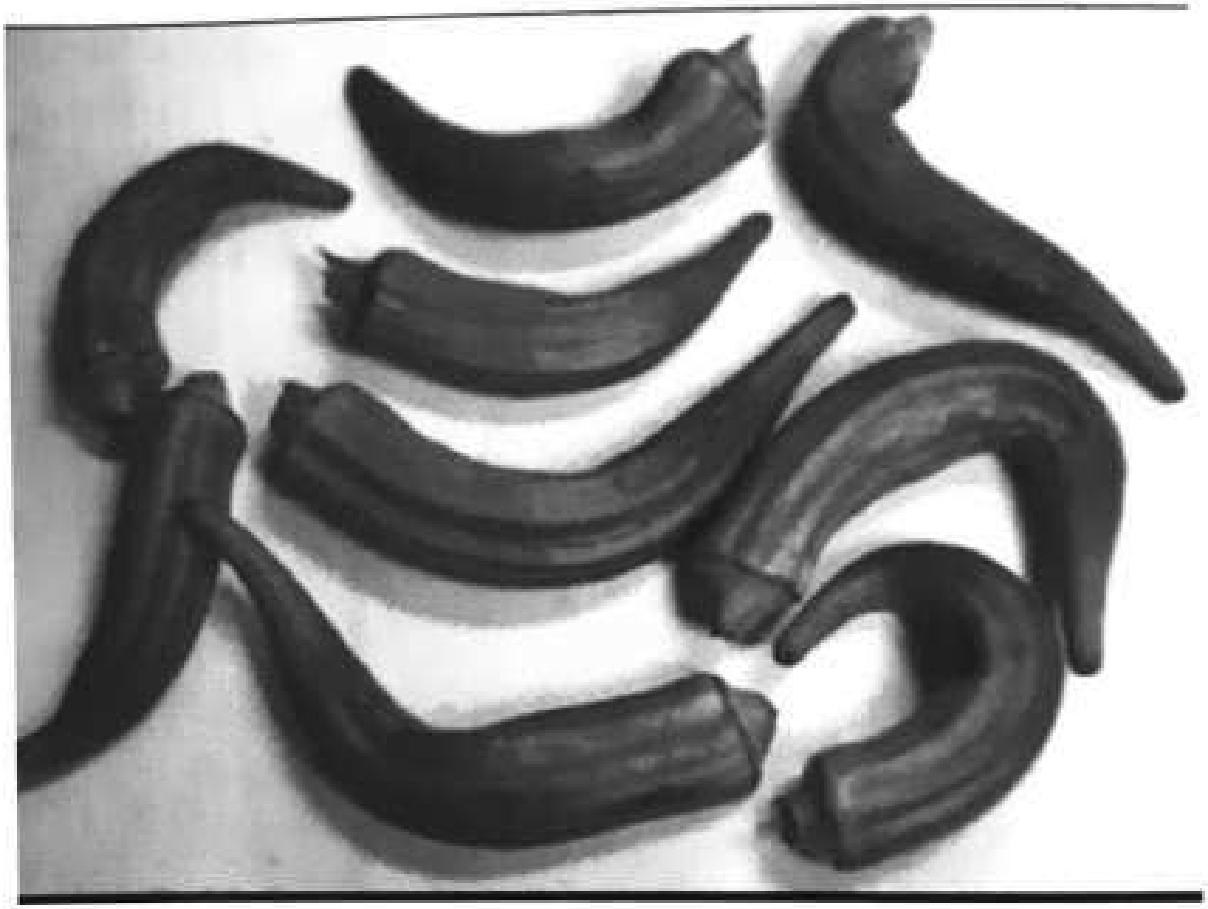
Germinating stage



Flowering stage

Lady's finger bearing

Harvesting stage



Climatic condition required for growing lady's finger:

Lady's finger need temperature above 20o c for normal growth and development germination percentage speed of emergency are optimal at 30 - 35o c. Lady's finger requires long warm growing season during its growing period.

It gives good yield in warm humid condition. It grows best within a temperature range of 22- 35o c. It can be suceesfully grown in the rainy season even in heavy rainfall area. Seeds fail to germinate when the temperature is below 20o c.

Crop plants grow best, where minimum and maximum temperatures are 18o C and 35o C, respectively. High day temperature 42o C may cause dropping of flowers due to increase rate of respiration. Therefore, according to present study, high seed yield of lady's finger can be harvested, when maximum and minimum temperature remains around 33.5 – 36o C, 19.5 – 24o C, 26.9 – 30o C, respectively with 65 – 75 percent relative humidity and 4.1 – 5.7mm daily evaporation.

Flower initiation and flowering are delayed with increase temperature. It is short day plant but, it wide geographical distribution (latitude of 35 - 40o c). Flower initiation and flowering are hardly affected by day length subtropical cultivar.

Pollination Requirements:

The lady's finger pollen grain is large with many pores, and every pore is a potential tube source; therefore, many tubes can develop from one pollen grain. Lady's finger is self – fertile, when the anthers come in contact with the stigmas, self – pollination may result; however, cross – pollination also occurs.

They also reported 4 to 18 percent cross pollination. If the anthers deposit an adequate number of pollen grains on the stigmas to fertilize all of the ovules, and outside agency is not needed to transfer the pollen. Pollen grain of lady's finger are heavy and sticky and could not be readily transferred by wind, therefore insect are responsible for the transfer of pollen grains and consequently cross pollinated in lady's finger plant.

Pollinators:

Lady's finger is not wind pollinated. Lady's finger produce nectars which attract insects to them. I was visited various insect such as butterflies, houseflies, honey bees were seen around the lady's finger flower. It is freely visited by honey bees, but the value of insect pollinator visitation is unknown.

Ant was observed to be the most prominent out of the various insects observed. Ant was observed to start visiting the flower immediately after opening is initiated. Ants can be very destructive to lady's finger pods. Ants feed on the moisture and sugar content of lady's finger. These cause discoloration or distortion of plant.

Studies should be made of seed production and pod development of bagged, and cross- pollinated lady's finger flowers to clarify the pollination requirements and needs for pollinators.

Propagation method:

Seed propagation (Sexual propagation)

Lady's finger plant is potential self pollinated (Autogenous) crop, but considered as often cross – pollinated due to its showy corolla and the extent of cross pollination (4-19%) in particular place depend up on cultivar, competitive flora insect population and season.

Since lady's finger is an often cross pollinated crop an isolation distance of 200m between cultivar is recommended for production of pure seed. Hybrid seed production of the hetero is exploited in lady's finger for production of F1 hybrids; generally hand emasculation and pollination are to produce hybrid seed in lady's finger. Emasculation of flowers of female parent is done before anther.

Emasculated flower are covered with butter paper bags. Pollination is done the next day morning and again covered with the bag. But hand emasculation and pollination are uneconomical due to less seeds/ fruit. Use of mail sterility can be induced by use of chemicals and irradiation.

Advantages of sexual propagation:

Simplest and most economical process among various type of plant propagation. Some plant vegetable species can only through sexual propagation like papaya and tomato.

Stronger and disease resistant and long life span method viral transmission can be prevented this type of propagation and sexual propagation responsible for production of large number of lady's finger crops and too with different varieties and the only propagation process in which result offspring have genetic variation and exhibit diversity of characters from parent crops.

This genetic variation have responsible for continuous evolution that keeps production of lady's finger better and offspring . Easy storage and transmit ion of seed.

Disadvantages of sexual propagation:

Seed take long time to turn plants and time interval between sowing and flowering is longer. Seeding propagated through sexual propagation is unlikely to have same genetic characteristics as that of parent plants. Some plant species do not produce seeds through sexual propagation and hence are unsuitable to propagation for same.

Medicinal and nutritional composition of lady's finger:

Lady's finger fruits have medicinal values, Alkaline reaction, soothes irritated membrane of the intestinal tract, lowering blood sugar, heal burn and any kind of skin rashes, mucilaginous texture soak up unhealthy cholesterol, toxin, mucous waste and clean them from the intestinal tract, acts as laxative that can heal ulcer and may reduce acid reflux, promote good cardiovascular and gastrointestinal health, antioxidant and anticancer.

Abelmoschus esculentus is also one the potential natural plant that been used to manage diabetes. There is no much report available on the bioactive properties of A. *esculentus* despite its wide usage as medicinal plant .Diabetes can be described as increases in glucose in the blood, careful control blood sugar are importance prevents diabetes.

Lady's finger also contains carbohydrates and vitamins. Carbohydrates are mainly present in the form of mucilage. Consumption of young immature lady's finger pods is important as fresh fruits and it can be consumed in different forms.

Dried lady's finger does not provide any beta carotene (vitamin A) or retinol. However, fresh lady's finger pods are the most important vegetable source of viscous fiber, an important dietary component.

Uses of lady's finger plant:

1. Its medicinal value has also been reported in curing ulcers and relief from hemorrhoids.
2. Lady's finger has found medicinal application as a plasma replacement or blood volume expander and also useful in urinary disorders, chronic dysentery.
3. The fruits of lady's finger have reawakened beneficial interest in bringing this crop into commercial production.
4. Its mature fruit and stems contain crude fiber, which is used in the paper industry.
5. Lady's finger helps to stabilize blood sugar as it curbs the rate at which sugar is absorbed from the intestine tract.
6. Lady's finger helps to prevent and improve constipation due to the absorbing water and ensuring bulk in stools.
7. It is a supreme vegetable for those feeling weak, exhausted and suffering from depression.
8. It treats lung inflammation, sore throats and irritable bowel syndrome.
9. Lady's finger is good for atherosclerosis and the fruit is also good for constipation.
10. It protects you from pimples and maintains smooth and beautiful skin.

Soil pH Test:

PH is an important criterion for the evaluation of soil quality measurement. Soil pH indicates the acidity, alkalinity and neutrality of soil solution. Alkaline soils have higher concentration of Na+ ions on the action exchange site whereas acidic soils results from the formation of organic and inorganic acids.

During the process of organic matter decomposition, leaching also contributes significantly to acidity. Based on the concentration of H+ ions in the solution, electrodes of the pH meter acquire electrode potential which can then be measured potential metrically against a calomel electrode. The potential difference between these two electrodes gives the pH value of the given solution.

PROCEDURE:

1. Warm up the instrument for 15 minutes before use.
2. Adjust the ph knob and zero adjustment knob to zero position and set the temperature control knob to the solution temperature.
3. Immense the electrodes in distilled water and see to it that pH knob and zero adjustment knob are pointing towards zero.
4. Now wipe the electrodes with a blotting paper, immerse the electrode into the buffer solution of pH 4.0
5. Keep the functional switch to the pH range of the buffer solution and adjust the pH knob to the exact ph of the buffer solution.
6. Similarly calibrate the pH for buffer solution 9.2
7. Now take 10g of soil in a 1000ml beaker.
8. Stir well and allow the soil suspension to settle.
9. Immerse the electrodes carefully into the soil suspension.
10. Take care to stir well the solution before taking the reading.
11. Adjust the functional switch to the particular ph range and record the PH.

PH of the lady's finger soil is – 7.98

SOIL BULK DENSITY:

Soil bulk density is ratio of the mass of the soil to the bulk volume expressed in grams per cubic cm (g/cm3) or tons per cubic meter (t/m3), which includes the volume of both solids and space at specified soil water content. The bulk density is dependent on conditions at sampling time.

Soil bulk density is the mass per unit volume of the soil, which varies with different soil types. Determination of bulk density is considered to be important because it restricts infiltration and makes soil impermeable. Also it prevents root penetration into deeper regions. The specific gravity of the soil is directly related to its bulk density.

PROCEDURE:

1. Homogenize the soil samples.

2. Dry the soil in an oven at 105 degree C till weight is obtained.

3. Weigh a 100ml graduated cylinder ad record its weight (W1)

4. Transfer the dried soil sample to the weighed measuring cylinder.

5. Keep adding soil and tapping the cylinder until a tapped soil volume of 100ml is obtained. Weigh the cylinder containing the soil, and record its weight (W2).

6. Do a duplicate analysis by repeating the entire procedure with another portion of soil. The average of the two analyse is the bulk density.

Soil sample - *Abelmoschus esculentus*

Volume of soil sample (cm3) - 50

Weight of soil sample (g) - 214.35

$$\text{Bulk density} = \frac{\text{weight of soil (gm)}}{\text{Volume of sample (Cm3)}}$$

$$= \frac{W2-W1}{100}$$

$$= \frac{214.35 - 146.58}{50}$$

Bulk density of lady's finger soil is = 1.35 g/cm3

MOISTURE CONTENT OF SOIL:

Moisture content of the soil is its water content. Presence of water in soil determines its texture and compactness which ultimately reflects its suitability to support life. Source of moisture in the soil is infiltration and irrigation. Persistence of moisture in soil depends on several factors such as water holding capacity, evaporation and plant uptake.

PROCEDURE:

1. Weigh 10g (M2) air dry soil into a previously dried (105) and weighed (M1) petridish.
2. Dry in an oven with the lid unfitted at 105 degree C.
3. Next, when the soil has dried, remove the container from the oven.
4. Cool it in a desiccators for 30 minutes and re-weigh (M3).

Sample - *Abelmoschus esculentus*

Initial weight
Of soil (g) - 36.91

Final weight
Of soil (g) - 46.21

$$\text{Moisture} = \frac{\text{Amount of Moisture In the soil}}{\text{Weight of dry soil}} \times 100$$

$$= \frac{M2 - M3}{M3 - M1} \times 100$$

$$= \frac{36.91 - 46.21}{46.21 - 32.06} \times 100$$

Moisture content of the lady's finger soil is = 5%

Conclusion:

Lady's finger is a medicinal plant of immense importance with large pharmacological applications. It has been used as an ingredient of many herbal formulations, which are used for the cure of various ailments, in particular the regulation of blood pressure, fat, diabetes, chronic dysentery genitourinary disorders, simple goiter and ulcer.

We found that application of organic amendments has a significant effect on germination of lady's finger seed compared to inorganic fertilizers. Vermicompost showed more positive effects on germination as well as plant growth and development.

However, farmyard manure had shown higher influence on germination than that of plant growth. While, biochar exhibited delayed seed germination but enhanced plant growth. Among the studied organic amendments, the use of vermin compost could be a better option to achieve enhanced growth of lady's finger seedling.

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A COLLECTION OF PLANT DISEASES IN THOOTHUKUDI

A short field project work submitted to

St. Mary's College (Autonomous)

Affiliated to

MANONMANIAM SUNDARANAR UNIVERSITY

In partial fulfilment of the requirement for the Degree of Master of science in Botany

By

N.MARIA REENA MAXILDA - 20APBO06

DEPARTMENT OF BOTANY

ST.MARY'S COLLEGE (AUTONOMOUS)

THOOTHUKUDI-628001

CERTIFICATE

It is certified that this short field project work entitled "**A collection of plant diseases in Thoothukudi district**" submitted to St. Mary's college (Autonomous) affiliated to MANONMANIAM SUNDARANAR UNIVERSITY in partial fulfilment of the requirements for the degree of Master of science in Botany, and is a record of work done in the Department of Botany, St.Mary's College (Autonomous), Thoothukudi during the year 2020 – 2021 by the following student.

GUIDE

HEAD OF THE DEPARTMENT (i/c)

EXAMINER

PRINCIPAL
St. Mary's College (Autonomous)
Thoothukudi - 628 001.

ACKNOWLEDGEMENT

We offer our praise and sincere thanks to the Almighty God, his for avalanche of graces and abundant blessings, enabling to complete this project.

We consider is a privilege to express our gratitude to Rev. **Dr. Sr. A.S.J. Lucia rose M.Sc., PGDCA, M. Phil., Ph.D., Principal,** St. Mary's College (Autonomous). Thoothukudi for giving permission for doing research and to utilize the facilities in the Department of Botany and her constant encouragement.

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We also thank the non-teaching staff members of Department of Botany St. Mary's College (Autonomous) for help in various stages of my work.

CONTENTS

INTRODUCTION

With the vast diversity of plants, there are a large number and kind of diseases that affect them. On an average, each kind of plant can be affected by one hundred or more plant diseases. Each kind of pathogen may affect anywhere from one variety to several dozen or even hundreds of species of plants. To facilitate the study of plant diseases, these can be grouped in some generalize categories. It also becomes essential for further identification and the subsequent control of any given plant disease. There are several criteria that may be used as a basis for classification of plant diseases. Plant diseases are classified according to:-

- Symptoms they cause (root rots, cankers, wilts, leaf spots, scabs, blights,
- anthracnoses, rusts, smuts)
- The plant organ they affect (root diseases, stem diseases, foliage diseases,
- fruit diseases)
- The types of plants affected (field crops diseases, vegetable diseases, fruit
- tree diseases, forest diseases, turf diseases, diseases of ornamental plants)
- The extent to which plant disease is associated with plant:-
  Localized disease: affecting only a part of the plant.
  Systemic disease: affecting the entire plant.
- Mode of natural perpetuation and mode of infection:-
  Soil borne: Inoculums of the diseases causing pathogen remains in soil and penetrate the plant resulting in diseased condition e.g. Root rot, wilt
  Air borne: The micro-organisms are spread through air and attack the plants causing diseases. e.g. Blight, rust, powdery mildew.
  Seed borne: The micro organisms are carried along with seeds and cause diseases when congenial condition occurs. e.g. Damping off.
- Occurrence and distribution of plant disease geographically:-

Endemic: When a disease is more or less constantly prevalent from year to year in a moderate to severe form in a particular country. e.g., Wart disease of potato is endemic to Darjeeling.

Epidemic or epiphytotic: A disease occurring periodically but in a severe form involving major area of the crop. It may be constantly present in locality but assume severe form occasionally e.g. Rust, Late blight,Mildews.

Sporadic: Diseases which occur at very irregular interval and location in a moderate to severe form e.g., leaf blights, wilt.

Pandemic: Diseases occurring throughout the continent or sub-continent resulting in mass mortality e.g., Late blight of potato.

However, the most commonly used criterion is the type of pathogen that causes

the disease. On the basis of causal factors, diseases are classified into two

groups.

1. Non parasitic disease: The causal factors of these are mainly physiological

or environmental like freezing injury caused by low temperature, high

temperature, unfavourable oxygen or soil moisture, mineral deficiency or excess mineral etc. Example: Black heart of potato caused due to high temperature, Bark necrosis of red delicious apple caused due to excess mineral, Red leaf of cotton and Khaira disease of rice is caused due to mineral deficiency.

2. Parasitic disease: The causal factors of the disease are parasitic micro or macroorganisms which need a host plant to survive or to complete the life cycle. Various fungi, bacteria, viruses, mycoplasma, algae and animal parasites such as nematodes etc. parasitize and cause disease in host plants.

Example: Bacterial blight of paddy, Club root of crucifer caused by mycoplasma, Tobacco mosaic by virus, ergot, smut and rusts caused by fungi, ear cockle of wheat caused by nematode etc.

- 1885-Bordeaux mixture : Downy Mildew of Grape was a great problem in the growing fields in France but there were no known ways of controlling it. The vineyards were also troubled with pilferers. They began applying a mixture of copper

sulfate and lime to the plants along the edges of the fields. It was also observed that these plants held onto their leaves throughout the season.

- 1900- White Pine Blister Rust caused by Cronartium ribicola. The pathogen was introduced on seedlings from European nurseries. White pines, especially young trees and plants belonging to the genus Ribes(currants and gooseberries) are susceptible to the disease. Although White Pine Blister Rust is occasionally a severe foliar disease on Ribes plants, on white pines it is lethal if allowed spreading from an infected branch into the trunk. This disease caused the first US quarantine in 1912.
- 1904-1940- Chestnut Blight caused by Cryphonectria (Endothia) parasitica. Chestnut seedlings imported from the orient brought with them the pathogen that killed off all the mature chestnuts in eastern North America. The disease devastated the people who relied on the chestnut tree for their livelihood. There are still some sprouts left in the forests, put up by the living root systems, but they too eventually succumbedto the blight.
- 1910- Citrus Canker caused by Xanthomonas axonopodis pv. Citri, was discovered near the Georgia border and was eradicated in 1931. The pathogen was found 400 miles awayin Dade County in 1912. The pathogen spread throughout the Gulf States and as far north as South Carolina. It took more than 20 years to eradicate that outbreak of citrus canker. In 26 counties, over 250,000 grove trees and over 3 million nursery trees were destroyed by burning. Subsequent outbreaks occurred in 1986 and 1995.
- 1930- Dutch Elm Disease caused by Ophiostoma ulmi. This disease devastated mall and roadside planting of American elm trees throughout much of the United States.
- 1941- Golden Nematode, Globodera rostochiensis, was discovered in 1941.It caused a slow decline in potato plants that eventually lead to death. As of 1955, the distribution was believed to be located only in Nassau and Suffolk countries in NYS. After decades of building their population levels, the Golden Nematode was capable of reducing the potato yield up to 70%.
- 1970- Southern Corn Leaf Blight caused by Helminthosporium maydis. Originally considered a minor disease, a change in the genetics of seed corn caused an epidemic. In 1970, the disease was reported in every state east of the Mississippi River, also in several states west of the Mississippi River. In some areas damage caused losses of 50-100%. Nationally losses averaged 20-30%. 1999- Plum Pox, caused by Plum

Pox Virus, is a disease of stone fruits caused by a viral pathogen called the Plum Pox Virus, also known as"Sharka". It was first discovered in an Adams County, Pennsylvania Orchard in 1999.

- 2004-Soybean Rust, is caused by two fungi named Phakopsora pachyrhizi and Phakopsora meibomiae. P. pachyrhizi appeared in the US in November 2004, apparently entering on winds of Hurricane Ivan. It was found in 9 States shortly thereafter. It was found in Florida early in 2005 on Soybean and new detections for that season remained in the South.

Plant pathology may call upon the basic techniques and knowledge of botany, mycology, bacteriology, virology, nematology, plant anatomy, plant physiology, genetics, biochemistry, horticulture, soil science, forestry, chemistry, physics, meteorology and many other branches of science. Plant pathology profits from advances in any one of these sciences, and many advances in other sciences have been made in the attempt to solve phytopathological problems.

A good knowledge of at least the basic facts of the related sciences is indispensable for efficient performance by any plant pathologist. Although plant pathology as a science attempts to increase our knowledge of the causes and the development of plant diseases, it is also a science with a more practical goal. The purpose is to develop controls for all plant diseases. The goal is to save the produce which today is destroyed by plant diseases and to make it available to the growers who work hard to produce it and to the hungry and ill-clothed millions of our increasingly overpopulated world. methods of cultivation, increased use of fertilizers, improved varieties, increased irrigation and crop protection.

the living entities and the environmental conditions that cause disease in plants; the mechanisms by which these factors produce disease in plants; the interactions between the disease causing agents and the diseased plant; and the methods of preventing disease,alleviating the damage it causes, or controlling a disease either before or after it develops in a plant.

Groundnut showing black spots

Leaves showing black spots

Blackspots showing spores under microscope

Tikka Disease of Groundnut

Symptoms:

All parts of the host plant above soil level are attacked by the disease. The first visible symptoms appear on the leaflets of lower leaves as dark spots which at a later stage, are surrounded by yellow rings. The spots are circular. They appear in a large number on the leaves. Mature spots are dark-brown to almost black, particularly on the upper surface of the leaflets .

Whereas, on the lower surface they are lighter in colour. The spots are few on the leaf petioles and stem. Sometimes spots coalesce resulting in the defoliation. The shedding of leaves is a characteristic feature of the disease. Due to excessive spotting and consequent leaf fall, smaller and fewer nuts are formed.

Causal Organism of Tikka Disease of Groundnut:

The spotting is due to the attack of Cercospora personata (Berk. & Curt.) Ell. & Ever., the conidial stage of Mycosphaerella berkeleyii Jenkins; and Cercospora arachidicola Hori, the conidial stage of Mycosphaerella arachidicola Jenkins.

Cercospora personata possesses mycelium which is entirely internal and ramifies intercellularly by developing haustoria in the palisade and spongy mesophyll cells of the host. The mycelium forms dense stroma which produces long septate to non- septate geniculate hypophyllous conidiophores .

The conidiophores emerge in tufts by rupturing host epidermis. Conidia are pale-brown, obclavate or cylindrical, septate, measuring 30-50 μ in length and 5-6 μ in breadth .

A ready and reliable means of distinguishing the spots produced by these two fungi are: spots due to C. personata are more circular and smaller than those produced by C.

arachidicola. These are the typical 'Tikka' disease spots which almost cover the entire leaf surface in an epiphytotic condition of the disease.

Again in the latter a yellow halo is present around spots from the beginning while in the former halo develops at maturity of the spots. Besides this, the colour of the spots on the lower surface of the leaflets produced by C. personata is carbon black and those produced by C. arachidicola are light-brown.

Although the spots produced by C. personata appear late, yet they are more dangerous than those of C. arachidicola being rapid in their development resulting in severe epiphytotic.

Disease Cycle of Tikka Disease of Groundnut:

The pathogen perennates through conidia on diseased plant debris lying in the soil. The conidia may also remain adhered to shell. They have also been found to remain associated with the seeds and are responsible for primary infection. A temperature range of 26°C. to 31 °C. with high atmospheric humidity is favourable for disease development.

Prolonged low temperature and dew also favour infection. The entrance of the pathogen in the host tissue takes place either by direct penetration through the epidermal cells or by way of stomata.

The leaf infection is largely through the upper surface of the leaflets. The fungus mycelium ramifies the host tissue in and around the infection court and aggregates underneath the epidermis and forms stroma.

During the development of stroma the epidermis is ruptured by the pressure developed in the host tissue and the conidiophores developed from the stroma emerge out, ultimately conidia are produced on them. These conidia form the secondary inoculum through which secondary infection is induced.

The disease is disseminated by wind which blows the conidia from leaf to leaf. Insects and splashes of rain have also been reported to play role in the disseminator of the disease.

Application of nitrogen and phosphatic fertilizers often makes host plants susceptible to infection.

Control of Tikka Disease of Groundnut:

(i) Burning of previous year's diseased plant debris will, to a great extent, reduce the source of primary infection.

(ii) Two to four years' crop rotation often cuts down the rate of infection.

(iii) (iii) Avoid late sowing to reduce infection rate.

(iv) Seed disinfection checks the disease incidence. Care should be taken to remove the shells thoroughly and the adhereing soil before seed disinfection. Treatment for half an hour in 0.5 per cent. copper sulphate solution is recommended for seed disinfection. Agrosan GN also is an effective disinfectant. Seed dressing with Thiram (1: 350) or Flit 406 (1: 500) before sowing prevents Aspergillus seed rot and pre-emergence losses.

The spread of secondary inoculum can be controlled by spraying the crop with different fungicides. Three sprayings of Bordeaux mixture (4:4: 50) with linseed oil as sticker at an interval of 15 days are effective in checking the disease. A similar treatment with Dithane Z-78 has also produced good results. Spraying with 0.15 per cent. Cupravit or 0.15 per cent. Perenox also produces effective results. To obtain best results, timely and thorough spraying of the crop should be done. Care should be taken that under surface of the leaves is thoroughly sprayed.

(vi) Dusting with sulphur (six applications at 10 days interval) or with sulphur containing 3.5 per cent, metallic copper has been found to be very useful for controlling the disease.

(vii) The use of resistant varieties is the most effective means to combat the disease.

Red rot of sugarcane

DESCRIPTION OF THE DISEASE

Red rot attacks the stalks, stubble rhizomes, and leaf midribs of the sugarcane plant. It may invade leaf-blade and leaf-sheath tissues and is capable of infecting sugarcane roots . but it is not important as a disease of these organs.

RED ROT IN THE STALK

Red rot is frequently not discernible from external examination of the sugarcane stalk unless it has so completely rotted the interior as to cause the rind to lose its natural bright color and become dull in appearance. Such an advanced stage of rotting of standing cane seldom occurs in the United States, although it has been observed in P. O. J. 2714 in southern Florida. Plants so affected may be detected by the yellowing, shriveling, and dying of the upper leaves. More certain identification of red rot may be made by splitting the stalk of standing cane, seed cutting, or stubble rhizome. The disease is recognized by the longitudinal reddening of the normally white or yellowish-white internal tissues of the internode, especially when this red color is interrupted by occasional white patches extending crosswise of the stalk . Unless these whitish patches are present, identification as red rot is often uncertain without microscopic examination or culturing of the causative fungus.

symptoms

RED ROT ON THE LEAF MIDRIBS

Louisiana and Georgia expressed that The lesions on the leaf midribs originate as dark reddish areas, which usually elongate rapidly and sometimes extend the entire length of the leaf. The young lesions are blood red in color with darker margins. The centers become straw-colored with age, and when fructification of the fungus begins, the lesions are covered with black powdery masses of conidia , The lesion from a single point of infection is usually continuous along the midrib, but sometimes it is broken up into a succession of red blotches alternating with Typical internal stalk symptoms of the red rot of sugarcane on a very susceptible variety, showing whitish patches extending across the rotted areas and matted mycelium in the pith cavity in one of the two diseased stalks, contrasted with the natural color of a healthy stalk.

Atkinson and Edgerton's demonstration of the movement of conidia in the stalks of sugarcane through the vascular bundles in the transpiration stream, lodging at intervals and initiating new points of infection, suggests that a similar movement m the leaves may be responsible for the discontinuous lesions that are commonly observed on many varieties

. 4. Disease Cycle of Red Rot of Sugarcane:

The sources of primary inoculum are the old fragmented stalks and leaves and other rubbish on which the fungus grows saprophytically; and unknowingly planted diseased stock during

cultivation. Ratoon crops also serve as a source of primary inoculum. Opinions differ whether the fungus is strictly saprophytic or parasitic.

The conidia that are produced in the acervuli developed along the midribs of the diseased leaves during primary infection, form the secondary inoculum. They are disseminated by wind, rain splashes, irrigation water and also by insects. The conidia germinate readily by germ tube which on coming in contact with any hard surface, e.g., soil particles or plant parts, forms appressorium from-which infection hypha is produced.

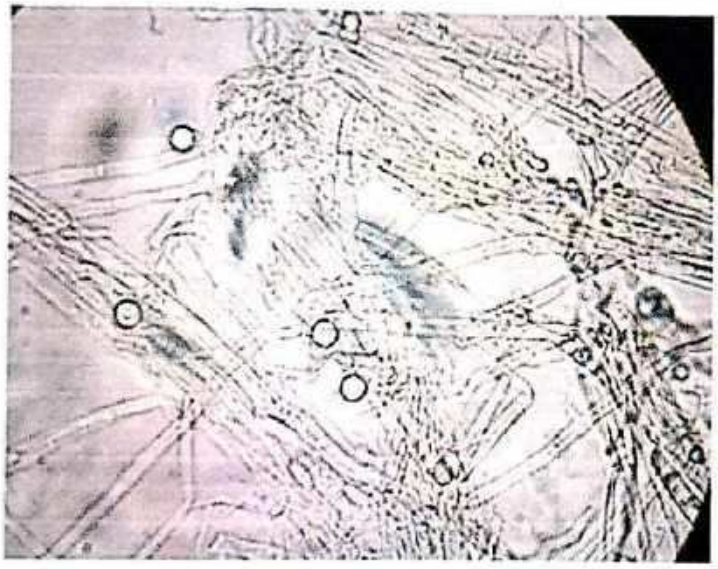
The pathogen may gain entrance through the nodes at the leaf scars, through any kind of wound, through root primordia and seed-cuttings. The diseased canes are frequently found to be injured by insects, especially borers, and no doubt these wounds facilitate the entrance of the fungus, which in turn does much more damage than the insects.

. Control of Red Rot of Sugarcane:

Red rot of sugarcane is hard to control because the stalk from which seeds are prepared has been largely affected from the time of planting, and fungicides cannot reach the infected tissues inside a diseased seed sett. Therefore careful selection of red rot-free seed setts is recommended for planting. Seed should always be taken from disease-free nurseries examined regularly by the cane protection staff.

Sugar cane nodes and internodes showing red rot

Colleotricum sp showing mycelium under microscope



Before planting, each seed sett should be carefully examined and those setts which show reddening should be discarded.

The spread of the red rot can be prevented during the growing season by timely rogueing and burning of the affected clumps with utilization of the green leaves for cattle fodder. In no case ratoons of sugarcane should be kept in the red rot affected fields. Attention should always be given to sanitation by digging out stubbles of diseased canes and burning them with other trash in the field.

Where facilities are available for hot water treatment of seeds, they can be utilized for controlling red rot of seed (treat in water at 50°C., for two hours). Treating seed with fungicides like Arasan (0.25 per cent.) is often effective.

The use of sugarcane varieties resistant to red rot is also recommended. Some of the resistant varieties are: Co. 975, 1148, 1158, 1336 and 6611; Co. S 561, 574; B.O. 3, 10, 47.

The possibilities of an epidemic is very much minimized with the practice of long crop rotations (2 to 3 years) where planting is done in plots.

One of the best ways to reduce the incidence of the disease is to raise healthy stock for planting in plots especially fertilized, cultivated, and kept disease-free by constant care.

Citrus canker

Causal organism

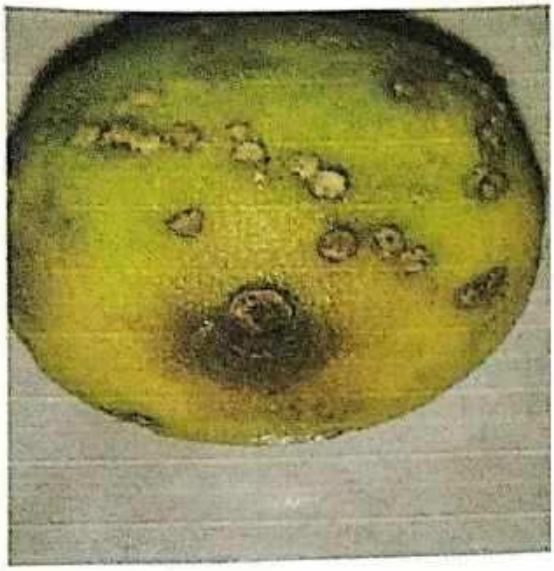
Different pathovars of Xanthomonas citri (syns. X. campestris, X. axonop-odis) have been reported to cause citrus canker disease. Both the pathogens haveapproximately similar symptoms; nevertheless, separation can be made based on host series, cultural and biochemical characteristics.

symptoms

Gottwald and Graham, 1992 suggested that On twigs and fruit, raised corky lesions surrounded by an oily or water-soaked margin . No chlorosis surrounds twig lesions , but may be present on fruit lesions . Twig lesions on angular young shoots perpetuate Xac inoculum in areas where citrus canker is endemic. Twig dieback, fruit blemishes , and early fruit drop are major economic impacts of the disease in advanced stages. If twigs are not killed back by girdling infections, the lesions can persist for many years, causing raised corky patches in the otherwise smooth bark.

(Das, 2003) expressed that The earliest symptoms on leaves appear as tiny, slightly raised blister-like lesions around 7 days after inoculation under optimum conditions. Optimum temperature for infection falls between 20 and 30°C (48). Under less than optimum infection and incubation conditions, symptoms may take 60 days or more to appear . As the lesions age, they first turn light tan, then tan-to-brown, and a water-soaked margin appears, often surrounded by a chlorotic halo . The water-soaked margin may disappear as lesions age, and is not as prominent on resistant cultivars . The center of the lesion becomes raised and spongy or corky . These raised lesions from stomatal infection are typically visible on both sides of a leaf. Eventually, the centers of leaf lesions become crater-like and may fall out, creating a shot-hole effect. Defoliation becomes a problem as the disease intensifies on a plant

Citrus fruit showing lesions



Leaves showing small brown spots

Disease cycle

The bacterium propagates in lesions in leaves, stems, and fruit. When there is free moisture on the lesions, bacteria ooze out and can be dispersed to new growth and other plants. Rainwater collected from foliage with lesions contains between 105 to 108 cfu/ml . Wind-driven rain is the main natural dispersal agent, and wind speeds 18 mph (8 m/s) aid in the penetration of bacteria through the stomatal pores or wounds made by thorns , insects such as the Asian leafminer , and blowing sand. The serpentine mines under the leaf cuticle caused by the larvae of the Asian citrus leafminer, a pest first detected in 1993 in Florida, provide ample wounding on new growth to greatly amplify citrus canker infection .

Water congestion of leaf tissues can be seen following rainstorms with wind. Citrus foliage can hold 7 microliters/cm2 of leaf area . Studies of inoculum associated with water congestion have demonstrated how as few as 1 to 2 bacterial cells forced through stomatal openings can lead to infection and lesion formation . Wind blown inoculum was detected up to 32 meters from infected trees in Argentina . However, in Florida, evidence for much longer dispersals .meteorological events such as severe rainstorms and tropical storms has been presented Pruning causes severe wounding and can be a site for infection

Multiplication of bacteria occurs mostly while the lesions are still expanding, and numbers of bacteria produced per lesion is related to general host susceptibility . The bacterium remains alive in the margins of the lesions in leaves and fruit until they fall and begin to decompose. Bacteria also survive in lesions on woody branches up to a few years of age . Bacteria that ooze onto plant surfaces die upon exposure to drying. Death of bacteria is accelerated by exposure to direct sunlight. Exposed bacteria survive only a few days in soil and a few months in plant refuse that is incorporated into soil. On the other hand, the bacteria can survive for years in infected tissues that have been kept dry and free of soil.

Control measures

- Brazilian regulation stipulates that any field that has a number of diseased trees greater than 0.5% of the total orchard must be eliminated.
- After eradication, the contaminated field should be sprayed with copper fungicide based on 1.5 kg of metallic copper per 1 mL of water (0.15% of metallic copper).
- The contaminated plantations are prohibited and are forbidden from marketing the production until eradication works are completed.

Powdery mildew of grapes

Causal organism

Grape powdery mildew is caused by the fungus Uncinula necator. This fungus has a narrow host range attacking only grape plants and a few related species. It is the most common and widespread disease of grapevines in the B.C. Interior. Popular wine grape varieties vary in susceptibility to powdery mildew.

Symptoms:

All succulent green tissues of the vine are susceptible to infection at some time in their development. The fungus penetrates only the epidermal cells, inserting haustoria (specialized feeding structures) inside the cells to absorb nutrients. The presence of fungal mycelia (vegetative structure) with conidiophores (sporeproducing structures) and conidia (spores) on the surface of the host tissue give infected vines a whitish gray, dusty, or powdery appearance

On occasion, the upper surface of infected leaves exhibit chlorotic or shiny spots that are similar in appearance to the oil spots of downy mildew. Young leaves that are infected may become stunted or distorted, and heavily infected leaves may turn dull, dry, and prematurely drop in autumn; infected petioles and cluster stems become brittle and break as the season progresses. When shoots are infected, fungal tissues appear dark brown to black in feathery patches, which later appear reddish brown on the surface of dormant canes.

The fungus produces its sexual structures – black spherical bodies called cleistothecia – on the surface of infected leaves, shoots, and clusters in older areas of infection. Cleistothecia wash onto the trunks of vines, where they overwinter.

Grape fruit showing powdery spores

Leaves showing powdery surface

Powdery surface under microscope

Disease cycle

Primary PM infections result from inoculum that overwinters on the vine as cleistothecia, formed during late summer and washed into cracks and crevices of the bark. Cleistothecia release spores starting near budbreak through the post-bloom period. Primary infections require at least 0.1" of rain and temperatures above 50° F. All green tissues are susceptible to infection. Grape leaves are most susceptible when very young and become resistant shortly after they are fully expanded. Berries are highly susceptible from immediately prebloom until fruit set, and then become less susceptible around 8° Brix; however lesion development from previous infections may continue.

Primary infections soon produce the whitish lesions appearing first on shoots near the trunk of the vine where cleistothecia have splashed via rain from the old wood. The whitish lesions are the characteristic that gives the disease its common name. Those lesions produce an abundance of conidial spores which are wind-blown to other susceptible tissue. Secondary infections by conidia do not require the presence of free moisture; however, high relative humidity favors disease development. Therefore, vineyards in close proximity to bodies of water or parts of the vineyards with poor circulation may have high disease severity.

Repeating cycles of infection, disease and conidial generation occur every 6 to 8 days at temperatures of 60 to 80° F. The rapid generation cycle accounts for PM's destructive, wildfire-like spread. Uncontrolled infections from bloom until shortly after fruit set usually result in severe disease on clusters and may increase the severity of Botrytis and sour rot, as well as Brettanomyces on fruit.

Cultural control:

Based on reducing humidity and improving air circulation and sunlight exposure. • Plant in sites with good air circulation and sun exposure (direct exposure to intense sunlight is detrimental to powdery mildew fungus)

. • Use training systems that allow good air movement through the canopy and prevent excess shading.

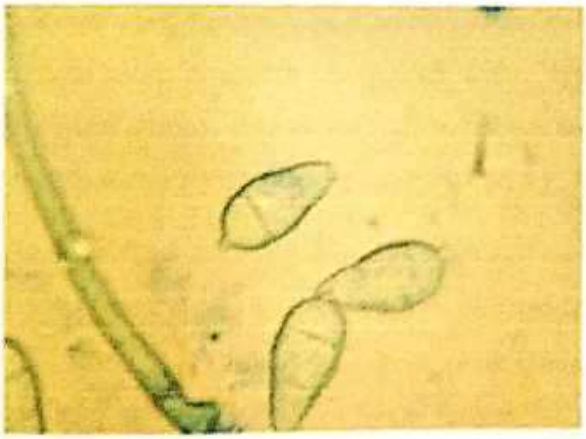
• Plant varieties less susceptible to powdery mildew.

• Practice canopy management that allows good sunlight exposure

• Scout for primary powdery mildew infections. Be sure to look inside the canopies where decrease sun exposure and increased humidity are favorable for powdery mildew development. Sulfur is relatively cheap and is highly effective, but has its limitations with sulfur-sensitive varieties or when air temperature exceeds 95° F or is below 65°F. • The sterol-inhibiting compounds (SIs) (Elite, Nova, Rubigan, and Procure) and QoIs (Abound, Flint, Sovran, and Pristine) are highly effective PM fungicides however; precautions must be taken to avoid the development of strains of PM that are resistant to those fungicides. Regardless of which SI or QoI fungicide you choose to use, they should be alternated with unrelated materials in a seasonlong program. Do not apply the SI or QoI fungicides to sporulating PM-diseased vines without the addition of sulfur. Use the higher end of a recommended product rate per acre and do not exceed the label's recommended interval for repeat applications.

Rice blast disease

Pyricularia sp. showing Under microscope



Rice blast disease

Causal organism

Causal organism Rice blast is caused by the Ascomycete fungus, Magnaporthe grisea Barr (anamorph Pyricularia grisea Sacc., synonym P. o ry z a e C a v.). Pyriform macroconidia, ca. 20 × 10 µm, (Figure 1) are produced on conidiophores which protrude from lesions on plants. These germinate and develop an appressorium (Figure 2) at the tip of the germ tube, which attaches to the surface of plant tissues; an infection-peg from the appresorium penetrates into plant tissues. The wall of conidiophores and appressorium are pigmented by melanin.

Symptoms

The fungus is able to infect and produce lesions on all organs of the rice plant except the root. Leaf blast . When the fungus attacks a young leaf, purple spots can be observed after an incubation period, changing into a spindle shape which has a gray centre with a purple-to-brown bord e r, and then surrounded by a yellow zone as time passes. Brown spots appear only on the older leaves or leaves of resistant cultivars. In young or susceptible leaves, lesions coalesce and cause withering of the leaves themselves, especially at the seedling and tillering stages. Lesion formation on the n-leaf (where n is the top developing leaf), causes shortening of the n + 1 leaf sheath and the n + 2 leaf blade, with consequent stunting of the whole plant. Neck rot and panicle blast Infection to the neck node produces triangular purplish lesions, followed by lesion elongation to both sides of the neck node – symptoms which are very serious for grain development.

When young neck nodes are invaded, the panicles become white in colour – the so-called 'white head' that is sometimes misinterpreted as insect damage. Later infection causes incomplete grain filling, and poor grain quality. Panicle branches and glumes may also be infected. Spikelets attacked by the fungus change to white in colour from the top and produce many conidia, which become the inoculum source after heading. Collar rot Infection at the junction of the leaf blade and the leaf sheath, i.e. the collar, readily occurs and causes browning of the tissues and withering of the leaves. Node blast During heading, the stem nodes which appeared from the leaf sheaths are attacked and sometimes cause lodging. Diseased nodes are brown or black in color.

Disease cycle

A disease cycle begins when a blast spore infects and produces a lesion on the rice plant and ends when the fungus sporulates repeatedly for about 20 days and disperses many new airborne spores. Under favourable moisture and temperature conditions (long periods of plant surface wetness, high humidity, little or no wind at night and night temperatures between 12–32 °C) the infection cycle can continue. In the canopy of rice plants, newly developed leaves act as receptors for the spores. The maximum number of spores produced was 20,000 on one lesion on leaves and 60,000 on one spikelet in one night. Lesions on leaves become an inoculum source for panicles.

Overwintering

The pathogen can continue to live in plants from one crop season to another in the tropics, or survive in the temperate zone on residues of diseased plants or seeds, or on ratoons of stubble. Weeds can act as alternative hosts for the disease in glasshouse tests, but their role in the field in unclear.

Lesion expansion

Exposure of the diseased plants to higher temperatures, e.g. around 32 °C, causes lesions to expand rapidly in the first 8 days and level off shortly thereafter, then a swift cessation of lesion enlargement takes place. On the other hand, the rate of enlargement is slow and constant over the 20-day period at lower temperatures, e . g . 16 °C. Lesions expand slowly and cessation occurs gradually in the intermediate temperature regime, 20–25 °C.

Control measures

Burning or composting of diseased tissues

Diseased straw and stubble must be burned or composted, otherwise they can become inoculum sources for the next crop season.

Healthy seed

To obtain healthy seeds, the seeds must be collected from the field located under unfavorable conditions for the pathogen, and fungicide must be applied if necessary. Gravity separation methods for seeds are useful. Salt solution, 200 g l–1, or ammonium sulfate solution, 230 g l–

1, is used to separate sufficiently matured seeds, followed by chemical treatment for seed disinfection against a range of pathogens.

Fertilizer management

Nitrogen and phosphorus content in the plants affects disease proneness. Excess nitrogen fertilizer encourage disease development, while silica application reduces disease development. Therefore the amount and type of fertilizer must be carefully decided upon according to the cultivar used, soil condition, climatic conditions and disease risk.

Cultural systems

Sowing into water eliminates disease transmission from seeds to seedlings because of the anaerobic condition that is unfavorable to the pathogen. On the contrary, sowing on wet soil allows seed transmission. Shade affects disease occurrence because of the longer wet condition.

Chemical control

Many fungicides are used against blast disease, including benomyl, fthalide, edifenphos, iprobenfos, tricyclazole, isoprothiolane, probenazole, pyroquilon, felimzone(= meferimzone), diclocymet, carpropamid, fenoxanil and metominostrobin, and antibiotics such as blasticidin and kasugamycin. Systemic fungicides are widely used to protect against leaf blast by seedling application and also to protect against panicle blast when applied more than 20 days before heading. The composition, amount, timing and application method of fungicide applied depends on the disease forecast or level of disease present.

Fungal spot of guava(Anthracnose)

Pathogen

Anthracnose is caused by Gloeosporium psidii . Sheld. which is now called Colletotrichum psidii Curzi.

The mycelium becomes intercellular, branched and light brown in colour. Brown to dark brown coloured acervuli are formed on the affected parts ofthe plants. Setae and conidia are formed in the acervuli. Conidiophores are hyaline and small, setae are long, tapering at the end, dark brown to black in colour. Conidia are formed at the tip of the conidiophore and are sickle-shaped, unicellular, hyaline measuring 11.24 x 4.5-5 mm. They germinate by germ tube. In moist weather, acervuli appear as black dots on twigs or fruits which later produce pinkish spore mass. Spores are disseminated by wind or rain to initiate fresh infection.

Symptoms

- Pinhead spots are first seen on the unripe fruits which gradually enlarge measuring 5-6 mm in diameter.
- They are dark brown to black in colour, sunken, circular and have minute, black stromata in the centre of the tissues.
- On leaves, the fungus causes necrotic lesions at the tip or on the margins. These lesions are usually
- ashy grey and bear fruiting bodies. The tender twigs are also infected which wither and die from the tip downwards giving it a wither-tip appearance.
- The disease was found to develop more rapidly at 30°C and 96.1 per cent relative humidity in both ripe and unripe fruits.

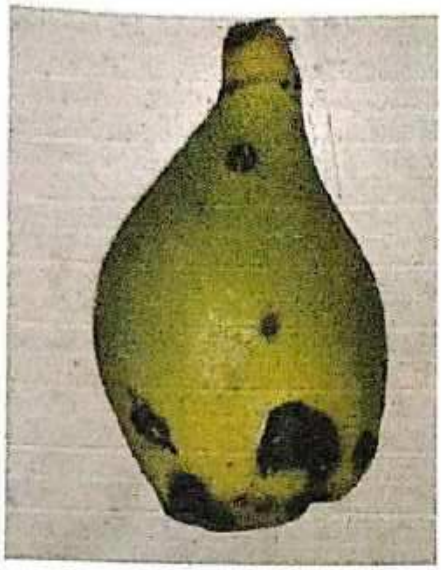
Management

- Effective control of anthracnose can be achieved by sprays of Bordeaux mixture (3:3 :50) at 7 days interval.
- Copper oxychloride and cuprous oxide also significantly control the disease (Tandon and Singh,1969).
- Monthly sprays of Difolatan (0.3%) closely followed by Dithane Z-78 (0.2%) were found effective.
- Bordeaux mixture and other copper fungicides caused russetting of fruits .

Fungal spots on the leaf and fruit

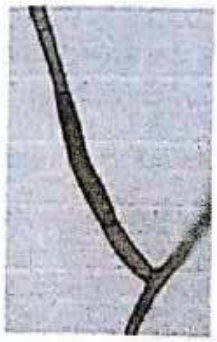
Fungal spot showing under microscope

Fruit on fungal spots



Leaves on fungal spot

Colleotricum mycelium under microscope



Fungal spot of mango fruit

Anthracnose

Anthracnose, the most important mango disease, is caused by the fungus Colletotrichum gleosporioides

.symptoms

Flower blight, fruit rot, and leaf spots are among the symptoms of this disease. Symptoms on the panicles (flower clusters) start as small black or dark-brown spots. These can enlarge, coalesce and kill the flowers , greatly reducing yield. On leaves anthracnose lesions start as small, angular, brown to black spots . If tissue is young when originally infected, spots can enlarge to form extensive dead areas . Lesions that begin in older leaves are usually smaller with a maximum diameter of 1/2 inch (6 mm); they appear as glossy dark brown to black angular spots.

Fruit infection commonly occurs and can result in serious decay problems in the orchard, in transit, at the market, and after sale. The fungus invades the skin of fruit and remains in a "latent" (a living but nonsymptom-producing) state until fruit ripening begins. Ripe fruit, either before or after picking, can then develop prominent dark-brown to black decay spots . These may coalesce and can eventually penetrate deep into fruit, resulting in extensive fruit rotting.

An interesting manifestation of anthracnose fruit damage is the symptom called "tear staining" that develops when spore-laden water droplets from infected twigs and panicles wash over and infect the fruit. Anthracnose is usually more serious in years when rain and heavy dews are frequent, from the onset of flowering until fruit are about half size.

Control measures

anthracnose, especially on very susceptible cultivars, such as 'Haden' and 'Irwin', centers on a diligent fungicide program. Effective control of even the postharvest phase of this disease is best accomplished by a good spray program in the orchard. Begin fungicide applications at the first appearance of panicles and continue spraying at recommended intervals until the pre-harvest waiting period is reached.

Sugarcane whitefly disease

symptoms

- Yellowing of leaves and later it shows pale in colour
- Leaf turns pinkish or purple and later gradually dry.
- Infested leaves look white and black dots.
- In severe cases it look like fiery appearance
- It shows very slow in growth of plant

Disease cycle

- **Egg:** Females lay eggs in a line near the midrib or any where on the lower surface of the leaves. Eggs are yellowish with a small curved stalk. Colour changes to black about two hours after the eggs are laid.
- **Nymph & Pupa:** Neonate nymphs are pale yellow in colour, flat and oval in shape, later turn shiny black. Its body is surrounded by fringes of wax.The fourth instar being the pupal stage, is flat, oval, greyish in colour and slightly bigger than the nymph. There is a 'T' shaped white marking on the thorax, which splits at the time of adult emergence.
- **Adult:** Pale yellow body with hyaline wings dusted with waxy bloom, exhibit brisk fluttering movements

control measures

Cultural method:

- Avoid water stagnation and provide proper drainage facilities
- Detrashing of cane at the 5th and 7 th month
- Avoid the excess application of fertilizers

Physical method:

- Detrashing the puparia bearing leaves and immediately disposing by burning or burying to prevent emergence of adult white flies.

- Ensure adequate irrigation which facilitates the soil movement and reduces the multiplication.
- **Chemical method**

- Spray fenitrothion 2 lit / ha (1000 lit spray fluid)
- Spray thiomethoxam 125g/ha. 750 litres of spray fluid

Leaves on pest disease

Whitefly insects under microscope

- Spraying acephate 2g per lit of water.the spray has to be repeated after a month to kill the nymphs emerging from eggs
- Application of chlorpyriphos 1250 lit spray fluid by using hand sprayer

CHEMICAL CONTROL

It is unlikelythat chemical control will be needed against the sugarcane whitefly. However, if necessary, use horticultural oil (made from petroleum), white oil (made from vegetable oils), or soap solution . White oil: 3 tablespoons (1/3 cup) cooking oil in 4 litres water, ½ teaspoon detergent soap. Shake well and use. Soap: Use soap (pure soap, not detergent). 5 tablespoons ofsoap in 4 litres water, OR 2 tablespoons of dish washing liquid in 4 litres water. Commercial horticultural oil can also be used.White oil, soap and horticultural oilsprays work by blocking the breathing holes of insects causing suffocation and death. Spraythe undersides of leaves; the oils must contact the insects. A second application ofsoap or oils may be necessary after 3-4 weeks. Use synthetic pyrethroid insecticides to kill ants if they are present, attracted to the honeydew.

Papaya black browny spot disease

Causal organism

The fungus on the infected papaya leaves was identified as A. caricae by comparing with the description and illustrations given by . Sporulation of A. caricae was hypophyllous, ranging from dark blackish brown to black. Stroma was well developed, and erumpent . Conidiophores were olivaceous brown, geniculate, smooth in dense fascicles with several prominent conidial scars at the tip up to 52 µm long × 6 – 9 µm wide . Conidiogenous is cells polyblastic with thickened and darkened scars. Conidia solitary, ellipsoidal, pyriform or clavate, 1-septate (mature), hyaline to mid pale brown, verrucose, 16–32 × 5–11 µm. Conidia are elliptic-oviod, rounded at the top, one or two septate, hyaline to brown in colour. Size of the conidia varies from 27-30 µm. Sporodochia of A. caricae was hypophyllous, dark blackish brown to black. stroma well-developed, erumpent. Conidiophores closely packed together and covering the surface of the stroma, usually unbranched, hyaline to olivaceous brown, with several prominent conidial scars at the apex, up to 45 × 69 µm.

Symptoms

- Affected leaves become necrotic, weak and subsequently die under severe disease pressure, which results in extensive defoliation.
- Black spot disease of papaya is very lethal and thus both leaves and fruits of papaya can be affected .
- Severe black spot infections can cause the leaves to curl and die prematurely which affects photosynthesis.
- The pathogen can cause direct damage by causing spots on the fruit. Therefore, management of this disease is very important Symptoms.
- initiated during cooler weather accompanied with rains, and the disease spread continued even after rains. Periods of wet weather may increase the development of the disease .

- There was a marked correlation between the severity of [illegible] spotting and the rainfall where spotting increased considerably with increased [illegible] rainfall while during dry spells the disease declined

Browny spot showing on fruit

Browny spots on upper leafy surface

- markedly . During prolonged dry weather condition, most infected trees recovered completely from the disease.

Control measures

- . Removal of infected leaves will increase air circulation and improve spray penetration through the fruit column [32]. Heavily infected leaf blades should be removed by cutting the petiole half way between the leaf blade and the trunk to protect insects and pathogens to enter the wound.
- It is also reported that high black spot pressure influence the efficacy of mancozeb and tebuconazole fungicides .
- Therefore, options of curative and protectant fungicides should be availed to control such economically important pathogen. In this regard, laboratory tests showed that A. caricae was more sensitive to difenoconazole than tebuconazole .
- Similarly it was reported that, Difenoconazole, Pyraclostrobin and Chlorothalonil were better in managing papaya black spot than Mancozeb and Tebuconazole . Fungicides viz.,
- Difenoconazole, Chlorothalonil, Propiconazole and Hexaconazole were very effective in managing this pathogen . It is advisable to look for signs of disease on the new growth since the fungicides protect the new leaves and fruits; but old damage cannot be undone

Castor mold disease

Causal organism

The fungus Botryotinia ricini (Godfrey) Whet. is morphologically characterised as grey, conidia dusty, conidiophores erect, seldom curved, septate, 11-23μ thick, wall blackish brown approaching the tip closely, and hyaline with numerous projection at the tip from which conidia are formed

Soares, 2012 suggested that Amphobotrys ricini is characterized morphologically and molecularly by its erect, cylindrical, light brown hyaline, straight, curved dichotomously, with uninflated and thin-walled conidiogenous cell; conidia globose developing synchronically or sterigmata, on shorter denticle, smooth, unicellular, sub-hyaline to light brown.

Symptoms

The primary targets of the fungus are the inflorescence and the capsules, in any development stage (Araújo et al., 2007; Dange et al., 2005; Gonçalves, 1936; Lima et al., 2001). Some authors (Drumond & Coelho, 1981; Batista et al., 1996) claim that the male flowers are the first to be infected, but it is not always the case because any part of the inflorescence can be infected, the female flowers being the preferential target.

Gray Mold of Castor: A Review 223 came from the fact that the male flowers are the first to be exposed, at the earlier stage of inflorescence formation; consequently such flowers are exposed longer to the infection units of the fungus. However, as soon as the male flowers suffer anthesis they are no longer a target and are hardly infected, contradicting the statement of Drumond & Coelho (1981) that "the fungus attacks first the male flowers because the anthers, being soaked with the rain water or dew, easily retain the fungus spores carried by the wind".

The first symptoms are visible as bluish spots on the inflorescences, on both female and male (before anthesis) flowers, and on developing fruits. On fruits, the symptoms

the occurrence of yellow ooze at the point of infection is frequent (Batista et al., 1996; Dange et al., 2005) as a result of the rapid enzymatic tissue degradation. The symptoms on the male flowers, before anthesis, are small, pale brown, necrotic spots, which can evolve to larger brown spots with a darker edge .

The infected flowers and young capsules became softened due the fungal colonization and mycelial growth is, at first, pale gray and later dark olivaceous. A profuse sporulation is usually observed in such stage . When the infection starts on immature capsules, they become rotten; if the infection starts later, with fully developed capsules, the seeds usually became hollow, with coat discoloration and weight loss (Dange et al., 2005).

224 Plant Pathology inflorescence, the male flowers can be infected first, but the fungus has a clear preference for the female flowers . Infection can lead to complete destruction of the raceme , particularly if it reaches the main stem and the weather conditions are favourable for the disease. Several other plant parts, e.g. leaves, petioles and stem can also be infected, mainly due to the deposition or fall of infected material from the inflorescence or racemes. On leaves, the lesions are usually irregular, but can assume an elliptic or circular pattern, the size is very variable, sometimes coalescing and resulting in a foliar blight .

Disease cycle

The disease starts with spore deposition on the host surface, followed by penetration and colonization of the host tissues. Soon after colonization, the fungus, under favourable conditions, sporulates profusely on the dead tissues, and then the conidia became the main inoculum source for new infection sites.

Although most authors recognized the major role of conidia after the disease had been established in the field, there is much speculation about the primary inoculum source of B. ricini. Godfrey (1923) claims that the fungus survives on soil or crop residues as sclerotia and, under the right conditions, these can produce sexual structures, which will be responsible for the initial infection.

However, there is no report of sexual reproduction under natural conditions, other than the original reports of Godfrey (1919), so the purported role of ascospores as the initial inoculum source remains unclear, despite the fact that apothecia are easily overlooked in the field. Under tropical climate, the initial inoculum source are probably the conidia from wild castor plants which grows spontaneously near the crop areas all year (Gonçalves, 1936).

Wild castor plants can produce flowers throughout the year and consequently new susceptible tissue will be available for the fungus to self perpetuate in its anamorphic state through the year. By infecting the first inflorescence under favourable conditions, the fungus produces abundant sporulation, thus allowing multiple rounds of re-infection, since this pathogen is easily spread by wind, rain splash and, probably, by insects.

Control measures

- the application of fungicides either by spraying or dusting is ineffective once the disease has been well established in the field. Fungicides with high specificity and distinction in their mode of action are effective in controlling many plant diseases (Chagas, 2009)

- . The use of two prophylactic sprays with carbendazim (0.05% and 0.1%) at the flowering stage and immediately after the appearance of the first symptoms decreases the disease spread (Sudhakar et al., 2010).

- Application of tebuconazole, iprodione, and procymidone are known to control fungal pathogen. Among these, procymidone and iprodione fungicides are highly effective against gray mold, only if they are applied at the start of disease development and on a weekly bases (Soares, 2012).

- It was observed that out of the two fungicide applications with nine treatments, propiconazole, which was applied at 1 mL/L recorded the lowest incidence (9.6%) of gray mold. Studies have shown that both Botrytis spp. and Botrytis ricini are phylogenetically and biologically similar as stated earlier (Walker, 2016)

- Bhattiprolu (2008), studied on the effectiveness of Trichoderma viride against B. ricini as influenced by culture medium pH (4.0 – 6.0), incubation temperature (25°C), fungicides and exposure to mutagenic agents (gamma rays). The isolate was found compatible at 10% leaf extracts of Azadirachta indica, Curcuma longa, Datura stramonium, Emblica indica, Lantana camera, Memordica dioica, and Ricinus communis and other fungicides such as mancozeb 0.25%, copper oxychloride 0.3%, and thiram 0.3% but not with benomyl 0.1%, carbendazim 0.1%, thiophanate-methyl 0.1%, and 0.2% hexaconazole. In the end, mutant TV4 obtained the highest inhibition of B. ricini of about 88% when compared to the wild castor isolate.

Bunchy top of banana

Causal organism

Banana bunchy top virus (BBTV) is a circular single-stranded (ss) plant DNA virus and the type memberof the genus Babuvirus in the family Nanoviridae. BBTVcauses bunchy top disease of banana and plantain. Itsintegral genome consists of at least six circular ssDNA genome components and is associated with all geographi-cal isolates of BBTV. BBTV is a phloem-limited virus thatis transmitted from plant to plant by the aphid vector, and long-distance spread is through the movement of infected plant material. BBTV is an economically important plant pathogen causing substantial damage to banana crops worldwide.

Symptoms

The keikis (suckers) which develop after a "mother" plant has been infected with BBTV are usually severely stunted, with leaves that do not expand normally and remain bunched at the top of the pseudostem. These leaves are stiff and erect, are shorter and narrower than normal leaves, and have chlorotic edges. Keikis with these symptoms will not bear fruit.

- **Maturing plants:**

  On mature plants infected with BBTV, new leaves emerge with difficulty, are narrower than normal, are wavy rather than flat, and have yellow (chlorotic) leaf margins. They appear to be "bunched" at the top of the plant, the symptom for which this disease is named. Severely infected banana plants usually will not fruit, but if fruit is produced, the banana hands and fingers are likely to be distorted and twisted.

- **Subtle disease symptoms**

  Some symptoms require close inspection to in order to see them. The ability to detect these symptoms enables earlier removal of diseased plants. These symptoms are referred to as "Morse Code Streaking" and "Green J-Hooks.

Morse code streaking

The initial symptoms of banana bunchy top virus consist of dark green streaks in the veins of lower portions of the leaf midrib and the leaf stem (petiole). The streaks also occur, but are less prominent, in the veins of the leaf blade (lamina). This symptom is sometimes referred to as "Morse code streaking" because the streaks are irregular and resemble a series of "dots" and "dashes." Rubbing away the waxy white coating that covers the petiole or midrib makes it easier to see the streaking.

Green J-hooks.

Also, dark green, hook-like extensions of the leaf lamina veins can be seen in the narrow, light-green zone between the midrib and the lamina. The short hooks point down along the midrib toward the petiole. They can best be seen by back-lighting the leaf against the sky.

Disease cycle

BBTV is the sole member of the genus *Babuvirus* in the family Nanoviridae. The genome of BBTV is made up of at least six circular, single-stranded DNA components, each about 1 kilo-base pair in length. Replication takes place by rolling circle replication, a unidirectional nucleic acid replication that can result in rapid synthesis of single-strands of DNA.

There are specific virus-like particles that have been proposed as the virions of BBTV but there are still discrepancies in the scientific world about the exact relationship between these virions and the single-stranded DNA virus. Nevertheless, it was demonstrated that the associated ssDNA molecules are transmitted with the disease and therefore are designated as the pathogen.

It is known that Banana aphid (*Pentalonia nigronervosa*) transmits the virus from infected to healthy plants by feeding. Aphids feed on the plant phloem tissues by injecting their thin, flexible stylet into the epidermis of the plant tissue until it reaches the phloem of the leaves. Then the aphid injects saliva and sucks the cell contents.

This ingestion of viral components is done inadvertently by the aphid. Vector transmission of the BBTV is circulative and non-propagative, meaning that

transmission of the virus occurs from and to the phloem tissues and the virus does not replicate within the aphid's midgut. Acquisition of the virus by the banana aphid requires about 18 hours of feeding and then the aphid can retain the virus for approximately two weeks.

The retransmission of this virus can happen after as little as two hours of feeding on a healthy plant however it takes about a month for the BBTV symptoms to appear after infection. To infect, the carrier aphid can feed on the banana plant for as few as 15 minutes, but more often a couple hours, as the longer feeding time will increase the odds of transmission

Stunted growth on banana stem

The damaged veins

The suckers produced on infected plants that would usually be used for planting the next season will also be diseased, which is one way the disease can spread from year to year Banana aphids also have the capability to feed on *Heliconia* and flowering ginger, however, these alternate hosts of the aphid vector are not hosts of the virus. The ability of banana aphids to feed on alternate hosts is important to keep in mind when attempting to control the virus.

CHEMICAL CONTROL

- The choice ofchemicals for killing aphids on diseased plants, before removal from the plantation, depends on whether they are grown for household use or for sale.
- Banana for home use: Use soap solution, white oil (vegetable oil) or horticultural oil (petroleum oil)
- . Kerosene can also be used as mentioned above. Aim to spraythe "throat", the V-shaped area where the leaves meet and where aphids hide.
- Commercial plantations: The following have been recommended for Pacific island countries: dimethoate (400 g/L), used at 75ml/100L; diazinon (200 g/L), used at 1.5ml/L; acephate (75%WP), used at 1.3 g/L. Note, in Australia, the use of dimethoate is restricted, and the use of the chemical remains under review.
- Diazinon use is also under review. Synthetic pyrethroids are likelyto be effective.

tortoise beetle leaf spot disease incurry leaf

symptoms

The longer second antennal segment the presence of hairs on theapex of elytral epipleura and the absent of prosternal groove for antennalreception separate this species from other Cassidinae. In additiory the livingbeetle has the peculiar habit of completely covering itself with a white coating. Length 1.G1.8 mm, width 0.5-0.6 mm. Head black. Pronotum greyish-black. Mesonotum, metanotum and abdomenyellowish-orange. The larva with15 pairs of spines on each sides, 8 pairs each on the thorax and abdomen.Pronotum with 3 pairs of spines, 2 pairs close at each other at the anteriorangles, and 1 pair in the middle. Disc depressed on each side of mid-dorsalline. Mesonotum with 3 pairs of spines, including one pair of shorter spinesin middle. Metanotum with 2 pairs of spines, including one pair of shorterspines at the anterior.

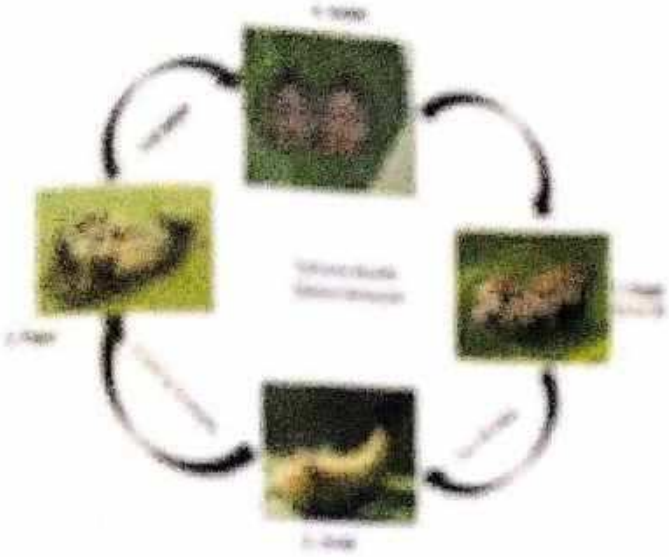
Disease cycle

Egg The creamy white eggs are housed in a papery golden brown ootheca of 10-25 layered membranes filled with three eggs each . The egg is elongated measuring 1.5 to 2 mm long. The size of ootheca ranges from 8-13mm long and 8-10 cm wide.

Larva Young larvae (first to third instar) are light brown with 2 rows of 5 square round black spots on the thoracic area and 7 rows of 2 narrow rectangular spots black spots on the abdominal region. The narrow rectangular spots split into two making 7 rows of 4 spots as the larva matures, and the colour of the body changes to golden brown. The body is surrounded with black spines. Mature larva measures 7-10 mm long and 3-5 mm wide.

Pupa The pupa is yellow with darker shade along the body margin. The lateral spines of the head, thoracic and the anterior part of the abdomen are reduced. The lateral spines of the abdomen are compressed towards the posterior end . The number of spots is reduce to one pair on the anterior most portion of the head, one on each side of the head, two pairs on the thoracic area and 4-6 spots on the posterior end. Pupa measures 10-12 mm in length and 5-7 mm in width.**Adult** The adult is a medium-sized golden tortoise beetle with broad transparent extension of the elytra, 10-13 mm long and 9-13 mm wide. The elytra contain 4 of broad black spots on the transparent extension of the elytra and 19 -23 small spots for the female and 15-17 small spots for the male with a dark golden yellow dorso-median.

[illegible] cycle



[illegible] leaves showing [illegible]

Control measures

- Leaf spot disease can be controlled by spraying Carbendazim 1 g/lit of water. Spraying Sulphur compounds should be avoided. At the end of first year 250-400 kg of leaves/ha can be harvested. line.

- Simply mix two tablespoons of neem oil and one tablespoon of dish soap with one gallon of water. Use a spray bottle to apply the mixture to areas where you see clusters of golden tortoise beetles, shaking the bottle occasionally to keep the oil from separating in the bottle.
- Continue to treat affected areas for three to five straight days or until the pests are gone. Spray the neem oil mixture on vines, stems and leaves, but try to avoid spraying directly on flowers so as not to drive away pollinators. Use the spray late in the evening when bees are less active and when the sun is not around to dry the solution up too quickly.
- Though insecticides are only recommended for the most extreme infestations and are rarely necessary, larvae and adult golden tortoise beetles are easily killed by spraying residual insecticides, like permethrin, directly onto the leaves of affected plants.

Ceratocystis paradoxa pest disease in pine apple

Causal organism

Ceratocystis paradoxa or Black Rot of Pineapple is a plant pathogen that is a fungus part of the phylum Ascomycota. It is characterized as the teleomorph or sexual reproduction stage of infection. This stage contains ascocarps, or sacs/fruiting bodies, which contain the sexually produced inoculating ascospores. These are the structures which are used primarily to survive long periods of time or overwinter to prepare for the next growing season of its host. Unfortunately, the sexual stage is not often seen in the natural field but instead the anamorph, or asexual stage is more commonly seen.This asexual stage name is Thielaviopsis paradoxa and is the common cause of Black rot or stem-end rot of its hosts.

symptoms

One of the most well-known diseases caused by Ceratocystis paradoxa is Black rot or stem-end rot of pineapple, but it can also infect tropical fruit plants such as banana and coconuts as well as sugarcane. The pathogen infects the fruits through wounds or other openings after harvest has already happened and the fruit is fresh.Symptoms for this disease are very obvious black lesions on the fruit, the main infection part of the plant. If the pathogen infects the plant while fruits are still on it, they will prematurely drop. Other symptoms include discoloration of leaves as well as the seeds. The lesions on the fruit evolve to become soft rot spots that produce a heinous odour. The fruit can even get to the point of breakdown

Disease cycle

The pathogen Ceratocystis paradoxa is the teleomorph stage of the inoculation and is uncommon in the natural environment. This is because the primary disease observed is caused by the anamorph stage which is due to Thielaviopsis paradoxa. Chlamydospores are the overwinter stage of the pathogen. Because pineapples are grown using pieces of fruit previously harvested pineapples, these chlamydospores can be present and can start the inoculation early on.theyare not present in the planting, then they must infect the wounds or natural openings on harvested pineapple.

Pest showing on pine apple fruit

Black spot showing on fruit surface

When the chlamydospores first infect the plant, they give rise to the mycelium, or hyphae network, which then lead to further spore infection. This gives rise to the black rot that is seen. If the infection is seen out in the field, the chlamydospores will over winter in the dead debris of the plants or in the soil.

Control measures

- If the disease has inoculated the fruit after the harvest has already happened, there are a few ways to limited the spread of the disease. One way is to soak the fruit in hot temperatures.
- Also, if the fruit is to be stored then it should be at cold temperatures to limit further spore production. It is also helpful to keep the fruit as clean as possible.
- If the disease begins in the soil from debris or chlamydospores from past fruit then it is best to change out the soil or to keep it as dry as possible to make sure the conditions are not ideal for the pathogen.
- Post-harvest fungicides are also useful in limiting the disease, however continued use could possibly lead to pathogen resistance. The fungicide may also be harmful to the consumers if it is directly sprayed onto the fruit.
- Most fungicides that can be bought retail are sold in a liquid form. A very common active ingredient is sulfur, present at 0.08% in weaker concentrates, and as high as 0.5% for more potent fungicides. Fungicides in powdered form are usually around 95% sulfur and are very toxic.
- Ziram is also a fungicide that is toxic to humans with long-term exposure, and fatal if ingested. A number of fungicides are also used in human health care.

Sooty Mould (Capnodium sp.) in sapota

Sooty mold is a collective term for different Ascomycete fungi, which includes many genera, commonly Cladosporium and Alternaria. It grows on plants and their fruit, but also environmental objects, like fences, garden furniture, stones, even cars. The mold benefits from either a sugary exudate produced by the plant or fruit, or if the plant is infested by honeydew-secreting insects or sap suckers.

Symptoms

The fungus itself does little harm to the plant; it merely blocks sunlight, and very rarely may stunt a plant's growth and yellow its foliage. Thus, sooty mold is essentially a cosmetic problem in the garden, as it is unsightly and can coat most of a plant in a matter of days or weeks.

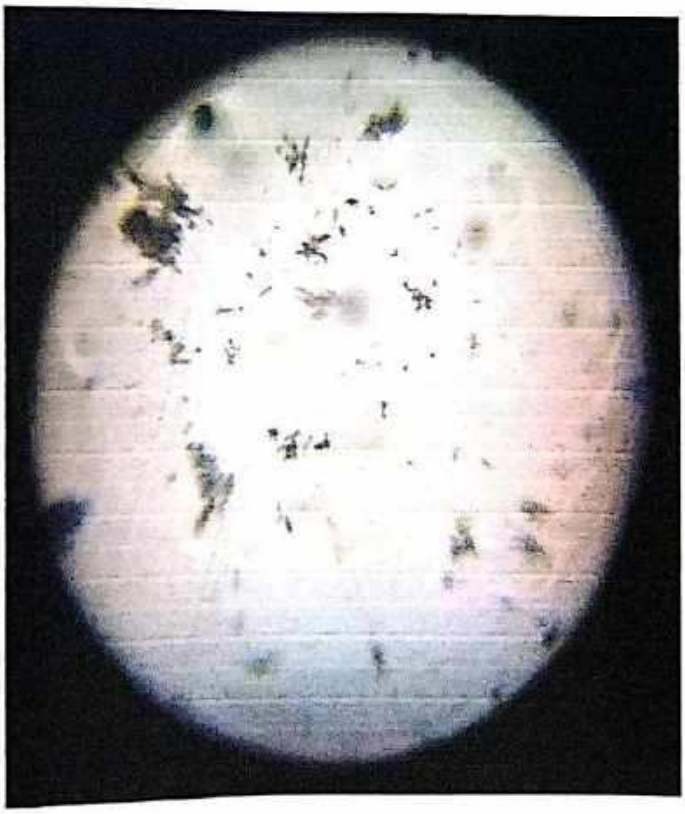
Control

- The simplest form of non-chemical control is to wipe and wash affected plant parts with lukewarm water and soap, insecticidal soap or dish soap, one tablespoon per gallon of water; strong soaps or detergents may damage the plant.
- This can also be sprayed if the plant is large but is much less effective than when combined with physical removal. After allowing the soap to sit for a while the sooty mold is rinsed off with a hose/ water. Sooty mold will regrow, unless the underlying reason for its growth is eliminated.
- Chemical control of sooty mold itself is not needed. If sap-sucking pests are responsible for the honeydew on which the mold is growing, there are several options:
- Using formulations of neem oil, which is an organic broad spectrum pesticide, insecticide, fungicide and miticide controls mites and insects such as whitefly, aphid, scale, and mealy bugs, and additional fungus diseases like black spot, rust, mildew, and scab. Neem oil can be used on house plants, flowers, vegetables, trees, shrubs and fruit indoors and outdoors. Neem oil is biodegradable and has not been shown to be toxic to mammals, birds, bees, earthworms, or beneficial insects.

- Synthetic insecticides such as the organophosphates acephate (orthene), malathion, or diazinon can be used in severe cases but read the labels for approved crops and the number of days to wait to harvest.

Leaf showing on sooty mold

Sooty molds under microscope



Dark pinpricks disease in phyllanthus emblica

Symptoms

Dark colored pinpricks on fruit surrounded by a lighter area that turns yellow or remains light green; stink bugs often carry pathogens in their mouthparts which can cause secondary infections and decay of fruit; adult insect is shield-shaped and brown or green in color; may have pink, red or yellow markings; eggs are drum shaped and laid in clusters on the leaves; larvae resemble the adults but are smaller

Cause

Insect - Stinkbugs (Brown mormorated stink bug) Halyomorpha halys

Adult insects overwinter under leaves, on legumes, blackberries or on certain weeds such as mustard or Russian thistle

Management

Remove weeds around crop which may act as overwintering sites for stink bugs and practice good weed management throughout the year; organically accepted control methods include the use of insecticidal soaps, kaolin clay and preservation of natural enemies; chemical treatments are not recommended for tomatoes that are to be processed for paste or canning unless secondary infections with other pathogens are a concern.

Dark lesions on fruit

Leaves of Helicomyia sp.

Results and discussion

- Some of the economically important plants were affected by macro and micro organisms such as (bacteria,fungi.virus,pest and nematodes) .
- Based on the micro organisms , the disease are classified into many groups,Disease involved in the study of causal organism, symptoms, disease cycle ,control measures.
- Exotic pests have caused immense economic and ecological damage, and, with few exceptions, are the only ones that merit serious attention. A few pathosystems that exhibit properties of both endemic and exotic diseases are often disturbed, or 'degenerate,' as a result of human intervention.
- If pesticides were used ,Nutrition and health improved Food safety/security Life expectancy increased Reduced maintenance costs.

- If antimicrobial agents used , such as sulfona-mides, for which the mechanism of action involvesblocking a specific metabolic pathway in bacteria (folicacid synthesis), inhibit the growth of susceptible bacteria but do not kill the organisms.

- disease control is reasonably successful for most crops. Disease control is achieved by use of plants that have been bred for good resistance to many diseases, and by plant cultivation approaches such as crop rotation, use of pathogen-free seed, appropriate planting date and plant density, control of field moisture, and pesticide use.
- Continuing advances in the science of plant pathology are needed to improve disease control, and to keep up with changes in disease pressure caused by the ongoing evolution and movement of plant pathogens and by changes in agricultural practices. disease control is reasonably successful for most crops.
- Disease control is achieved by use of plants that have been bred for good resistance to many diseases, and by plant cultivation approaches such as crop rotation, use of pathogen-free seed, appropriate planting date and plant density, control of field moisture, and pesticide use. Continuing advances in the science of plant pathology are needed to improve disease control, and to keep up with changes in disease pressure

caused by the ongoing evolution and movement of plant pathogens and by changes in agricultural practices.

Conclusion

The diseased [illegible] in the [illegible] crop plants [illegible]. The [illegible] field [illegible] collection of diseased plants, the affected plant parts brought to lab and preserved through herbarium. For further study on the disease [illegible] 35 types of disease were identified.

FIELD STUDY ON AGRICULTURE AND PROBLEM ASSOCIATED WITH FARMER AND FORMING IN RAMANATHAPURAM DISTRICT – A SURVEY

A short field project work submitted to

St. Mary's College (Autonomous)

Affiliated to

MANONMANIAM SUNDARANAR UNIVERSITY

In partial fulfillment of the requirement for the Degree of Master of science in Botany

By

M. MUTHULAKSHMI - 20APBO07

DEPARTMENT OF BOTANY

ST.MARY'S COLLEGE (AUTONOMOUS)

THOOTHUKUDI-628001

CERTIFICATE

It is certified that this short field project work entitled "**FIELD STUDY ON AGRICULTURE AND PROBLEM ASSOCIATED WITH FARMER AND FORMING IN RAMANATHAPURAM DISTRICT – A SURVEY**" submitted to St.Mary's college(Autonomous) affiliated to MANONMANIAM SUNDARANAR UNIVERSITY in partial fulfilment of the requirements for the degree of Master of science in Botany, and is are cord of work done in the Department of Botany , St.Mary's College (Autonomous), Thoothukudi during the year 2020 – 2021 by the following student.

By

M. MUTHULAKSHMI - 20APBO07

M. Glory
GUIDE

M. Glory
HEADOFTHEDEPARTMENT

EXAMINER

Lucia Rose
PRINCIPAL

St. Mary's College (Autonomous)
Thoothukudi - 628 001.

FIELD STUDY ON AGRICULTURE AND PROBLEM ASSOCIATED WITH FARMER AND FORMING IN RAMANATHAPURAM DISTRICT – A SURVEY

A short field project work submitted to

St. Mary's College (Autonomous)

Affiliated to

MANONMANIAM SUNDARANAR UNIVERSITY

In partial fulfillment of the requirement for the Degree of Master of science in Botany

By

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DECLARATION

Hereby declare that the desertain entitled **"FIELD STUDY ON AGRICULTURE AND PROBLEM ASSOCIATED WITH FARMER AND FORMING IN RAMANATHAPURAM DISTRICT – A SURVEY''** is the original work and it has not been submitted for the award of any Degree, Diploma , fellowship or any other similar title and that the dissertation represents independent and original work on the partial fulfillment of the requirements for the degree of MASTER OF SCIENCE IN BOTANY during 2020-2021, submitted to St.Mary's College (Autonomous), Thoothukudi-628 001.

Place; Thoothukudi-628 001

Date; 17.04.2021

M.Muthulakshmi

M.MUTHULAKSHMI(20APBO07)

ACKNOWLEDGEMENT

We after our praise and sincere thanks to the Almighty God, his for avalanche of graces and abundant blessings, enabling to complete this project.

We consider is a privilege to express our gratitude to Rev. **Dr. Sr. A.S.J. Lucia roseM.Sc.,PGDCA,M.Phil.,Ph.D.,Principal,**St.Mary's College(Autonomous),Thoothukudi for giving permission for doing research and to utilize the facilities in the Department of Botany andher constant encouragement.

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INTRODUCTION

Concerned by the slow growth in the Agriculture and allied sectors, the National Development Council(NDC) ,resolved that a special Additional Central Assistance Scheme ,named National Agriculture Development Programme (NADP/RKVY) be launched. The NDC also felt that Agriculture Development strategies must be reoriented to meet the needs of farmers and called upon the Central and State governments to evolve a strategy to rejuvenate agriculture with a commitment to achieve four per cent annual growth in the agricultural sector during the 11th plan.To implement this, formulation of action plans by means of developing District Agriculture Plans (DAP) is recommended. It is of the view that such plans would also reflect the felt needs of the farmers and stake holders .Such District Agriculture Plans aim at moving towards projecting the requirements for development of Agriculture and allied sectors of the district including animal husbandry and fisher, mino-irrigation projects ,rural development works ,agricultural marketing schemes and schemes for water harvesting and conservation, etc .keeping in view the natural resources and technological possibilities in each district..These plans thus, present the vision for Agriculture and allied sectors within the overall development perspective of the district apart from the financial requirement and the source so financing the agriculture development plans in a comprehensive way.

Once the preparation of District level agriculture planning exercise is completed, the operationalization of such plan is essential. This follows the preparation of a comprehensive State Agricultural Plan (SAP)by integrating the above District level agriculture plans. The DAP therefore could integrate multiple programmes that are in operation in the district concerned, include the resources and activities indicated by the state, combine the resources available from the other programmes and finalize the plan. With this in mind, the District Agriculture Plan for each district of Tamil Nadu is prepared.

GENERAL DESCRIPTION OF THE DISTRICT

Ramanathapuram District is located in the Southern part of Tamil Nadu State on the East Coast of India. Its geographical location is spread between 9° 05' and 9° 50' of North Latitude and 78° 10' and 79° 27' of East Longitude. It is bordered by Pudukkottai and part of Sivaganga districts on the northern side, by Sivaganga district on the North Western side and by Viruthunagar district on the west. The district has the East coast line as its eastern boundary parting the district from the Bay of Bengal. Hence the Palk Strait is guarding the district on the eastern side and Gulf of Mannar on the South. The district has a long history from the medieval kingdom of Pandiya which was in rule in the SouthTamil Nadu. The district takes the honour of being the birth place of Dr.A.P.J. Abdul Kalam , the previous President of India.

District Administration

There are two Revenue Divisions *viz.*, Ramanathapuram and Paramakudi in the district. Details of total number of hamlets, revenue villages, firkas and taluks in each division are presented .Further, details of blocks and number of panchayats and hamlets in each block are presented in Table 2.2.

Details of Revenue Divisions and Taluks of Ramanathapuram District

Name of the Division	Taluks	No. of firkas	No .of Revenue Villages	No. of Hamelet villages
Ramanathapuram	Ramanathapuram	7	67	529
	Tiruvadanai	7	98	635
	Rameswaram	1	2	31
Paramakudi	Paramakudi	6	93	367
	Mudukulathur	6	46	207
	Kamuthi	5	49	352
	Kadaladi	6	45	241
Total		**38**	**400**	**2362**

Details of Blocks and Panchayats in Ramanathapuram District

Sl.No.	Block Name	No. of Panchayats	No. of Hamlets
1	Tiruvadanai	47	310
2	R.S.Mangalam	35	325
3	Paramakkudi	39	163
4	Bogalur	26	91
5	Nainarkoil	37	113
6	Kamudi	53	346
7	Mudukulathur	46	169
8	Kadaladi	60	285
9	Ramanathapuram	25	120
10	Tiruppullani	33	240
11	Mandapam	28	200
	Total	**429**	**2362**

Similar to revenue divisions, number of municipalities in the district are also two and they are Paramakudi and Ramanathapuram. These two municipalities comprised nine towns.

Geology

Most of the area is covered by the unconsolidated sediments of Quaternary age except in the northwestern part, where isolated patches of Archaen Crystallines and Tertiarys and stone are exposed. The Archaeans are mainly represented by the Charnockite group of rocks comprising garnet ferrous granulite and the Khondalite group of rocks made up of quartzite of genesses.

The Tertiary sandstone (Cuddalore Formation) comprise pinkish, yellowish, reddish (variegated colours) medium to coarse grained sandstone and claystone. It is

Overlain by thin alluvium and exposed towards north of Vaigai River. Detached exposures of laterite and lateritic soils are seen in the north western part of the district.

A major part of the district is covered with the fluvial, fuvio-marine, Aeolian and marine sediments of Quaternary age. The fluvial deposits which are made up of sand, silt and clay in varying degrees of admixture occur along the active channels of Vaigai, Gundar, Manimuthar and Pambar rivers. They have been categorized into levee, flood basin, channel bar/ point bar and paleo-channel deposits. The paleo channel deposits comprise brown coloured, fine to medium sands with well preserved cross-beddings.

The fluvio-marine deposits are exposed in the Vaigai delta as deltaic plain, paleo-tidal and dune flat deposits. The deltaic plain and dune flats comprise medium and grey brown sands. The paleo tidal flat deposits include black silty clay, black clay and mud. In Rameswaram Island, the fluvio-marine deposits include in durated sand and dune sands.

The Aeolian deposits comprise red sands which are in nature of ancient dunes and occur over a 3.2 Km wide and eight Km long stretch and lie parallel to the sea coast. These are separated by marshy deposits of black clays. The sands are underlain by calcareous hardpan. In Rameswaram Island also brown sand deposits occur around Sambaimadam on either side of NH49 west of the town.

The marine formation comprises coastal plain deposits of sand and clay in varied proportions. Marine calcareous hardpan occur a slow terraces and platforms, with admixture of quartz, limonite and garnet concentration.

Mineral Resources

Gypsum, limonite, garnet sand, lime shells, salt, clays and building stones are the known mineral potential of the area. The entire occurrence is of local nature only and is not of any economic significance.

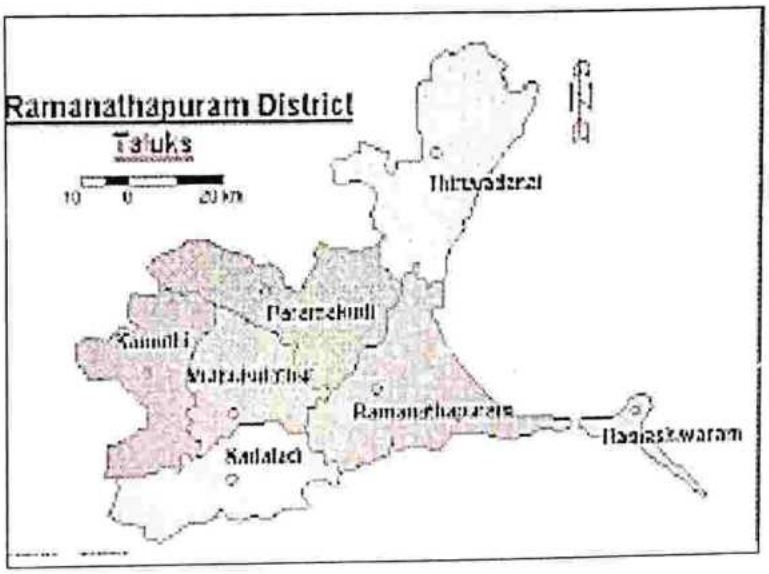


Figure1.Taluk Map of Ramanathapuram District

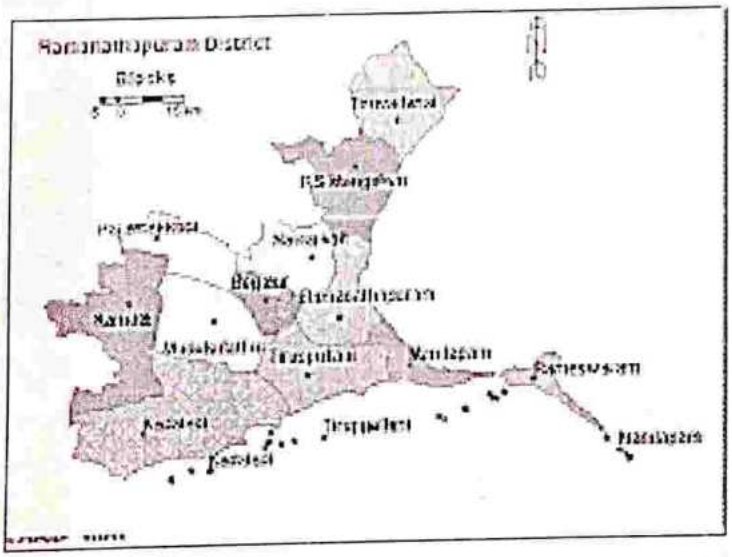


Figure2.BlockMap of Ramanathapuram District

Geomorphology and Geohydrology

Major part of the district is a gently sloping plain except for remnant hills in the western area. Recent Quaternary studies have brought out various erosional and depositional landforms of fluvial and marine regimes. The fluvial landforms comprise flood plains of Vaigai, Varshalei, Pambar, Kottakkarai and Gundar rivers. The marine landforms comprise sand mounds (Teri's) and barrier dunes along the present coast. The erosional processes are manifested in the form of pediment sand pedipal in around Kamuthi.

Geohydrologically, the district has been divided into three zones with reference to laterite, flood basin and coastal plain areas respectively. Further, the area is demarcated into Manimuthar – Pambarbasin , Vaigai delta and Vaipar - Gundarbasin. The Northwestern part of the Ramanathapuram district exposes isolated patches of Archaeans crystallines and Cuddalore Sandstone capped by laterite/lateritic soil. The yields of bore wells of 60 to 90 m depth in the crystallines vary from 3 to 400 l pm draw down of 10 to12m water head. The saline aquifers in coastal tracts occur to a depth range of 80m from ground level followed by fresh water aquifers. The quality of groundwater varies from alkaline to high saline types in the district.

Hydrogeological Conditions

In most places, ground water available at a depth beyond 6 to 7m is saline. The fresh water available within 6 to 7 m depths dries up quickly within 2 to 3 months after monsoon. There is acute drinking water short age in most part of the year. Hydrogeologically, the district can be classified as Omtofoir zones as detailed below:

i) Shallow Fresh Zones

Areas covered by sand dunes, beach ridges, pockets of strand plains, pockets of natural levees, pockets of Palaeo channels, pockets of pediments and valley fills covered by crystallines and tertiary sand stones. The depth of water level varies from few cm to 5M.

ii) Deep and Confined Fresh Water Zones

It occurs in the northern part of the district in Thiruvadanai taluk. The thickness of the cretaceous aquifer is in the order of 20 to 30 M. This is underlined by crystalline basement. In the artesian belt area of Thiruvadanai taluk of Ramanathapuram district, fresh ground water is available at a depth range of 350m – 450m in and around Thiruvadanai, Neerkundram, Vellaiyapuram and in some other places of Thiruvadanai taluk.

iii) Moderate Quality Ground Water Zone

This occurs in certain pockets of river course, pockets of Palaeo channels, parts of pediment sand valley fills and in major parts of stand plains.

iv) Saline Water zones

This is marine and fluvial marine origin. The formations explored upto 780m is found to be unsuitable for any purpose.

Soil

The soils of Ramanathapuram district can be assorted into the main types *viz.,*. ,clay, coastal alluvium, sandy loam, alluvium, sandy and red soil, clay and black cotton soil and the same were believed to have been derived from the Archaen gneisses where calcareous formation are abundant. Calcium carbonate concretions of various sizes and shapes are present in majority of the black soil area and this affects the fertility of the soils. Clay soil, as a whole, constituted about 45 per cent of the total soil. Details are presented in Table 2.3. River alluvium includes alternate layers of sand and clay for a huge thickness. River alluvium occurs in areas bordering the Vaigai river. Coastal alluvium occurs in Kadaladi, R.S.Mangalam, Mandapam, Ramanathapuram, Thiruppullani and Thiruvadanai blocks. There are vast stretches of saline and alkaline soils found in the coastal blocks. Rameswaram Island contains mainly sandy soil.The fertility status of soil showed that nitrogen status of soil is low in all block sand phosphorus status of soil is also low in all blocks with the exception of Thiruppullani,

Kamudhi and Kadaladi blocks. The potash content of soil is high in all the blocks. The mineral resources of the soil include gypsum, limestone and magnesium. While Mudukulathur and Keelakarai regions account for sizable deposits of gypsum, Rameswaram Island contains large quantities of limestone deposits.

Distribution of Soil Type in Ramanathapuram District

(in hectares)

S.No.	Soil Type	Area	Percentage
1	Sandy Soil	7328	1.79
2	Clay soil	182463	44.62
3	Sandy clay soil	22138	5.40
4	Alluvial soil	43769	10.70
5	Sandy loam soil	63602	15.54
6	Coastal Alluvial soil	71357	17.45
7	Red soil	18390	4.50
	Total	408957	100.00

It could be noticed from the table that about 45 percent of the area is clay soil followed by coastal alluvial soil (17.45 percent), sandy loam soil (15.54 percent) and alluvial soil (10.70percent) in that order.

Area under different Problem Soil Categories

In spite of alluvial soil present in the district, scenario of agricultural production is not showing an encouraging trend because of prevalence of problem soils. It could be understood from Table 2.4 that out of 2,06,290 ha. of area, about 54.42 percent alone could be considered as normal soil, 29.28 percent as moderately acidic and 12.40 percent as moderately alkaline soil types.

Details of Problem Soils in Ramanathapuram District

(inhectares)

S.No.	Details of soil	Area	Percentage
1	Normal soil (pH7.5 – 8.5)	112263	54.42
2	Moderately alkaline soil (pH8.6–9.0)	25589	12.40
3	Alakaline soil (pH>9.0)	691	0.33
4	Moderately acidic soil (pH6.0 – 6.5)	60399	29.28
5	Acidic soil (pH > 6.0)	1614	0.78
6	Moderately Saline (EC1.0-3.0)	2121	1.04
7	Saline Soil (EC>5.0)	3613	1.75
	Total	**2,06,290**	**100.00**

Soil Erosion

Yet another problem noticed in the district is soil erosion. From the data presented in Table 2.5, it could be seen that 13.77 percent of the soil area has been identified as severely eroded.

Details of Eroded soils in Ramanathapuram District

(inhectares)

S. No.	Details of eroded soil	Area (ha.)	Percentage
1	Slightly eroded soil (<1percentslope)	280587	85.17
2	Moderately eroded soil	3476	1.06
3	Severely eroded soil	45371	13.77
	Total	**3,29,434**	**100.00**

Micro Nutrient Status

Micronutrient status presented in Table 2.6 shows that in general soil status is low in zinc and high in manganese. Taluks like Paramakudi, Kamuthi and Kadaladi have high content of copper, iron and Managanese. Ramanathapuram,Thiruvadanai and

Mudukulathur taluksare deficit(low)inzinc,copperandiron.

Micronutrient Status of Soil in Ramanathapuram District

S.No.	Taluk	Zinc	Copper	Iron	Manganese
1	Ramanathapuram	Low	Low	Low	High
2	Thiruvadanai	Low	Low	Low	High
3	Paramakudi	Low	High	High	High
4	Kamuthi	Low	High	High	High
5	Mudukulathur	Low	Low	Low	Low
6	Kadaladi	Low	High	High	High

Distribution of Different Soil Structures in Ramanathapuram District

(Areainhectare)

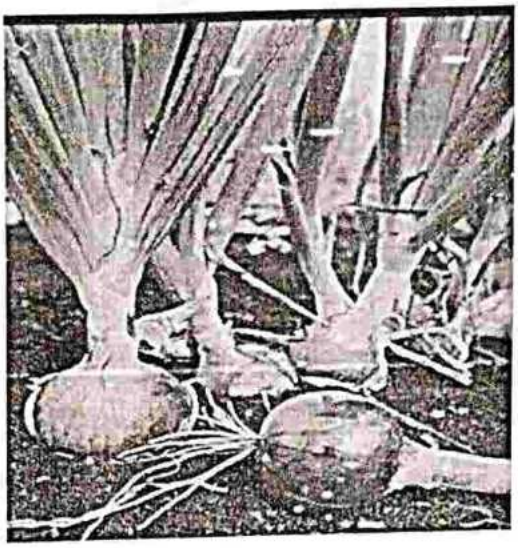
SoilDescription	Area(ha.)
Deep, fine, mixed, Alfisols	1,18,833.30
Deep, fine, montmorillonitic,Vertisols	31,243.00
Verydeep, fine, montmorillonitic,Vertisols	30,148.09
Deep, finesilty, mixed, Inceptisols	22,807.06
Verydeep, coarseloamy, mixed, Entisols	19,436.67
Verydeep, coarseloamy, mixed, Inceptisols	16,341.37
Deep, fineloamy, mixed, Inceptisols	11,773.43
Verydeep, fineloamy, mixed, Entisols	11,003.18
Verydeep, fineloamy, mixed, Alfisols	8,990.90

Contd... (Areainhectare)

Soil Description	Area(ha)
Verydeep, coarseloamy, mixed, Alfisols	8,742.37
Verydeep, veryfine, montmorillonitic, Inceptisols	8,593.71
Deep, very fine, montmorillonitic,Vertisols	7,345.98
Deep, fine, mixed, Inceptisols	7,324.96
Verydeep, fine loamy, mixed, Inceptisols	6,246.87
Moderately shallow, fine loamy, mixed, Inceptisols	6,190.82
Deep, contrasting particle size, mixed, Inceptisols	6,104.92
Very deep, fine, montmorillonitic, Inceptisols	5,157.07
Verydeep, contrasting particle size, mixed, Inceptisols	5,089.75
Moderately deep, fine, mixed, Inceptisols	5,065.44
Very deep, sandy, mixed, Alfisols	4,871.80
Moderately shallow, fine loamy, mixed, Alfisols	4,406.27
Moderately deep, fine, mixed, Alfisols	2,643.78
Very deep, fine, kaolinitic, Alfisols	2,561.55
Deep, coarse loamy, mixed, Entisols	1,562.22
Very deep, clayey skeletal, kaolinitic, Alfisols	1,461.33
Very deep, sandy, mixed, Entisols	1,208.75
Moderately deep, very fine, montmorillonitic,Vertisols	946.28
Deep, sandy, mixed, Entisols	745.45
Moderately deep, fine, montmorillonitic, Inceptisols	468.60
Deep, fineloamy, mixed, Alfisols	422.76
Shallow, clayey, mixed, Alfisols	12.49
Deep, coarse loamy, mixed, Alfisols	0.36

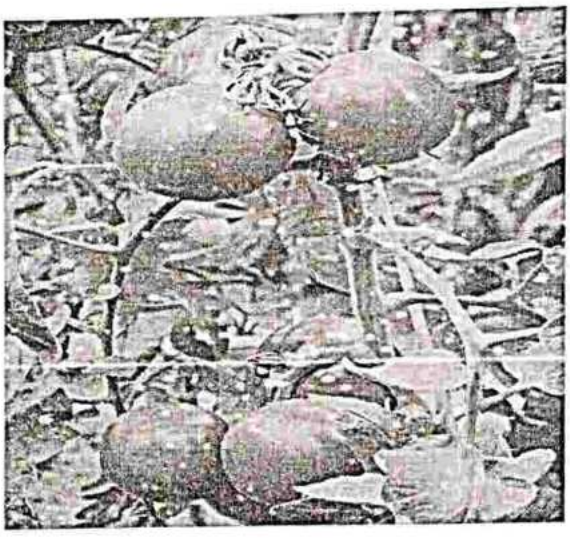
S.NO	BOTANICAL NAME
1	*Oryza sativa* – Paddy
2	*Capsicum annuum* - Chilli
3	*Solanum lycopersicum* - Tomotto
4	*Gossypium herbaceum* - Cotton
5	*Solanum melongena* - Brinjal
6	*Allium cepa* - Gnion
7	*Arachis hypogaea* - Goundnut
8	*Cocous nucifera* - Cocount
9	*Cyamposisteragonoloba* - Cluster beans
10	*Coriandrum sativa* - Coriander
11	*Pennisetum glaucum*– Millet
12	*Momordica charantia* - Bitter Gourd

¶ *Arachis hypogaea*



Allium cepa

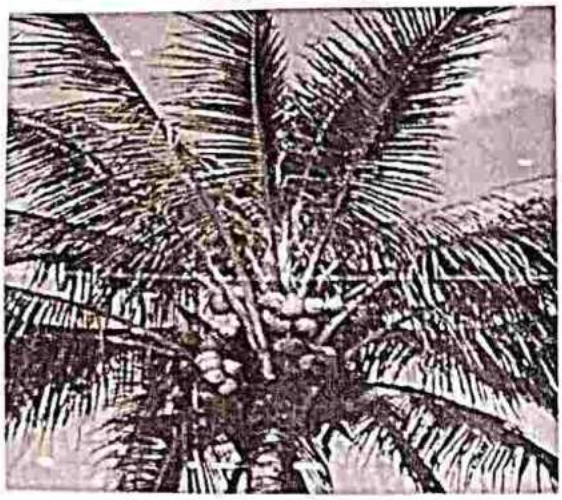
Capsicum annuum



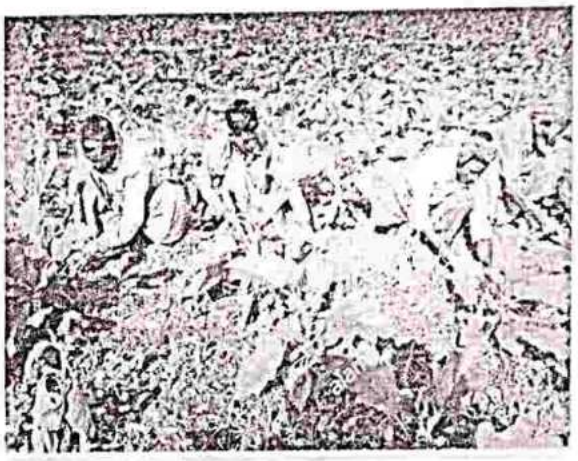
Solanum lycopersicum

Coriandrum sativa

Pennisetum glaucum



Cocous nucifera

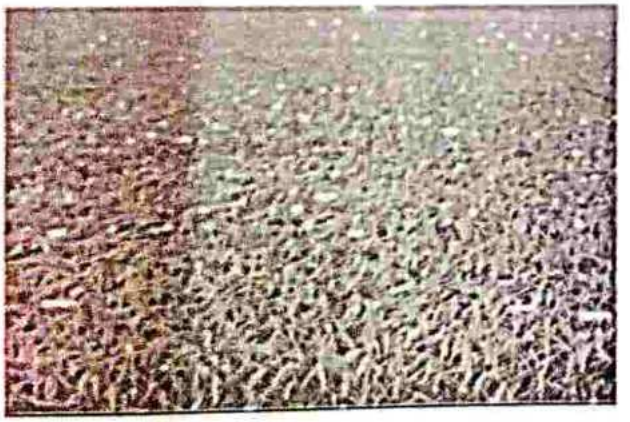


Solanum melongena

Gossypium herbaceum

Momordica charantia

Oryza sativa



Land Holding Pattern

Blockwise distribution of marginal and small farmers and the irrespective area are presented in Table 2.9. Percentage of marginal farms to total in the district was found to be the largest in Kamuthi, Muthukulathur, Kadaladi and Thiruvadanai blocks;

Percentage of smallfarms was more in Muthukulathur, Kadaladi, Kamuthi and Paramakudi blocks. Similarly, percentage of agricultural labourers was found to be high in Thirupulani, Muthukulathur, Kamuthiand Kadaladi blocks.

S. No	Block	No.of Marginal Farms	No. of Small Farms	Total area under Marginal Farms		Total area under Small Farms		No.of landless Agrl. labourers
				Wet	Dry	Wet	Dry	
1	Ramanathapuram	21410 (9.43)	2466 (3.93)	1150 (4.32)	4610 (6.14)	970 (5.81)	2310 (4.38)	2180 (4.38)
2	Thirupulani	13808 (6.08)	3534 (5.64)	1120 (4.21)	5102 (6.80)	760 (4.55)	3950 (7.50)	6294 (12.66)
3	Mandapam	12264 (5.40)	2102 (3.35)	954 (3.59)	4220 (5.62)	298 (1.78)	2031 (3.85)	1560 (3.14)
4	Thiruvadanai	29807 (13.13)	6193 (9.88)	3027 (11.38)	8129 (10.83)	2274 (13.62)	5084 (9.64)	4109 (8.26)
5	R.S.Mangalam	18758 (8.26)	3424 (5.46)	3868 (14.55)	4496 (6.00)	2130 (12.76)	3066 (47.60)	3181 (6.40)
6	Paramakudi	17286 (7.61)	7046 (11.25)	3077 (11.57)	4789 (6.38)	2126 (12.74)	3882 (5.81)	4714 (9.48)
7	Bogalur	16870 (7.43)	4389 (7.00)	1975 (7.43)	5536 (7.38)	1139 (6.82)	3324 (6.30)	1868 (3.76)
8	Nainarkoil	14822 (6.53)	5871 (9.37)	1427 (5.37)	4765 (6.35)	1309 (7.84)	3894 (7.38)	2608 (5.24)
9	Kamuthi	40145 (17.68)	9855 (15.73)	2178 (8.19)	11962 (15.94)	1383 (8.29)	10301 (19.53)	4991 (10.04)
10	Mudukulakthur	38628 (17.02)	10483 (16.73)	4887 (18.38)	11503 (15.33)	2823 (16.91)	6444 (12.22)	7143 (14.37)
11	Kadaladi	33189 (14.62)	7292 (11.64)	2925 (11.00)	9924 (13.22)	1476 (8.84)	7575 (14.36)	11070 (22.27)
	Total	**226987 (100.00)**	**62653 (100.00)**	**26588 (100.00)**	**75038 (100.00)**	**16690 (100.00)**	**52731 (100.00)**	**49718 (100.00)**

Details of Land Holding Pattern in Ramanathapuram District

Rainfall Pattern

Season wise distribution of rainfall in the district is presented in Table 2.10. It could be seen from the table that major rainy season in the district was the North East monsoon season wherein 60.65 percent of the normal annual rainfall was received. Next rainy season was the South West monsoon season which received nearly 16 percent and about 15 percent of annual rainfall was received during summer months.

Seasonwise Rainfall Distribution in Ramanathapuram District

(inmm)

S.No.	Season	2003	2004	2005	2006	2007	Normal	Percentage
1	Winter	-	5.3	25.5	43.0	89.5	67.4	8.15
2	Summer	108	147.4	346.9	91.2	31.4	122.7	14.84
3	SouthWest Monsoon	65.1	225.2	66.7	54.1	125.5	135.3	16.36
4	North EastMonsoon	393.8	796.2	810.5	743.9	622.2	501.6	60.65
	Total	**566.9**	**1174.1**	**1243:2**	**931.9**	**868.6**	**827.0**	**100.00**

Ground Water Potential

Paramakudi and Kamuthi blocks have been identified to be in semi critical stage of ground water exploitation (60-85 percent exploitation) in the district (Table2.14).

GroundWaterPotentialinRamanathapuramDistrict

S.No.	Over exploited(100percent)	Critical (55-100percent)	Semi Critical (60-85percent)
1	-	-	Paramakudi
2	-	-	Kamuthi

Agriculture

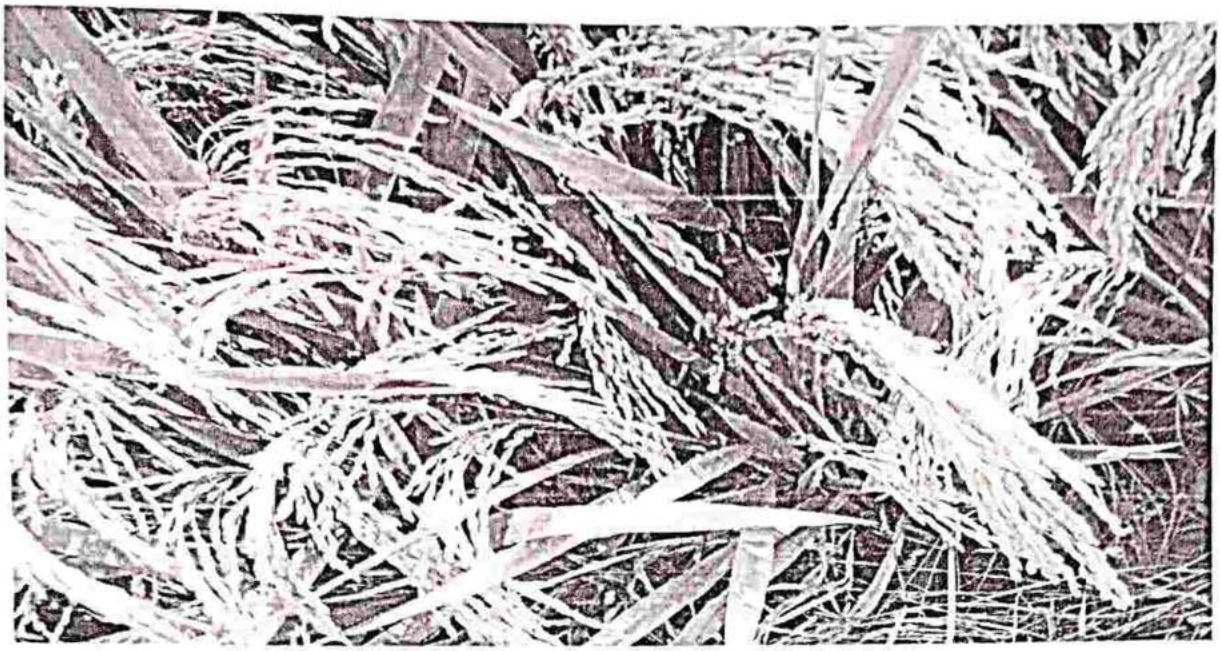
Like any other district in the State, Ramanathapuram also agriculture is the back bone of the district' seconomy. Out of the total cropped area of 183651ha, about 37.64 percent receive any form of irrigation, and hence mosily the crops are be in raised under rain fed condition (62.36percent).

Area under Important Crops

Distribution of different crops in Ramanathapuram district is presented in Table. It is clear from the table that about 67 percent of net sown area in the district is under paddy followed by chillies (12percent). Other major crops cultivated are coconut, oilseeds, cotton, millets and pulses.

General Cropping Pattern of the District below

Paddy



In Ramanathapuram district, paddy is the main food crop cultivated in more than63 percent of the net area sown. It is cultivated both as irrigated and rainfed. Rainfed sowing generally would commence from August and will extend upto October.In early sown area, farmers used to raise medium and long duration varieties of paddy.There is no marked area for late sowing, but when the monsoon rains delayed, the sowing will bealso taken up lately. In the late sown areas like, medium and short duration paddy varieties are sown. Farmers are having 10 local paddy varieties in addition to high yielding varieties with the duration ranging from 105-130 days and they will choose varieties according to their needs. Red gram is sown as a mixed crop in rain fed areas and also grown in garden land limited extent. In tank fed in specific area, irrigated paddy is sown generally in August to November. Sometimes, sowing will be further extended up to December depending on accumulation of rainwater in the tanks and also release of water from Vaigai Dam to the Vaigai fed system tanks.

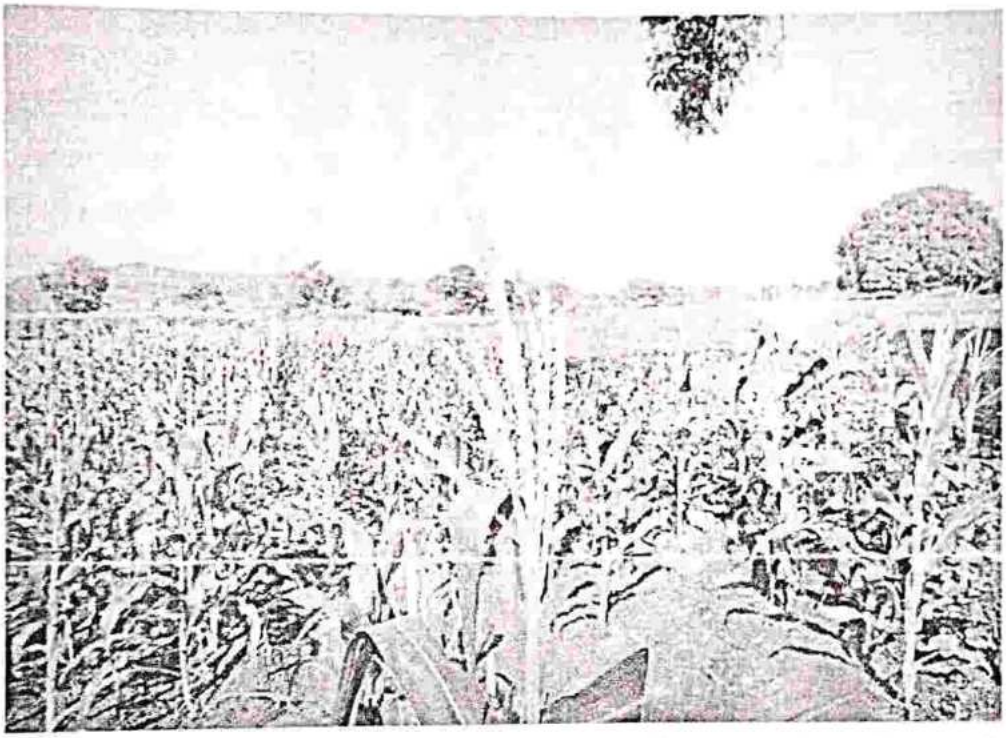
Crop Wise Area Cover age in Ramanathapuram District

(inhectares)

S. No.	Crop	2001-2002	2002-2003	2003-2004	2004-2005	2005-2006	Average	Percentage
I	Agriculture							
	1) Paddy	124517	126152	121031	126607	127395	125140	67.30
	2)Millets	3691	3899	6857	7004	6801		
	3)Pulses	2375	2375	3937	3055	2738		
	4)Oilseeds-edible	9194	8437	14662	10404	9681		
	Oilseeds-nonedible	--	--	110	132	200		
	5) Cotton	4415	1529	3310	5413	3733		
	6)Sugarcane	597	685	451	260	361		
	7)Coconut	8111	9120	8343	8472	8526		
	Total	152900	152197	158701	161347	167961	158567	85.28
II	Horticulture							
	a)Spices -							
	1)Chillies	18559	18115	22742	23126	19021	20312	10.92
	2) Coriander	1560	1130	1921	1684	1518		
	3)Others	205	219	238	223	274		
	Total	20324	19464	24901	25033	20813	22107	11.90
	b)Fruits							
	1)Banana	175	169	147	131	88		
	2)Mango	122	121	128	122	148		
	3)Guava	46	54	69	49	96		
	4)Cashew	98	124	230	261	375		
	5)Others	31	67	156	165	190		
	Total	472	535	730	728	897		
	c) Vegetables	94	150	152	191	165		
	d) Flowers							
	1)Jasmine	68	80	64	99	68		
	2)Others	2	5	22	2	--		
	Total	70	85	86	101	68		
	e)Drugs & Norcotics (Betel wine & others)	23	33	36	32	33		
	HorticultureTotal	20983	20267	25905	26085	21976	23043	12.39
III	Palm yrah	3985	4457	4435	3850	3904		
IV	Fodder crops	307	321	527	433	204		

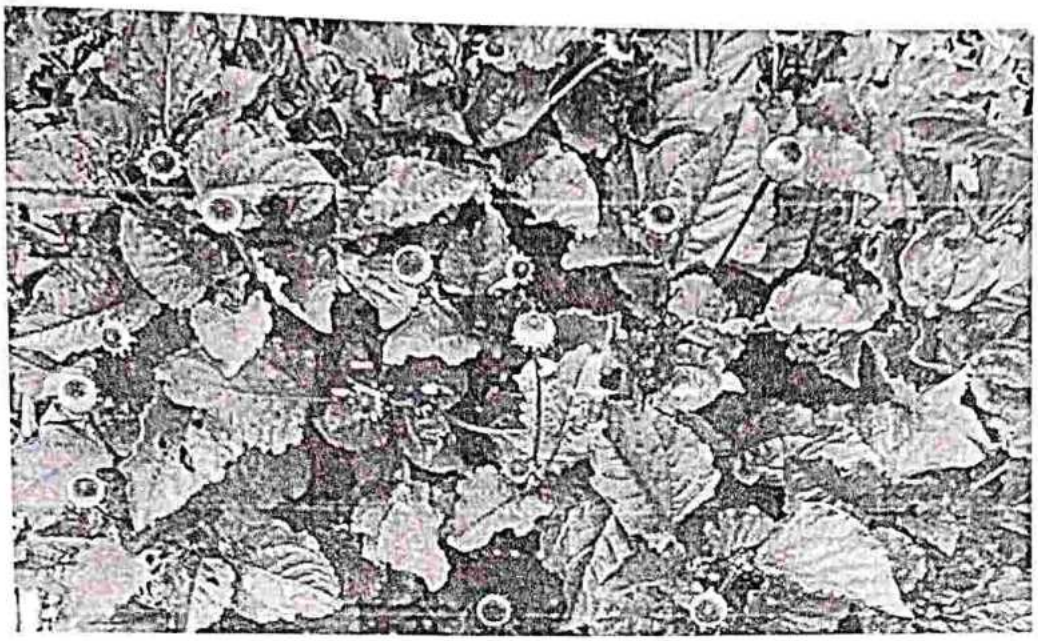
V	Other miscellaneous on food crops	4769	1853	700	108	44		
	Net Area Sown	182945	179102	190268	191823	185563	185940	100.00

Cholam



Rain fed cholam sowing is taken up in dry lands between July and September. Beyond September, there would not be any sowing of cholam crop and cumbu crop will be sown as alternate crop in those areas. Lab pulses is also sown as mixed crop.

Cumbu



Rain fed cumbu sowing is generally taken up between September and November. Only in Ramanathapuram taluk, the sowing will be extended up to December. Irrigated cumbu is taken up from February, March toJune, July.

Ragi

Rain fed ragi sowing is taken up during September and October. Irrigated ragi is mainly sown in September to October in East Ramanathapuram where the crop is sown in tank fed ayacut areas.

Minor Millets

Minor millets are generally sown between July and November and the area is spreaded over throughout the district.

Cotton

Rainfed cotton sowing is taken up in September-October.The sowing will be extended sometimes upto December depending upon rainfall. Rice fallow cotton is generally sown in Paramakudi and Kamuthitaluks during January-February months.

Pulses

Red gram is sown in June - August. Black gram, green gram and cowpea are sown as rain fed crops in September and October months. Red gram is sown as mixed crop with millets and groundnut. Black gram and Green gram are sown as pure crop as well as mixed crop with cotton and sugarcane. The Cowpea is sown as pure crop and also in some places as mixed crop with millets.

Groundnut and Gingelly

Groundnut and gingelly are cultivated mostly under rain fed conditions, during the months of December-January and April–May.

Chillies

Chillies are cultivated in both rain fed and irrigated conditions. They are directly broadcasted in the month of September. The transplanted chillies will be taken in the fort night of November.

Input Distribution in Agriculture

Supply of farm input sand their usage by the farmers is a pre-requisite in achieving targeted growth rate in agriculture. Hence status of input use by the farmers of Ramanathapuram district is presented in the following table. It could be noticed from the table that on an average 442.21tonnes of seeds are distributed annually and almost 84 per

Cent of them was paddy seeds followed by oilseeds (11.62 percent). In the case of mineralnutrientmixture,19.50 percent of the average quantity of 29.73 tons distributed annually, is used in paddy cultivation and about seven per cent in coconut cultivation and only two percent in pulses cultivation.

Chemical fertilizer uses owed that on an average12,047 tons of NPK fertilizers is being used by the farmers and in this about 64 per cent was nitrogenous fertilizers; 24 per cent was phosphoric and 12 per cent was potash. Plant protection liquid chemicals are distributed to the tune of 6400 litres per year and the quantity of dust chemicals was four M.T. On an average 23,000 coconut seedlings were distributed and in this 76 percent was Tall variety and the remaining 24 percent was Tall XD warf variety.

Details of Inputs Distribution

Sl. No.	Name of Input	Unit	2005-06	2006-07	2007-08	Average	Percentage
	Seed Distribution						
1	Paddy	Tonnes	327	363.4	417.5	369.30	83.51
2	Millets	Tonnes	4.13	6	6	5.38	1.22
3	Pulses	Tonnes	8.3	6.635	6.16	7.03	1.59
4	Oil Seeds	Tonnes	61.98	56.645	35.57	51.40	11.62
5	Cotton	Tonnes	9.5	8.29	9.52	9.10	2.06
	Total						**100.00**
	Bio Fertilizer		**128263**	**154215**	**138982**	**140486**	
	Mineral Nutrient Mixture						
1	Paddy	Tonnes	18.3	23.7	16.45	19.48	65.53
2	Millets	Tonnes	0.4	0.175	0.934	0.50	1.69
3	Pulses	Tonnes	0.031	0.094	6.05	2.06	6.92
4	Cotton	Tonnes	0.129	0.193	0.35	0.22	0.75
5	Coconut	Tonnes	6.785	7.382	6.616	6.93	23.30
6	Gingelly	Tonnes	0.315	0.412	0	0.24	0.82
7	Groundnut	Tonnes	0.235	0.19	0.45	0.29	0.98
	Total						**100.00**

Table2.16Contd....

Sl. No.	Name of Input	Unit	2005-06	2006-07	2007-08	Average	Percentage
	Chemical Fertilizer						
1	Nitrogen	Tonnes	7414	8842	6823	7693	63.86
2	Phosphorus	Tonnes	3084	2943	2496	2841	23.58
3	Potash	Tonnes	1331	1431	1777	1513	12.56
	Total	Tonnes	**11829**	**13216**	**11096**	**12047**	**100.00**
	P.P. Chemicals	Tonnes					
1	Liquid	Litres	31120	35181	18467	28256	6389.72
2	Dust	Tonnes	18.36	23.373	11.16	17.63	3.99
	Distribution of Coconut Seedlings						
1	Tall	Lakhs	0.217	0.149	0.159	0.18	76.09
2	Tall X Dwarf	Lakhs	0.046	0.061	0.058	0.06	23.91
	Total	**Lakhs**	**0.263**	**0.21**	**0.217**	**0.23**	**100.00**

Composite Index of Agricultural Development of Ramanathapuram District

Agricultural Development of a district is a comprehensive multidimensional process involving large number of related indicators. Hence, it can be well represented by composite indices which are used as yard sticks not only to gauge the development of each district but also to compare its performance in relation to other districts. These indices help to classify the sub-regions based on a set of large multivariate data. The information contained in the large set is transformed into a small set of indices which would provide a convenient method for classification. There are many methods of classification based on multi variate data. Among them, one method which is statistically sound is that developed by Iyengar and Sudarshan (1982). This method is simple and easy to apply and it helps to classify the districts into various stages of development, *viz.*,, 'highly developed', 'developed', 'developing', 'backward' and 'very backward'. In this method for each district a 'composite index' is constructed. The index lies between 0 and 1 with 1 representing 100 per cent development and 0 representing no development at all.

It is assumed that there are'n' districts and 'm' development indicators and that X_{id} Is the observed value of i^{th} Development indicator forthe d^{h} district (i=1,2,3...m, d = 1,2,3...n). First these values of development indicators for each district is to be standardized. When the observed values are related positively to the development (as in the case of cropping intensity), the standardization is achieved by employing the formula

$$y_{id}=(X_{id}- MinX_{id})/(Max\ X_{id}- MinX_{id})$$

where $MinX_{id}$ and $MaxX_{id}$ are the minimum and maximum of $(X_{i1},X_{i2},.....X_{in})$ respectively. When the values of the case of are a under wastelands, problemsoilsetc.,) the standardized values will be computed by the formula Obviously the standardized indices lie between 0 and 1. The indices are then used to determine the weights of individual variable and then they are subjected to further statistical analysis by fitting suitable probability distribution to determine the cut-off points for classification of the districts into five categories as mentioned above. The detailed methodology can be found in Iyengar and Sudarshan (1982).

The data base for the current study on Ramanathapuram district is taken

from various government publications like Season and Crops Report and Economic Appraisal of Tamil Nadu for the four periods 1990-91, 1995-96, 2000-01 and 2005-06. In all, 25indicators of agricultural development as given in Table 3.1 were used for estimating the composite index of development for the district. The 25 indicators were grouped into six different 'components': i) Crop-Area-Variables (10) ii)Irrigation (7) iii)Livestock (3) iv)Fisheries (1) v)Fertilizer (3) and vi) Cultivators and Labourers(2).

The analys is showed that Ramanathapuram district was classified as 'verybackward'in agricultural development in all the four periods. In terms of overall agricultural development its rank among the 29 districts of Tamil Nadu varied from 27 to28 during the 1990-91 to 2005-06. As for as the individual components of agricultural development are concerned, its rank in the above periods are summarized in Table 3.2.The table shows that except fisheries, in all other components, its performance in the period of study is not satisfactory . For example, in irrigation it ranks between 28th and29th in all the four periods. Similarly in crop variables also it occupied ranks between 27thand28th ranks.

Note on Crop Varietie sand their Performance

Crop: Paddy **Variety: RMD(R)**

Seed Distribution Details

Year	Quantity(Kg)	Type of seed	Purpose
2006-07	400	TFL	Research trails of FLD &OFT
2006-07	350	TFL	Rock feller foundation scheme
2007-08	690	TFL	Seed Village Scheme
2007-08	350	TFL	Rock feller foundation Scheme
2008-09	1500	TFL	Tobe distributed to the farmers

Development/Interventions needed and Identified

S.No	Crop	Problem	Interventions
1.	Brinjal	The color of the Mahycohybrids of brinjal is the major constraint to market the produce.	Suitable hybrids will be suggested.
2.	Chillies	No processing industries	Processing industries on value addition of chillies
3.	Vegetable crops Tomato, Chilli, Brinjal & Fruitcrops	Lack of cold storage facilities	Cold storage facilities can be introduced

State Department of Agriculture under the Directorate of Agriculture has implemented several schemes for the welfare of farmers raising paddy, millets, pulses (redgram, black gram and green gram), oilseeds (ground nut and gingelly) and cotton. In order to boost the yield of crops cultivated, mostly as rain fed, following interventions are considered to be worth

- Quality seed supply,
- Organic manuring,
- Vermicompost production,
- supply of farm inputs like zincsulphate , gypsum, biofertilizers, micro nutrient mixtures and powertillers.

Besides, there is a need to train the farmers on modern technologies through village campaigns audio visuals and to establish community structures like rural god owns, thrashing

floors and drying yards. These activities would deliver the complete output in full spirit only when the harvested grains and products are protected from rain and shine. Hence the department officials have proposed supply of tarpaulins at subsidized rates to the farmers in need of them. Considering the importance of Farm Yard Manure in maintaining soil health, such interventions as popularizing preparation and application of enriched Farm Yard Manure to different rain fed crops of millets, pulses, oilseeds and cotton were also recommended.

All these activities are combined as the components of a separate proposal entitled, **"Dry Farming Technology for Rain fed Crops".** Ramanathapuram district has already grabbed its share towards establishing seed testing laboratory which is a State level endeavor to ensure supply of quality seeds to farmers of Tamil Nadu. Altogether the department has proposed the following schemes for implementation during the period2008-2012.

- Increasing the production of rice in Ramanathapuram District.
- Increasing the production of millets in Ramanathapuram District
- Increasing the production of pulses in Ramanathapuram District
- Increasing the production of oil seeds in Ramanathapuram District
- Increasing the production of cotton in Ramanathapuram District
- Dry Farming Technology for Rain fed Crops

Obviously the standardized indices lie between 0 and 1. The indices are then used to determine the weights of individual variable and then they are subjected to further statistical analysis by fitting suitable probability distribution to determine the cut-off points for classification of the districts into five categories as mentioned above. The detailed methodology can be found in Iyengar and Sudarshan (1982).

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Conclusion

Ramanathapuram is one of the coastal districts bounded on the north hy Sivaganga and Padikal distrets, on the east and south by the Bay of Bengal, and on the west by Thoothukudi and Virudhunagar districts with an area of 4.175 km. Ramanathapuram district comprises of 7 taluks 11 blocks and 2362 villages With regards to the hierarchy of administrative arrangement are 2 municipalities 7 town panchayat und 429 village panchayats in the district.Most of the soil type in clay (45%) followed by coastal alluvial soil (179) and sandy lam (15%) and there no scope for large scale mining in the district. Ramanathapuram district is deficient in rainfall. In Ramanathapuram District, paddy is main lood crop cultivated in more than 6% of the net area sown.There are IN east areas in Ramanathapuram district am using a total areas of 5.356.85 has Designated a Biophite Reserve. The Gulf of Mannar is one of the biologically richest coastal regions in all of mam land of India with corals sea grasses, mangroves and their important fora and Gulf of Mannar Biosphere Reserve. (GOMBR) was declared in 1989 as the first marine Biosphere Reserve in the country.The district has 2 mens viz. Vaigai and Gundar, but they are not . Ramanathapuram district has 271 km of coastal line of which 130 km in Palk bay and 140 kr in Gulf of Mannar . The district is considered as an industrially backward areas as there and no major industries in the district. Ramanathapuram district is highly drought prone be caine of the lack of in and rivers. The island of Sri Lanka acted as a high barrier during taunami a kong with coral reefs mangroves and sea grasses of both Gulf of Mannar and Palk Bay, Coral and sea grass restoration has been done by Sagathi Deavadan Marine Research Institute in Gulf of Mannar cost of the district Mangrove restoration has been dine by the Forest Department.

A study on Manufacturing process of cotton in spinning Mill

A short field project work submitted to

St. Mary's College (Autonomous)

Affiliated to

MANONMANIAM SUNDARANAR UNIVERSITY

In partial fulfilment of the requirement for the Degree of Master of science in Botany

By

R. SANKARA ESWARI - 20APBO08

DEPARTMENT OF BOTANY

ST.MARY'S COLLEGE (AUTONOMOUS)

THOOTHUKUDI-628001

CERTIFICATE

It is certified that this short field project work entitled "**A study on Manufacturing process of cotton in spinning Mill** submitted to St. Mary's college (Autonomous) affiliated to MANONMANIAM SUNDARANAR UNIVERSITY in partial fulfilment of the requirements for the degree of Master of science in Botany, and is a record of work done in the Department of Botany, St.Mary's College (Autonomous), Thoothukudi during the year 2020 – 2021 by the following student.

By

R. SANKARA ESWARI - 20APBO08

GUIDE

HEAD OF THE DEPARTMENT i/c.

M. Glory
EXAMINER

Lucia Rose
PRINCIPAL
St. Mary's College (Autonomous)
Thoothukudi - 628 001.

DECLARATION

Hereby declare that the dissertation entitled **"SHORT FIELD PROJECT"** as the original work and it has not been submitted for the award of any Degree, Diploma, Fellowship or any other similar title and that the dissertation represents independent and original work on the partial fulfillment of the requirements for the degree of **MASTER OF SCIENCE IN BOTANY** during **2020-2021,** submitted to St.Mary's College (Autonomous), Thoothukudi - 628 001.

Place : Thoothukudi - 628 001

Date :

R. Sankara Eswari.

R. SANKARA ESWARI (20APBO08)

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Table of Contents

Introduction

The purpose of this project if about thread textile, from the raw material cotton yarn to thread and about the machineries. Yarn is long continuous length of inter lock fiber. Which is suitable for use in the production of textile sewing knitting viewing etc. The first known tool used to make a thread from yarn was these spindle then the used the spinning wheel. In the 17th century flying shuttle and spinning jenny was invented which made work easier, with the lesser man power. Yarn are of the different types such as animal fiber, plant fiber and synthetic.

In this project, I have discussed about cotton yarn (plant fiber to dhoti thread). Her I have collected the few information from SRS Textiles which is situated Sathyamangalam. Which specialized in the making of cotton dhoti thread. India is one of the leading cotton manufactures, and textile is one of the industries which provide a source of income and job opportunity to many in India.

Yarn and tools

I] HISTORY OF YARN:

Its origin cannot trace for some ancient European yarn. Artifacts have been carbonated roughly 2000 years. Going back to the stone age. we can see it was made from animal's hides, grasses and trees. As ages passed; Ancient Egyptians were making the best linen at that time. While was India working on cotton and china on silk and is still working. central Asia was profiting of wool and making across the world the textile industry only grew and benefiting of trait roots.

Early versions of the spindle were simple asterisk with small notch and a rock. It predates invention of the wheel by 1000 years. Hands spindle would have been made from be borne, antler, sticks or any materials that was available to them at that age. A spinning wheel was invented in India at around 500-1000CE. Thus new versions of spinning wheel which was mechanical gave a steady spinning rate and made it easy to manage the fiber by reliving both hands. This mechanical instrument was slowly adapted to meet the needs of local fibers throughout the world

Spindle

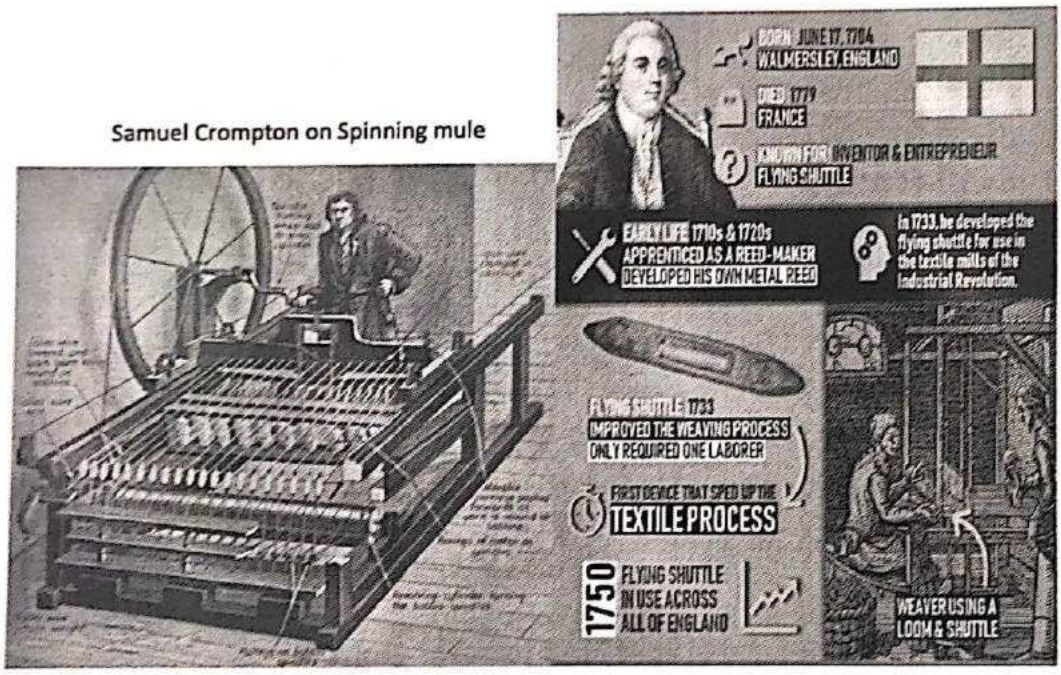
Spinning wheel

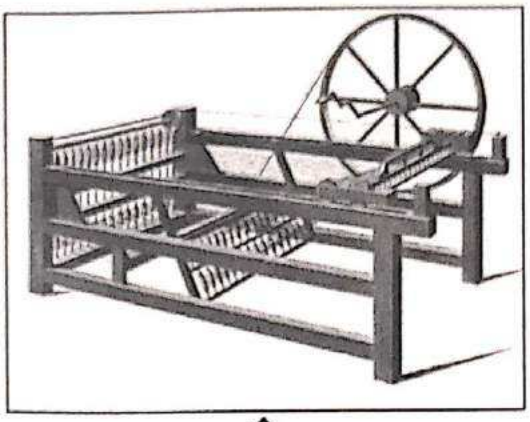
There was many ways yarn could be made up into something once it was made weaving is the craft that was dated back to Paleolithic Age Era or stone age. In Egypt it was dated back to 5000 BCE. In weaving 100m in weaving the basic or still in used today but there were changes overtime to make this process easier.

Knitting has a history that is fuzzy. The oldest item that was knitted, found item was the pair of socks which was from 11th century CE Egypt. Knitting moved to Europe at around 13th Century with the end to nalbinding. Nalbinding was, or knitted with one needle was use by the Vikings. One piece of mitten was discovered, which dated back to 1000 CE in Finland.

Moving on from one history to another. In France at about 1500 Crochet was used by nuns to create decoration for churches. Crochet means hook in French and it needed special kills by 1700-stambour Crochet or modern date embroidery started to came popularity by the 1800-S Crochet evolved to shepherd knitting and Irish Crochet which did in need the fabric to Crochet on.

Samuel Crompton on Spinning mule





The Spinning Jenny

The Ring Frame

Through the 18th Century yarn making and yarn product a common job. Not only in particular places but throughout Europe Asia and part of America. People made the own way of making yarn with advancement in these spinning mill weaving room it was easy to produce to large scale items such as ropes and sheep masts. In the year 1733 the flying shuttle was invented which made weaving easier on a large scale. In the year 1766 the invention of the spinning jenny made work easier. In the area of man power; were 8 people looks was done by a single person at the around Samuel Crompton invented the spinning mule. It was a water power a spinning machine with multiple spindle. Come 1780 stream power and the rest of the industrial revaluation took over and turned yarned into what we know today. James thought invented the ring frame. Which involves 100 of upright spindle. It creates one long piece of span yarn and another method is open-end. Which is the when fiber added to as spinning drum and drawn up creating a heavier and fuzzier fabric create for denim and towels.

II]Types of Yarn:

1. Animal fiber- Based Yarn

   - Animal Fiber are textile fiber obtained from animal.
   - Animal fiber basically hair or fur or skin or secretion of animals
   - Animal fiber are then woven are nectar are felted to calm beautiful
   - Animal Fabric are ultimately made into soft and warm jackets ponchos by lazars wraps, shawls, coat and other morphing and accessory. Copper rugs and blanket made with refer fiber.
   - Sheep, camel, Goat and rabbit are the commonly used animal for providing animal fiber which are very soft in texture horse, cow, pig gives straight fiber which are left soft.
   - The feather of birds is a fiber tool

2. **Plant fiber- Based Yarn**

   - ❖ Plant Fiber are constituted of cellulose fiber, consisting of helically wound cellulose micro fibers, born together by amorphous lignin matrix
   - ❖ Few plants fibers are hemp, jute, banana, kenaf, bamboo, cotton, etc.

3. **Synthetic fiber - Film Yarn**

   - Acrylic Yarn
   - Novelty Yarn
   - Polyester Yarn
   - Self – Stripping yarn

Cotton Yarn

Cotton Yarn is a fabric which is woven from cotton thread. The plant from which cotton is gathered is a shrub native to tropical and subtropical regions around the world including the America, the Africa, Egypt and India. In Mexico wild cotton species is found in great quantity

I] About cotton:

Cotton is a stable fiber, which means it is composed of different, varying length of fiber cotton is made from the natural fibers of cotton plants, which are from the genus Gossypium. It is primarily compound of cellulose an in soluble organic compound crucial to plant structure and is the soft and fluffy material. The cotton plant need lots of sun, along period without frost, and a good amount of rain. The term "Cotton" refers to the part of the cotton plant that grows in the boll. The encasing for the fluffy cotton fibers. Cotton is spun into yarn that is the woven to create a soft durable fabric.

The first cotton gin which is a tool that separates the cotton fluffy from the plant seeds, was invented in India in the 13th century.

Cotton is one of the most important fibers and a cash crop of India and plays a dominant role in the industrial and agricultural economy of the Country. Cotton is the most important fibers crop not only of India but of the entire world. It provides the basic raw material (Cotton fibers) to the cotton textile industry. Cotton in India provides a direct lively hood to 6 million farms and about 40 – 50 million people are employed in the cotton trade and it's processing.

II] Types of Cotton

Cotton was first domesticated from wild plant in India around 4000 BCE and around same time in Mexico. The cotton plant is shrub that prefer tropical and subtropical climates. The spinning fiber coming from boll or seed pot that are surround by flub by fiber until the invention of the cotton gen in the later 1700-S all cotton processing done by hand.

There are many pieces of cotton grown throughout the America, Africa, Asia and Australia. China is leader in cotton product a united state leads the world in cotton. Exports other leading cotton producing countries include Uzbekistan India Turkey, Pakistan and brazil there are over 50 Species cotton but the species that are grown for production around two main types old world cotton of India, Pakistan and new world cotton of America's the united states mainly grew variety of appellant cotton which account for 95% cotton marker old world. Cotton is 5% Percentage of the global cotton market. Cotton came in many color from tans, browns to blues green pink. The white is the only color that is mass produce commercial use.

III] There are four different types of cotton each with its own characteristics.

(i) **Pima cotton:**

Considered the finest type of cotton in the world, pima cotton's fibers are extra soft and extra-long. The cotton is native to south America and the America south east pima cotton fabric is very highly – sought after, as it is resistant to fading, tearing and wrinkling.

(ii) **Egyptian Cotton:**

Egyptian cotton is very similar to pima cotton. The two are even in the same Scientifics class.

Gossypium barbadence:

It has the same resistant qualities, but it is grown in the Nile valley in Egypt.

(iii) **Upland Cotton:**

Upland cotton has very short fibers and makes up about 90 % of the world's total cotton production. The crop is native to and grown in central America, Mexico, the Caribbean and southern Florida.

(IV) **Organic cotton:**

Organic cotton is any type of cotton that is grown without chemicals and from plants that are not genetically engineered.

IV] Characteristics of Cotton:

Cotton has a number of distinguish characteristics that make it such a popular fiber in the textile industry.

Softness:

The cotton plant is soft and fluffy and results in a fabric often retains that soft feel.

Durability:

The cotton plant's cellulose structure is strong, creating a tough and wear and tear resistant fabric.

Absorbent:

Fabric because there is a lot of space between the cotton fibers.

Holds dye well:

Due to its absorbent nature cotton takes dye very easily and can be made into a wide variety of collars.

Breathability:

The fiber structure of cotton makes it more breathable than synthetic fibers.

No static cling:

Cotton does not conduct electricity their fare static is not an issue with cotton.

Cotton has many uses, across number of different industries.

Woven fabrics:

Cotton is used to make a variety of woven fabrics, including canvas, denim, damask, flannel, and more

Clothing:

Cotton is a fixture of the textile industry as a result of its makes production, soft feel, durability and absorbency. Cotton is frequently used for T- Shirts, Blue jeans, dresses, sweat, dhoti and so much more Bedsheets and towels.

Since cotton is extremely soft and absorbent is an ideal fabric for bed room linens and to sop up moisture.

Underwear:

For the same reasons, Cotton makes comfortable and durable undergarments.

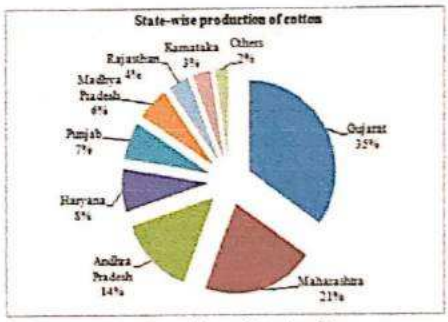
Home décor:

Cotton is also used throughout the home for upholstery, curtains, rugs, pillows.

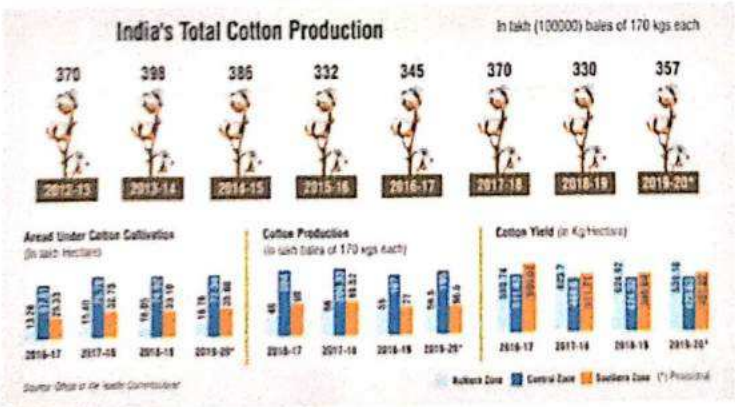
Cottonseed oil:

Cotton seed is byproduct of the cotton production process and the seeds are used to manufacture cotton seed oil, which is used for salad dressing and margarine. It can also used in makeup soap, candles and more.

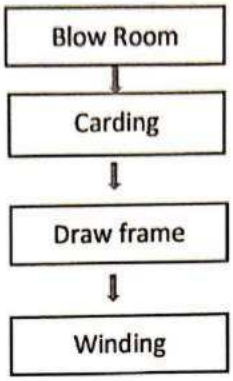
V] Map and chart showing cotton production



Source: Ministry of Agriculture, GoI



VI] Visited textile's cotton process flow chart:



BLOW ROOM

- The blow room is the first step of yarn production in the spinning mills.
- It consists of a number of machines used in succession to open and clean the cotton fiber to the required degree. About 40% to 70% trash is removed in this section

ACTON IN BLOW ROOM

- **Action of opposite spike (Opening)**

  The action of opposite spikes is opening the cotton fiber. By this action, the large pieces of cotton have been reduced in size.

- **Action of air current (transport and cleaning)**

  During processing, the movement of cotton from machines to machine is done by air current. It also helps the separation lint and trash

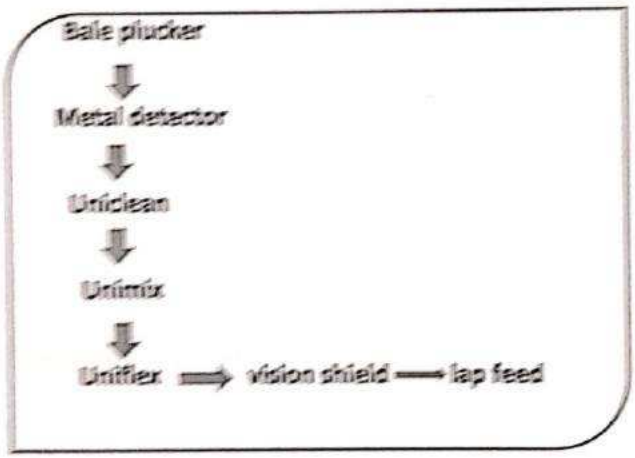
- **Action of beaters (Cleaning and opening)**

Beaters are responsible for removing almost all the impurity extracted in the below room. Beaters also helps in opening of cotton fiber

- Action of regulating motion (uniform output)

  The action of regulating motion gives the uniform output of cotton fiber by the help of swing door and swing paddle

FLOW CHART OF BLOW ROOM



Precautions

- Proper humidification inside the Hall.
- Functioning of all the exhaust fans.
- Proper housekeeping and cleaning.
- Prevention of flying of cotton dusts in open air.
- Use of mask by the operators.
- Proper maintenance of the machines will help in maintaining the dust level

Carding

Necessity:

- it is always considered by the experts that the card is the heart of the spinning mill
- The statement "Well-carded is half-spun" demonstrate the immense significance of the carding for the final of the spinning operation
- Carding is an operation where the tuft condition of the fibers is converted into an individual fiber form
- The carding is a very important process because unless the fibers are separated into individuals, they cannot be spun into smooth and uniform yarns neither can they be blended properly with other fibers.

Basic requirement of carding process

In carding m/c tow basic actions are taking place b/n tow wire covered surfaces, these are:

1. Carding action
2. Stripping action

Draw Frame

Actions involved in Draw Frame

Drafting: it is the process of increasing length per unit weight of silver, it is mainly due to peripheral speed of the rollers.

Doubling: The process of combing two or more silvers into a single form is called doubling

Draw Frame: In the cotton industry the term is applied exclusively to processing on the draw frame, where the operation is one of doubling and drafting.

Draw Frame = Drafting + Doubling

Objectives of Draw Frame

Equalizing

One of the main tasks of the draw frame is improving evenness over the short, medium and – especially – long term

Parallelizing

To obtain an optimal value for strength in the yarn characteristics, the fibers must be arranging parallel in the fiber stand. It is mainly the draw frame's task to create this parallel arrangement. It fulfills this task by means of the draft, since every drafting step lead so straightening of the fibers.

Blending

In addition to the equalizing effect, doubling also provides a degree of compensation of raw material variations by blending, which occurs simultaneously.

Dust removal

Dust is steadily becoming a greater problem both in processing and for the personnel involved. It is therefore important to remove dust to the greatest practical extent at every possible point within the overall process.

Winding

- Winding is one of the most important operation which is mainly occurred in spinning section.
- The creation of large yarn package that can be easily unwound, is called winding
- This makes using the yarn on subsequent machines both easier and more economical
- Three zones of winding
  1. Unwinding zone
  2. Tension and clearing zone
  3. Winding zone

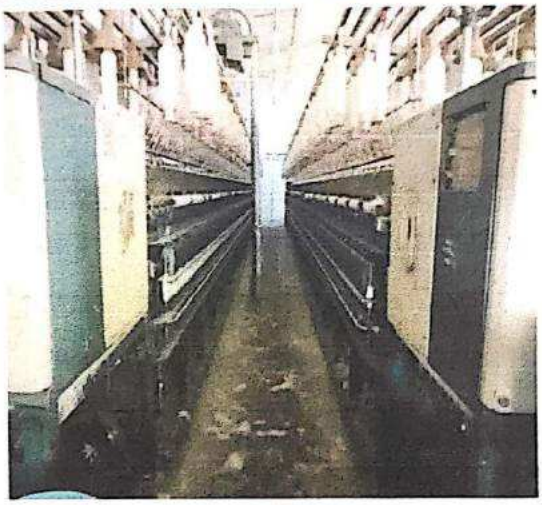
WHY WINDING?

- To transfer yarn from one package to another suitable one.
- Clearing of yarn
- Package the yarn
- Inspect the yarn
- Remove faults
- To improve quality
- Store in a suitable form
- Preparing soft packages for dyeing

The blow room

Carding

The draw frame



Winding

Visitation to textile for

Information collection.

Name of company	: SRS Textiles
Proprietor	: Mr. S.R.Selvam
No of workers	: 16 Members per shifts
No of Shifts	: 3
Protective gear	: Face Masks (small particles of cotton dust)
Qualification	: Experience Based
Raw Materials	: Cotton (Gossypium)

Order of raw materials are based on demand and supply.

Place of purchase of raw materials: Karnataka

Grade of cotton The grade is 40*s

The type of cotton is like towel clothes

Transportation: By road through trucks.

Finished goods: Per day 200 to 250 kg of cotton thread.

Output to : Erode dhoti textiles.

Conclusion:

Cotton plays significant role among the most useful material in India. Cotton and cotton textiles industries are central to the economic growth of both develop and developing countries. It is the raw material of wealth, industries industrialization and development. It is fast closing up due to competition from manmade fiber. Textile product are one of the basic essential human requirements next to food.

The main purpose of doing this project is to know textile industry. Which use plan products as raw material and the main objective of the studies to know how much crop cultivation is important in today's world.

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