STUDY ON PRELIMINARY PHYTOCHEMICAL, FTIR ANALYSIS AND ANTIBACTERIAL ACTIVITY OF TWO SPECIES OF EUPHORBIA

A short term project work submitted to

St. Mary's College (Autonomous)

Re accreditation with A++ Grade by NAAC affiliated to

MANONMANIAM SUNDARANAR UNIVERSITY

in partial fulfilment of the requirement for the

Degree of Bachelor of Science in Botany,

St.Mary's College (Autonomous), Thoothukudi.

By

AARTHI. S	-	**16AUBO01**
ABITHA. S	-	**16AUBO02**
ALAGU SATHIYA	-	**16AUBO03**
AMIRTHA ROSELIN. N	-	**16AUBO04**
ANCI. S	-	**16AUBO05**
ANITHA NANCY MARY. J	-	**16AUBO06**

DEPARTMENT OF BOTANY

ST. MARYS COLLEGE (AUTONOMOUS)

THOOTHUKUDI – 628 001

APRIL 2018-2019

CERTIFICATE

It is certified that this short term project work **"STUDY ON PRELIMINARY PHYTOCHEMICAL,FTIR ANALYSIS AND ANTIBACTERIAL ACITIVITY OF TWO SPECIES OF EUPHORBIA"** submitted to St. Mary's college (Autonomous) affiliated to **MANONMANIAM SUNDARANAR UNIVERSITY** in partial fulfilment of the requirements for the degree of Bachelor 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 2018 – 2019 by the following students.

By

AARTHI. S	-	**16AUBO01**
ABITHA. S	-	**16AUBO02**
ALAGU SATHIYA	-	**16AUBO03**
AMIRTHA ROSELIN. N	-	**16AUBO04**
ANCI. S	-	**16AUBO05**
ANITHA NANCY MARY. J	-	**16AUBO06**

B. Maria Sumi

GUIDE

29/3/19

HEAD OF THE DEPARTMENT

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

3/4/19

EXAMINER

Lucia Rose

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

ACKNOWLEDGEMENT

It is our humble attempt to present this **"STUDY ON PRELIMINARY PHYTOCHEMICAL, FTIR ANALYSIS AND ANTIBACTERIAL ACTIVITY OF TWO SPECIES OF EUPHORBIA".** First and foremost our sincere gratitude belongs to **Dr. Sr. A.S.J. LUCIA ROSE M.Sc., PGDCA., M.Phil., Ph.D.** principal, St. Mary's College (Autonomous) for providing an opportunity to do this project.

With deep sense of thanks to **Dr. Mrs. M. GLORY M.Sc., M. Phil., Ph.D.** Head of the Department of Botany, St. Mary's College, Thoothukudi for her encouragement and support.

We take great pleasure in expressing our heartful thanks to **DR. Mrs. B. Maria Sumathi M.Sc., M.Phil., Ph.D.** Lecture in Botany, St. Mary's College, Thoothukudi for suggesting this topic, for providing necessary information, timely suggestions, guidance and sustained interest throughout the period of investigation and for the perusal of this report.

Thanks are also due to guiding hands of all the staff members and the laboratory assistants of Botany, and also my friends for their encouragement

We wish to thank Ms. Santhiya, Research Scholar, Department of Chemistry, V.O. Chidambaram College, Thoothukudi, for her help in the FTIR spectrum studies.

Above all we humbly bow in gratitude to the GOD LORD for showering abundant graces on us and for helping us to yield fruitful results.

CONTENTS

INTRODUCTION

CHAPTER-I

INTRODUCTION

The plant kingdom is really a potential source of medicinal properties. (McChesney *et al.*, 2007). Chemical constituents of plants may be acted as defence agents against predator. Most of the chemical compounds also have therapeutic value for human. Plants with ethno botanical backgrounds are usually used for single-goal screening (using a specific bioassay technique) (Atta-ur-Rahman *et al.*, 2001).

The medicinal plants play an important role in supporting health care in India. The use of herbs to treat disease is almost universal and is now recognized by WHO as an essential building block for primary health care. According to World Health Organization, 80% of the people living in the rural areas depend on medicinal plants as primary health care system, particularly the developing countries.

Study on natural products is always an interesting target for scientists over decades, especially on plants. Since the beginning of human civilization, medicinal plants have been used by mankind for its therapeutic value. Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources. Many of these isolations were based on the uses of the agents in traditional medicine. The plant-based, traditional medicine systems continues to play an essential role in health care, with about 80% of the world's inhabitants relying mainly on traditional medicines for their primary health care (Owolabi *et al.*, 2007). Historically, plants (fruits, vegetables, medicinal herbs, etc.) have provided a good source of a wide variety of compounds, such as phenolic compounds, nitrogen compounds, vitamins, terpenoids and some other secondary metabolites, which are rich in valuable bioactivities like antioxidant, anti-

inflammatory, antitumor, antimutagenic, anti-carcinogenic, antibacterial, or antiviral activities. (Maridass and Britto, 2008).

Plant derived medicines are believed to be risk free, milder and superior to chemically synthesized drugs for human health. Human body recognizes components that occur in plants and has sophisticated mechanism for metabolizing such plant materials. The bioactive compounds naturally available in plants may have lower potency than allopathic (synthesized) drugs. However, as they are traditionally consumed in significant amounts through diet, they may provide long term physiological benefits without any detrimental side effects (Espin *et al.*, 2007). Consumption of foods rich in photochemical and other bioactive food components have been clearly linked to the prevention and reduction of cancer (Steinmetz and Potter, 1991), cardiovascular diseases (Duthie and Brown, 1994) and to improvement in immune system (German and Dillard, 1998).

The family Euphorbiaceae includes about 4000 species and 200 genera. The members of the family secrete an acrid juice of varying colours and density. Some species are slightly narcotic acrid and other aromatic. Most of the genera like *Phyllanthus, Jatropha, Acalypha, Euphorbia*are used in modern allelopathic and homeopathic systems of medicine.

The genus *Euphorbia* is the third largest genus of flowering plants, with almost 2000 species. Its exceptional diversity of growth forms and near-cosmopolitan distribution have attracted human interest since ancient times. Plants of the genus Euphorbia are prolific producers of diterpenes of great biomedical interest. *Euphorbia* species are used in the treatment of digestive and respiratory complaints,

inflammation and injuries (Madeleine *et al.*, 2015). For the present study, the two taxa - *Euphorbia hirta L* and *Euphorbia milli* Des Moul. are selected

Euphorbia hirta is a medicinal plant which is important in ethno medicine. It is used in the treatment of gastrointestinal disorders, bronchial and other respiratory diseases conjunctivitis, to increase milk flow in lacting women and for other female diseases. (Akomas *et al.*, 2015; Saeed *et al.*, 2013). It is also used to treat worm infestations in children and for dysentery, gonorrhea, jaundice, pimples, digestive problems and tumours. (Sandeep *et al.*, 2009). The plant has a reputation an analgestic to treat severe headache, toothache, rheumatism, colic, and pains during pregnancy. It was also used for intestinal parasites, diarrhea, peptic ulcers, heart burn, vomiting, amoebic, hay fever, emphysema, coughs, cold, kidney stones, menstrual problems, sterility, venereal diseases, skin and mucous membranes diseases, including (warts, scabies, fungal afflictions, measles) as an antiseptic to treat wounds, sores and conjunctivitis. (Ping and Darah *et al.*, 2013).

Euphorbia milli is a medicinal plant. It is most important source of medicine. It is used in the treatment of warts .The various extract of the plant and its parts have been found to possess antimicrobial, molluscicidal, antioxidant and antitumor activity. (Shi *et al.*, 2008; Murugan *et al.*, 2007)

SCOPE AND OBJECTIVES

SCOPE AND OBJECTIVES

The aspiration of current study was to assess the preliminary phytochemical, FTIR analysis and anti-bacterial potential of the whole plant extract of *Euphorbia hirta* L. and infloresence extract of *Euphorbia milli* Des Moul. In this work the following objectives are focused.

- Collection of whole plant from *Euphorbia hirta* and infloresence from *Euphorbia milli* for extracts preparation.
- To qualitatively screen the presence of different phytochemicals of acetone, methanol, and aqueous extracts of whole plant of *Euphorbia hirta* and infloresence of *Euphorbia milli.*
- To identify and compare the functional group of whole plant of *Euphorbia hirta* and infloresence of *Euphorbia milli* by Fourier transform infrared spectroscopy (FTIR) analysis.
- To evaluate the anti-bacterial potential of acetone, methanol, and aqueous extracts of whole plant of *Euphorbia hirta* and infloresence of *Euphorbia milli.*

LITERATURE REVIEW

CHAPTER-II

LITERATURE REVIEW

Plant produce a variety of compounds which may be considered as primary metabolites and secondary metabolites. Primary metabolites form the major portion of the plant material that include carbohydrates, protein and lipids etc. These are common to most plant species. Secondary metabolites are of more biological interest. They can be considered as specific to individual plant species. Secondary metabolites are synthesized by plant for self protection and survival. These compounds help to protect the plants from bacterial, fungal or insect attack or grazing animals. 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).

The phytochemical screening of different parts of the *Jatrophacurcus* 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 racemosa* (Euphorbiaceae). This study revealed the presence of alkaloid, tannin, flavanoid and terpenes.

Ravindranath (2003) has been isolated a novel macrocyclicditerpene–Jatrophenone from the whole plant of *Jatropha gossypifolia.* This compound possesses significant antibacterial activity.

Nwokocha *et al.* (2011) studied the comparative phytochemical screening of *Jatropha curcas*, *Jatropha gossypifolia, 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.

Phytochemicals are non-nutritive plant chemicals that contain protective and disease preventing compound (Chaturbhuj shah *et al.*, 2004).

The secondary metabolites of plants i.e., phenols and related alcohol, tannin, flavanoid, glucosides and their derived glycones, terpenes, and alkaloids showed antibacterial activity (Mitscher, 1974).

Alkaloids are commonly found to have antimicrobial properties (Omulokol *et al.*, 1997) against both Gram-positive and gram negative bacteria (cowan, 1990). Alkaloids which are one of the largest groups of phytochemicals in plants have amazing effects on humans and this has led to the development of powerful pain killer medications (Igbinosa *et al.*, 2009).

Phytosterols or plant sterols lower the serum cholesterol concentration by competing with dietary and biliary cholesterol for intestinal absorption (Clifton *et al.*,

2004). Phytosterol chemically acts as an antioxidant, a modest radical scavenger and physically a stabilizer in the membranes (Yasukazu and Etsuo, 2003)

Flavonoids, the largest groups of phenolic compounds are known to contain a broad spectrum of chemical and biological activities including antioxidant and free radical scavenging properties includes antimicrobial, anti-inflammatory, anti-feedent and hemolytic effects (Xu *et al.*, 2002).

Flavonoids are potent antioxidants and epidemic studies indicate that high flavonoid intake is correlated with decreased risk of lifestyle diseases like diabetes and cardio vascular diseases (Kaur *et al.*, 2008).

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)

Saponins are known to produce inhibitory activity on inflammation and are key ingredient of -traditional Chinese medicine and thus responsible for the most of the observed biological effects (Liu and Htenkel, 2002).

Phenolic substances possess many biological effects. These effects are mainly attributed to their antioxidant activities in scavenging free radicals, inhibition of peroxidation and chelating transition metals (Nickavar *et al.*, 2007).

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.* (2007) 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 *Eclipta prostratea*. 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 *Aerva lanata* using spectroscopic method. Thangarajan Starlin *et al.* (2012) detected the elements and functional groups in the ethanol extract of whole plant *Ichnocarpus frutescens* using FTIR spectroscopic method. Parag A. Petnekar (2013) carried out the FTIR spectroscopic analysis of methanolic leaf extract of *Ampelocissus lantifolia* for antimicrobial compounds.

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 cause 2 log cycle reduction of cell population in 90 min.

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 21 mm) and *Salmonella typhi* (17 and 19 mm) were found to be sensitive to both the extracts. This indicates that gymnemic acid, an active component of *Gymnema sylvestre* 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 17 mm. *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.

Ghassan Mohammad Sulaiman *et al.* (2013) synthesized silver nanoparticles from leaves extract of *Eucalyptus chapmaniana* and then tested the antimicrobial effect of silver nanoparticles against different pathogenic bacteria, yeast, and its toxicity against human acute promyelocytic leukaemia (HL-60) cell lines. Test for antimicrobial activity of silver nanoparticles was assessed by agar well diffusion method against different pathogenic microorganisms *Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Proteus vulgaris* (Gram negative), *Staphylococcus aureus* (Gram positive) and *Candida albicans* (Yeast). Cell viability was evaluated by MTT colorimetric method. The antimicrobial effect was dose-dependent and was more against gram-positive bacteria than gram-negative bacteria.

Singhal *et al.* (2011) described biosynthesis and antimicrobial activity of silver nanoparticles using *Ocimum sanctum.* They showed that *O. sanctum* leaf extract can reduce silver ions into silver nanoparticles within 8 min of reaction time. Biosynthesized silver nanoparticles are in the size range of 4-30 nm and possessed antimicrobial activity. They showed silver nanoparticles were exhibit more antimicrobial activity on gram-negative microorganism than gram-positive ones and also showed synthesized silver nanoparticles have stronger activity than silver nitrate and standard antibiotic ciprofloxacin.

Elampariti and Boominathan (2011) state the antibacterial activity of individual cruds extract and fraction and combination active fractions of *Camellia sinesis*. For antibacterial test, disc diffusion technique was used against human pathogenic bacterial strains. The significant inhibition of ethanol mixed methanol active fraction was inhibit 7 mm zone against *Streptococcus pnemoniae*, and 8mm zone against *Staphylococcus aureus*, it was higher activity than all individual fractions and crude extract.

Yusha'u, *et al.* (2011) studied antibacterial activities 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 α- haemolytic *Streptococci* using disc diffusion and microbroth dilution techniques. Sensitively test results showed that water fraction of the plant was active on *Stephylococcus aureus* and *Streptococcus pnemoniae* (10 mm) at 50 µg/disc concentration while ethanolic extract of the plant was active, *Streptococcus pnemoniae* and *Proteus species* at 200 µg/disc concentration with zone diameter formed by *Klebsiella pnemoniae* (11 mm) being wider than that formed in response to standard Augmentin disc (06 mm).

Shiv Shanker Gautam *et al.* (2012) studied the antibacterial potential of various extracts (petroleum ether, acetone, methanol and aqueous) of Nepetaciliaris against three gram-positive (*Staphylococcus inureus, Streptococcus pnemoniae* and *Streptococcus pyogenes*) and one gram-negative (*Pseudomonas aeruginosa*) bacterial pathogens. The agar well diffusion method was adopted to examine antibacterial and minimum inhibitory concentration values of most effective extracts against the susceptible bacteria. Erythromycin was used as positive control to determine the

sensitivity of the strains. Out of the four bacterial species tested. *S. pnemoniae* was the most susceptible. The *N. ciliaris* is potentially a good source of antimicrobial agents.

Souad Akroum and Korrichilalaoui (2012) tested the antimicrobial activity of their ethonolic and methonolic extract of *Viciafaba L. Vaccinium macrocarpon, Punica granatum, Lavandula officinalis, Artemisia absinthiam, Linum capitatum* and *Camellia sinensis* on some pathogen bacteria, then their ability to in vivo inhibit the growth of Streptococcus pneumonia. The phytochemical screening has given the composition of the most active extracts. According to the obtained results, the ethonalic extract of *lavendula officinalis* and *A. absinthium* has shown as inhibition of all the tested bacteria. The ethonalic extract of *L. officinalis* has given the highest activity against *S. pneumonia* followed by the methonalic extracts of *C. sinensis* and *P. granatum*. The phytochemical screening showed that the most active extracts contained mainly phenolic compounds.

Sirajahmedkakar *et al.* (2012) studied the crude methonal extracts of four plants of Balochistan (*Berberis baluchistani ca, Seriphidiumquertense, Iphiona aucheri,* and *Ferula costata*) have been tested for a wide array of antimicrobial activity against three gram positive bacteria *Staphylococcus aureus, Streptococcus pnemoneae, Streptococcus pyogene* and four gram negative bacteria *Escherichia coli. Salmonella typhinarium, klebsiella pnemoneae, Psuedomonas aeraginosa.* All the plant extracts were found to be effective against all the tested bacteria.

Inderjit Kaur *et al.* (2012) studied the antimicrobial activity of aqueous and methonalic extracts of *adhatoda vasica* were evaluated against the bacteria isolated from the spurum samples of asthmatic patients. The showed broad spectrum of antibacterial activities against Gram-positive *Staphylococus inermis* and

Streptococcus pneumoneae bacterial species in compansion to the Gram-negative E.coli and *Klebsiella pnemoneae* bacterial species.

Deepti *et al.* (2012) studied the antimicrobial activity and phytochemical analysis of *Morinda tinctoria* leaf extracts. The results showed that the leaf extracts contain a broad spectrum of secondary metabolites: Alkaloids, Phytosterols, Flavonoids, Phenols and terpenes in major proportion. Methonal extract was shown to be more effective against all the organisms followed by Ethylacetate, Chloroform and Hexane extracts. *Proteus vulgaris* (24 mm) was found to be most sensitive organism followed by *Klebsiella pneumonia* (21 mm).

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.*

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.

MATERIALS AND METHODS

PLATE I

MATERIALS AND METHODS

Materials

Botanical name : *Euphorbia hirta* L.

Family : Euphorbiaceae

Common name : Asthuma weed, Common spurge

Description of the plant : A small erect or ascending annual herb reaching up to 50 cm, with hairy stems. The leaves are opposite, elliptical, oblong-lanceolate, with a faintly toothed margin and darker on the upper surface. The flower are a small, numerous and crowded together in dense cymes about 1 cm in diameter. The fruits are yellow, three-celled hairy, keeled capsules, 1-2 mm in diameter, containing three brown four-sided angular wrinkled seeds.

Distribution of the plant : The plant is distributed in **Northern America**: (United States, Mexico); **Southern America**: (Brazil, Antigua and Barbados, Dominica, Grenda, Guadeloupe, Martinique, Montserrrt, St. Kitts and Nevis, St. Lucia, St. Vincent and Grenadines, Trinidad and Tobago, Belize, Costa Rica, El Salvador, Guatemala, Honduras, Nicaragua, Pannama, French Guiana, Guyana, Suriname, Venezuela, Argentina, Chile, Paraguay, Bolivia, Colombia, Ecuador, Peru); **Africa**: (Tanzania, Uganda, Cape Verde, Chad, Djibouti, Eritrea, Ethiopia, Somalia, Angola, Malawai, Mozambique, Zambia, Zimbabwe, Botswana, South Africa, Liberia, Egypt, Mali, Nigeria, Senegal, Sierra Leone, Togo, Cameroon, Central African Republic, Equatorial Guinea, Gabon, Rwanda, Zaire, Madagascar, Mauritius, Reunion, Seychelles); **Asia**: (Omen, Yemen, Taiwan, Palestine, Lebanom, Syria, Bhutan, India, Nepal, Pakistan, Sri Lanka, Myanmar, Thailand, Indonresia, Malaysia, Papua New Guinea, Philippinres) and **Australasia**: (Australasia, New Zealand).

PLATE 2

MATERIALS AND METHODS

Material

Botanical name : *Euphorbia milli* Des.Moul

Family : Euphorbiaceae

Common Name : Crown of Thorns or christ plant

Description of the plant : *Euphorbia milli* is a dense shrub up to a metre (3 feet) or so tall, it has 2 cm (0.8 inch) thick dark brown stems armed on all sides and at frequent intervals with sharp spines of varying length (mostly around 1-2cm (0.4-0.8 inch)). Cluster of bright green, elliptic, 5-6cm (2-2.4 inch) leaves, which are produced near the growing tips of the stems, last for at least several months before dropping off, leaving the plant's spiny stems permanently bare. Old leaves are not replaced and new ones will appear only on new terminal growth. The flower are tiny, but each is surrounded by a pair of 2 cm kidney-shaped, bright red bracts, which look rather like petals. Clusters of from two six of these paired, flower-like bracts appear on 5 cm (2 inch) stalk at the ends of actively growing spiny stems. They are not produced on the old stems. A sticky substance on the flower stalks adheres to the finger if touched . The main flowering season normally last from early spring through late summer, but flowering can be continuous if plants get exceptionally good light.

Distribution of the plant : *Euphorbia milli* is a species of flowering plant in the spurge family Euphorbiaceae, native to Madagascar. It is a climbing shrub with densely spiny stems. The straight, slender spines help *Euphorbia milli* scramble over other plants. The leaves are found mainly on new growth and are obovate. The flowers are small, subtended by a pair of conspicuous petal-like bracts, variably red, pink or white.

CHAPTER-III

MATERIALS AND METHODS

Collection and processing

The whole plant samples of *Euphorbia hirta* L. and inflorescence of *Euphorbia milli* Des Moul were collected from Thoothukudi District, Tamil Nadu respectively. 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 the extraction of active constituents of the plant material.

Preparation of extracts for phytochemical screening

The powder was steeped in acetone, methanol petroleumether and water (5gm/100ml) in a closed flask for twenty hours separately, shaking them frequently during six hours and allowed to stand at room temperature. The clear supernatant of the each extract was decanted and used to determine the phytochemical constituents.

Qualitative Phytochemical analysis of different extracts. (Harbrone , 1998)

The different solvent extracts of whole plant samples of *Euphorbia hirta* and inflorescence of *Euphorbia milli* were subjected to the following tests were carried out.

Test for Steroids

Libermann-burchard's test

A few ml of the test solution in chloroform s treated with a few drops of acetic acid, acetic anhydride, two drops of Conc. H_2SO_4 and heated gently. Blue or green colour showed the presence of steroid.

Mayer's test

To the powder/extract, 2 ml of Mayer's reagent was added; a dull white precipitate reveals the presence of alkaloids.

Test for Tannin

The test solution was mixed with basic lead acetate solution. Formation of a white precipitate indicates the presence of tannins.

Test for Phenol

The powder / extract, 2 ml of distilled water was added followed by few drops of 10% aqueous ferric chloride. Appearance of blue or green colour indicates the presence of phenols.

Quinones

To 1 ml of extract 1ml of con.H_2SO4 was added. Appearance red indicator the presence of quinine.

Test for Flavones (Shinadow's test)

A few ml of the test solution was treated with magnesium turnings and few drops of Conc. HCl. Red or pink colour indicated the presence of flavonoid.

Test for Saponin

The test solution was shaken with water. Copious lather formation indicates the presence of tannins.

Test for Terpenoids(Noller's Test)

To 1 ml extract with tin (one bit) and thionyl chloride (1 ml) were added. Appearance of pink colour indicates the presence of terpenoids.

coumarine

A few ml of the test solution was treated with alcoholic 'NaOH'. Yellow colour indicated the presence of coumarin.

FT-IR analysis

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

ANTIBACTERIAL ASSAY

Extraction of plant materials

The plant powder was extracted with acetone, methanol, petroleum ether and water. 25 g 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 $^{\circ}$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 three gram negative bacteria *Escherichia coli*, G-ve, MTCC 50, *Salmonella typhi* G-ve, 1357, and *Klebsiella pneumonia* G-ve, MTCC 3384 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 (5 mm) 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 + 37°C 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

CHAPTER-II

RESULT AND DISCUSSION

Plants 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).

Euphorbia hirta and *Euphorbia milli* are the important medicinal plants in Euphorbiaceae. Both the plants are selected for the present study. Both the plants are used for the local people for various ailments like relief pain, inflammation, dysentery and fever etc., These plants have some active principle which has this medicinal value.

QUALITATIVE ANALYSIS

Preliminary phytochemical analysis of the various solvent extracts of whole plant of *Euphorbia hirta* and inflorescence of *Euphorbia milli* showed different results. The alkaloids, phenols, tannins, saponins, quinones, flavonoids, terpenoids and coumarins were predominantly present in different parts of different solvent extracts. (Table 1-2).

Johnson *et al.* (2012) reported the methanolic 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, phenols and terpenoids in *peltophorum pterocarpum* flowers.

Alkaloids are commonly found to have antimicrobial properties (Omulokoli *et al.*, 1997) against both Gram-positive and gram negative bacteria (Cowan, 1999).It

is also used in the elimination and reduction of human cancer cell lines. (Nobori *et al.*, 1994).

Flavonoids serve as health promoting compounds as a result of their anion radicals (Hausteen, 1983). Several authors reported that flavonoids, sterols/terpenoids and phenolic acids are known to be bioactive antidiabetic principles (Oliver-Bever, 1986).

Phenolics have antioxidative, antidiabetic, anticarcinogenic, antimicrobial, antiallergic, antimutagenic and anti-inflammatory activities (Arts *et al.*, 2005; Mithraja *et al.*, 2011).

Steroidsare used to suppress inflammation. Phytosterols may reduce cholesterol level in human (Pollak, 1953). Phytosterols may inhibit lung, stomach, ovarian and breast cancers. (Woyengo *et al.*, 2009).

Saponins are known to produce inhibitory effect on inflammation (Just *et al.*, 1998). Plant saponins have been shown to inhibit cholesterol absorption from the intestinal lumen in experimental animals, and consequently to reduce the concentration of plasma cholesterol. (Hosttetmann and Marston, 1995). Saponins also show wide-ranging cytostatic effects against cancer cells. (Francis *et al.*, 2002).

Tannins are useful in treating inflammation, Ulcers and remarkable activity in cancer prevention and anticancer activities (Li *et al.*, 2003; Akinpelu *et al.*, 2009). It controls the irritation in the small intestine (Cheng *et al.*, 2002).

Our results showed alkaloids, Phenol, flavonoids, saponins, tannins, steroids, anthroqninones, coumarine, terpenoids and were predominantly present in organic solvents of different parts of *Euphorbia hirta* and *Euphorbia milli.* So the medicinal

values of this plant could be attributed to the presence of one or more of the detected metabolites.

FTIR

Fourier Transform Infrared spectroscopy was used to analyse the functional group present in the whole plant of the *Euphorbia hirta* and infloresence of the *Euphorbia milli.*

The FTIR spectroscopy analysis of *Euphorbia hirta* whole plant obtained peaks at 3422.52 cm^{-1}, 2921.54 cm^{-1}, 1627.23 cm^{-1}, 1449.66 cm^{-1}, 1022.14 cm^{-1}, 668.72 cm^{-1}. These absorption peaks are known to be associated with the stretching vibration for N-H in Urethanes, C-H in Alkane, C=N in Guanidine, C-H in Aromatic compound, C-O in Ether and N-H in primary amine. (Fig. 1, Table 3).

The FTIR spectroscopy analysis of *Euphorbia milli* infloresence obtained peaks at 3409.15 cm^{-1}, 2919.32 cm^{-1}, 2850.49 cm^{-1}, 1620.03 cm^{-1}, 1449.66 cm^{-1}, 1205.79 cm^{-1}, 1022.14 cm^{-1}, 668.72 cm^{-1}. These absorption peaks are known to be associated with the stretching vibration for N-H in Aliphatic amine, C-H in Alkane, C-H in Esters, N-H in Amine, C-H Aromatic compound, C-O Tertiary Alcohol, C-O Ether, N-H Primary Amine. (Fig. 2, Table 4).

From the spectral data presence of C=O, C-H, C-N, N-H were identified. These bonding are responsible for the presence of alkyl group, aldehyde group, methyl group, alcohol, ether, carboxylic group and aliphatic nitro 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 edema and rheumatic joint pain. Amides, amine and amino acid are the main group

which are involved in protein synthesis. The study revealed that the whole plant of *Euphorbia hirta* and inflorescence of *Euphorbia milli* contain a considerable amount of secondary metabolites and it may considered in future to be used human disease management.

ANTIBACTERIAL ACTIVITY

In the present study, antibacterial activity of different solvents (acetone, methanol, petroleum ether and water) using *Euphorbia hirta* and *Euphorbia milli* were tested against four human pathogenic bacteria (*Bacillus substilis, Escherichia coli, Salmonella typhi* and *Klebsiella pneumonia*) presented in (Table 5-6). The diameter of the inhibition zones against these species ranged from (2 to 15 mm).

The different solvents (acetone, methanol, petroleum ether and water) from whole plant extract of *Euphorbia hirta* exhibited maximum activity against different bacterial species, *E. coli* (5-7 mm), *Bacillus substillis* (2-7 mm), *Salmonella typhi* (2-5 mm), *Klebsiella pneumonia* (2-15 mm) inhibition zone. (Plate 3)

The different solvents (acetone, methanol, petroleum ether and water) extracts of infloresence of *Euphorbia milli* exhibited maximum activity against different bacterial species, *E. coli* (2-6 mm), *Bacillus substillis* (3-13 mm), *Salmonella typhi* (2-10 mm), *Klebsiella pneumonia* (3-11 mm) inhibition zone. (Plate 4).

The maximum activity was found to be 15 mm zone of inhibition obtained by acetone extract of *Euphorbia hirta* whole plant against *Klebsiella pneumonia*. The acetone extract of *Euphorbia hirta* whole plant exhibited more or less same zone of inhibition compared to standard antibiotics streptomycin.

The maximum activity was found to be 13 mm zone of inhibition obtained by methanol extract of *Euphorbia milli* infloresence against *Bacillus substillis.* The methanol extract of *Euphorbia milli* flower exhibited higher zone of inhibition compared to standard antibiotics streptomycin.

The antibacterial activity of *Euphorbia hirta* and *Euphorbia milli* 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 *Euphorbia hirta* and *Euphorbia milli* showed the extracellular of saponins. The antimicrobial activity of these plants may be due to the presence of saponins. The presence of tannins were also reported in *Euphorbia hirta* and *Euphorbia milli.* 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.*, 1988). Hence these plants stand as a potential candidate as a source of ingredients in drug formulations for the treatment of bacterial infection.

SUMMARY AND CONCLUSION

CHAPTER-V

SUMMARY & CONCLUSION

Euphorbia hirta L. and *Euphorbia milli* Des Moul.well known plants of family Euphorbiaceae is used as a therapeutic agent. The herb *Euphorbia hirta* is specially used in the treatment of severe headache, toothache, rheumatism, colic, intestinal parasites, diarrhea, peptic ulcers, venereal diseases, skin and mucous membranes diseases. (Ping and Darah *et al.*, 2013). *Euphorbia milli* is a medicinal plant. It is used in the treatment of warts .The various extract of the plant and its parts have been found to possess antimicrobial, molluscicidal, antioxidant and antitumor activity. (Shi *et al.*, 2008; Murugan *et al.*, 2007).

The phytochemical study revealed the presence of steroids, flavonoids, alkaloids, saponins, terpenoids, coumarine, phenol and tannins. The preliminary phytochemical tests are helpful in finding chemical constituents in the plant 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 genuity of the drug.

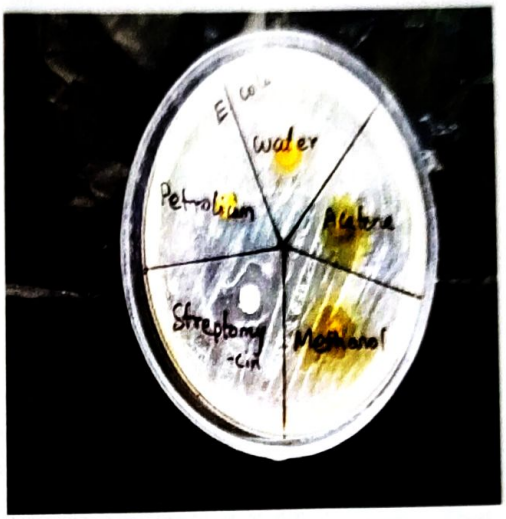
The FTIR spectrum of *Euphorbia hirta* showed strong IR bands characteristics of Alkane (2921.54 cm^{-1}), Guanidine (1627.23 cm^{-1}), Ether (1022.14 cm^{-1}), primary amine (3422.52 cm^{-1}) functional groups and in *Euphorbia milli* showed strong IR bands characteristic of Aromatic (1449.66 cm^{-1}), Ether (1022.14 cm^{-1}), Primary Amine (668.72 cm^{-1}), Esters (2850.49 cm^{-1}) functional groups. From the spectral data, presence of C=O, C-H, C-N, N-H were identified. These bonding are responsible for the presence of alkyl group, methyl group, alcohol, ether, carboxylic group and aliphatic nitro 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 edema and rheumatic joint pain. Amides, amine and amino acid are the main group which are involved in protein synthesis.

The different solvent extracts of whole pant of *Euphorbia hirta*, inflorescence of *Euphorbia milli* and streptomycin were used for antibacterial studies against four organism, *E. coli*, *Salmonella paratyphii*, *Bacillus subtilis*, *Klebsiella pneumonia*. These extracts showed ranging degree of antibacterial activity. The maximum activity was found to be 15 mm zone of inhibition obtained by acetone extract of *Euphorbia hirta* whole plant against *Klebsiella pneumonia*. The antibacterial activity of *Euphorbia hirta* and *Euphorbia milli* may be due to presence of various phytochemicals which are known to be synthesized by plants in response to microbial infection. The present study concluded that the whole plant of *Euphorbia hirta* and infloresence of *Euphorbia milli* extracts could be used as a potential source of antimicrobial agents. So the ingredients of this extracts can serve as source for drug formulations in future.

PLATE 3 : ANTIBACTERIAL ACTIVITY OF DIFFERENT SOLVENT EXTRACTS OF EHUPHORBIA HIRTA WHOLE PLANT

(a) Bacillus subtilis



(b) Escherichia coli

(c) Salmonella typhi

(d) Klebsiella pneumonia

TABLE 1 : PRELIMINARY PHYTOCHEMICAL SCREENING AND DISTRIBUTIO OF SECONDARY CONSTITUENTS IN *EUPHORBIA HIRTA* L.

	Alkaloids	**Flavanoids**	**Saponins**	**Phenols**	**Quinone**	**Tannis**	**Steriods**	**Terpenoids**	**Coumarins**
Methanol	+	–	+	+	–	+	+	+	+
Acetone	+	+	–	+	+	–	+	–	–
Ethanol	+	–	–	+	–	–	+	+	–
Petroleum	–	–	–	–	–	+	–	–	+
Chloroform	–	+	–	+	–	+	–	+	–

TABLE 2 : PRELIMINARY PHYTOCHEMICAL SCREENING AND DISTRIBUTION OF SECONDARY CONSTITUENTS OF *EUPHORBIA MILLI.*

	Alkaloids	Flavanoids	Saponins	Phenols	Quinone	Tannis	Steriods	Terpenoids	Coumarins
Methanol	–	+	–	+	+	–	–	+	+
Acetone	–	+	+	+	+	–	–	+	+
Ethanol	+	+	+	+	–	–	–	+	+
Petroleum	–	+	–	+	–	+	–	+	+
Chloroform	–	+	–	+	+	–	+	+	+

TABLE 3 : FTIR SPECTROSCOPY ANALYSIS OF EUPHORBIA HIRTA

Peak Value	Bond	Functional Group
3422.52	Medium Urethanes	N-H
2921.54	Strong Alkane	C-H
1627.23	Strong Guanidine	C=N
1449.66	Weak Aromatic Compound	C-H
1022.14	Strong Ether	C-O
668.72	Strong Primary Amine	N-H

FIGURE 1

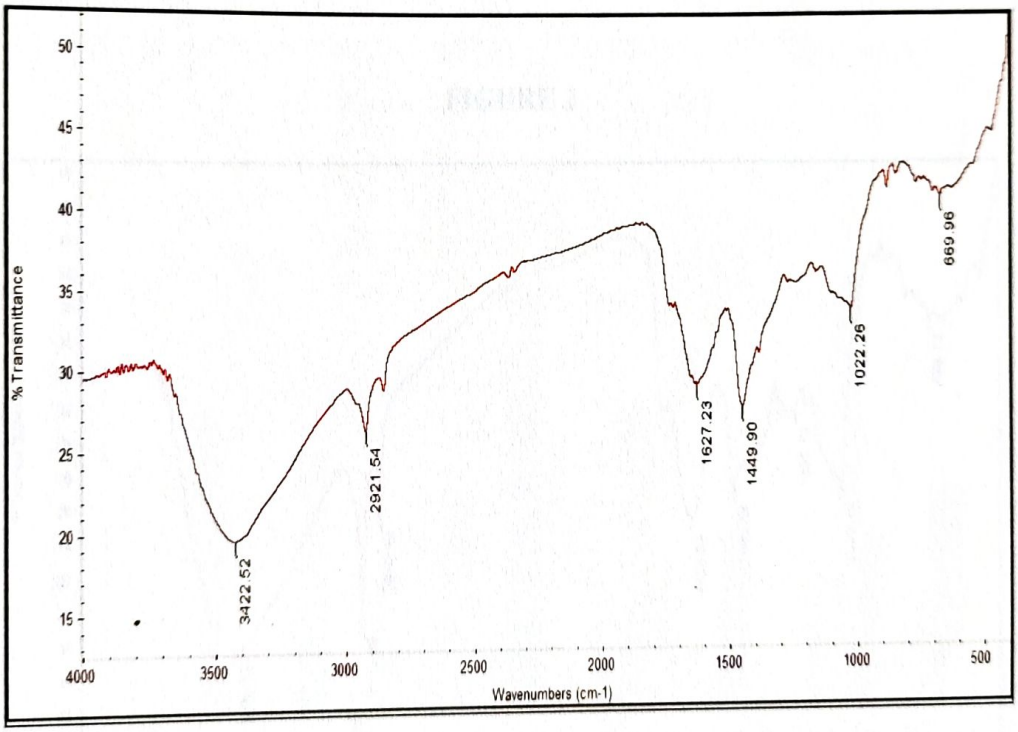


TABLE 4 : FTIR SPECTROSCOPY ANALYSIS OF EUPHORBIA MILLI

Peak Value	Bond	Functional Group
3409.15	Medium Aliphatic Primary Amine	N-H
2919.32	Strong Alkane	C-H
2850.49	Medium Esters	C-H
1620.03	Medium Amine	N-H
1449.66	Weak Aromatic Compound	C-H
1205.79	Strong Tertiary Alcohol	C-O
1022.14	Strong Ether	C-O
668.72	Strong Primary Amine	N-H

FIGURE 2

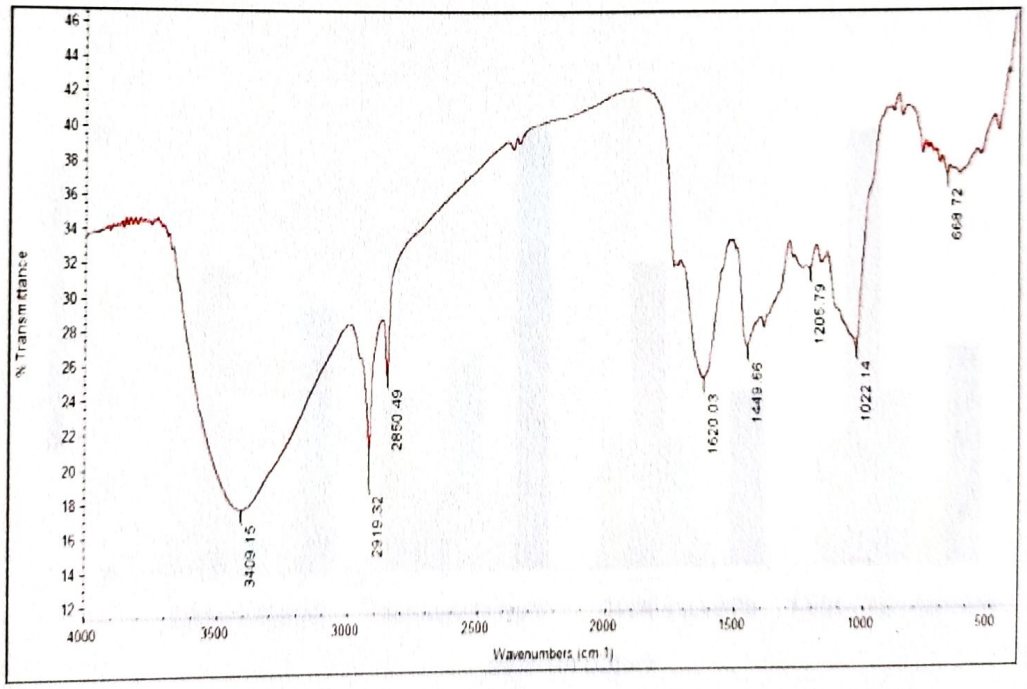


TABLE 5 : ANTIBACTERIAL ASSAY OF *EUPHORBIA HIRTA* WHOLE PLANT

S. No.	Solvent	Incubation zone in (mm)			
		Escherichia coli	*Salmonella Typhi*	*Bacillus substilis*	*Klebsiella pneumonia*
1	Methanol	7	4	3	3
2	Acetone	7	2	7	10
3	Petroleum ether	5	5	3	4
4	Water	5	2	2	2
5	Streptomycin	6	4	4	15

FIG. 3 : ANTIBACTERIAL ACTIVITY OF DIFFERENT SOLVENT EXTRACT OF *EUPHORBIA HIRTA*

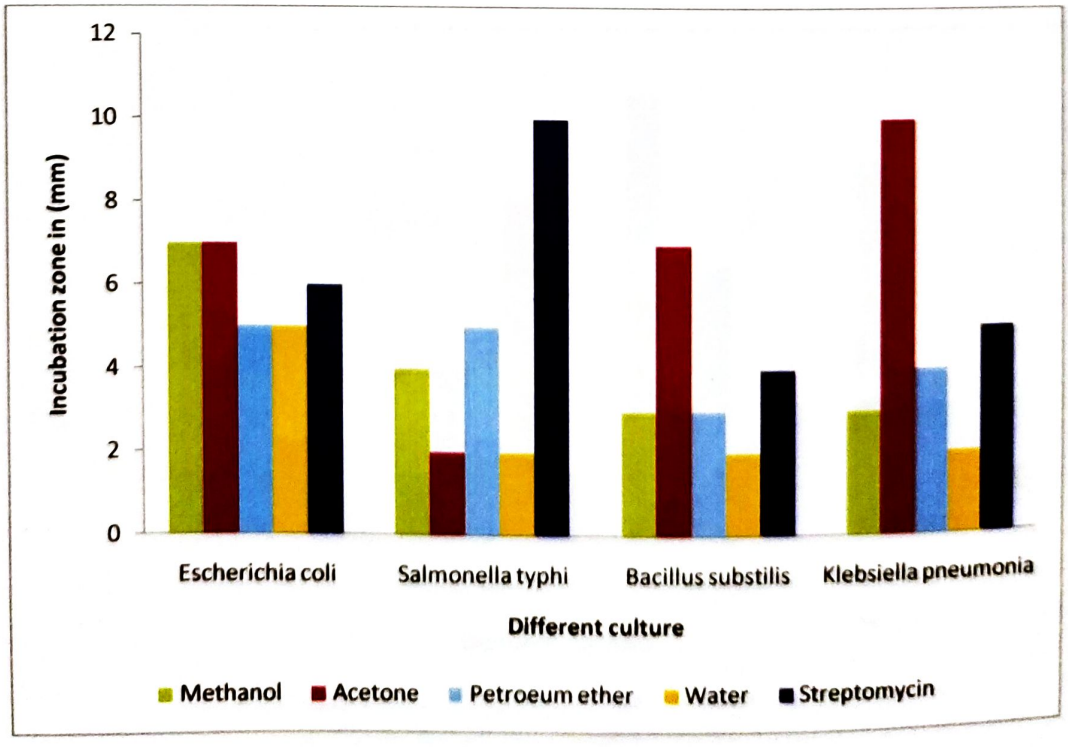
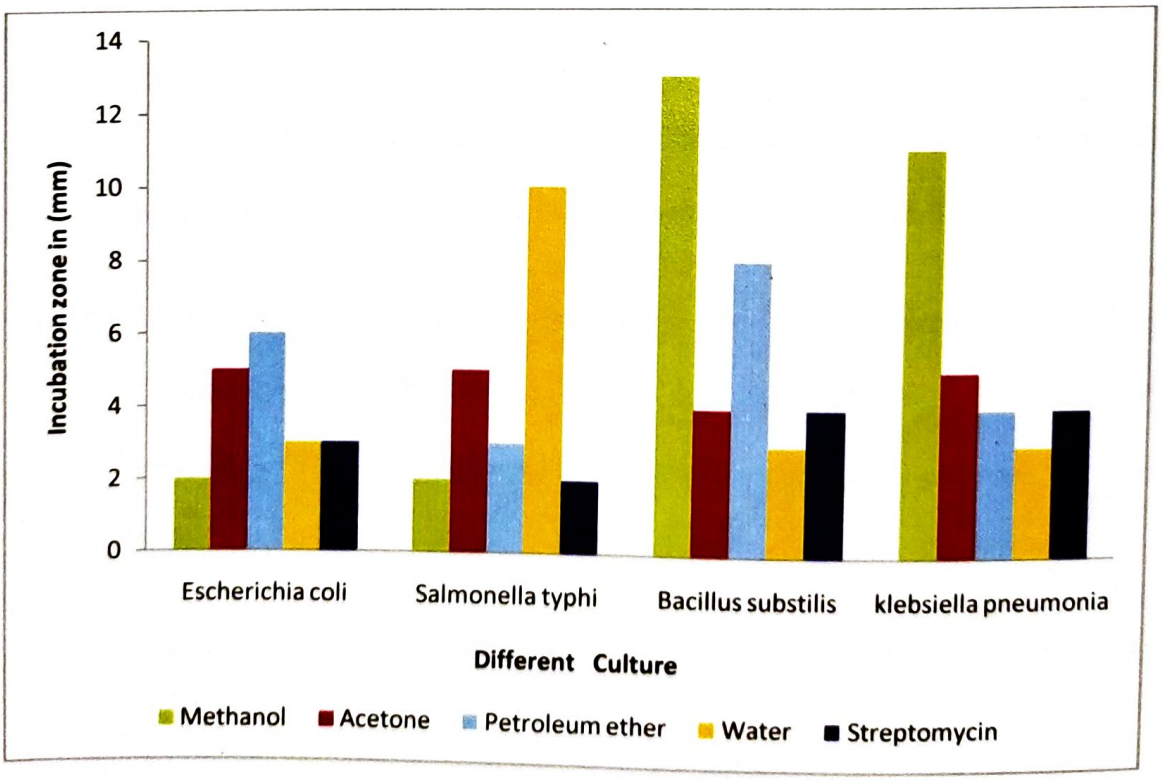


TABLE 6 : ANTIBACTERIAL ASSAY OF *EUPHORBIA MILLI* INFLOERESENCE

S.No.	Solvent	Inhibition zone in (mm)			
		Escherichia coli	*Salmonella typhi*	*Bacillus substilis*	*Klebsiella pneumonia*
1	Methanol	2	2	13	11
2	Acetone	5	5	4	5
3	Petroleum ether	6	3	8	4
4	Water	3	10	3	3
5	Streptomycin	3	2	4	4

FIG. 4 : ANTIBACTERIAL ACTI ITY OF DIFFERENT SOLVENT EXTRACT OF *EUPHORBIA MILLI*



REFERENCE

REFERENCE

Akinpelu, D.A., Olayinka, A., Aiyego, O. and Okoh, A. 2009. The bioactive potentials of two medicinal plants commonly used as folklore remedies among some tribes in West Africa. *Afr. J.* Biotech., 8(8): 1660-1664.

Akomas, S.C., Ijioma, S.N. and Emelike, CV. 2015. Effect of *Euphorbia hirta* on haematological and biochemical indicates in albino rats. *App. J. Hygine,* 4(1):1-5

Arts, I.C. and Hollman, P.G. 2005. Polyphenols and disease risk in pidermological studies. *Am. J. Clin. Nutr.,* 81:3175–3255.

Atta-ur-Rahman, Chou dhary, M.I. and Thomsen, W.J. 2001. Bioassay Techniques for Drug Development, *Harward Academic Publishers*, 67-68.

Chauturbhuj, S., Shah, Qadry, J.S. and Shah, 2004. *Quadry'spharmacognosy Twelth Revised Edition.,* 6-7.

Cheng, H.Y., Lin, C.C. and Lin, T.C., 2002. Antiherpes simplex virus type 2 activity of casuarinin from the bark of *Terminaliaarjuna* Linn. *Anti. Res.,* 55:447-455.

Clifton, P.M., Manny, N., Donna, R., Andriana, F., Paul, N. and Marja, C. 2004. High Dietary Intake of Phytosterol Esters Decreases Carotenoids and Increases Plant Sterol with No Additional Cholesterol Lowering, *J. Lipid Res.,* 45:1493-1499.

Cowan, M.M., 1999. Plant products as antimicrobial agents. *Clin. microbio. rev.,* 12(4):564-582.

CSH, Ya, Gaffney, T.H. and Haslam, E. 1988. Carbohydrate polyphenol complexation. In Hemingway RW and Karchesy JJ, Edition, Chemistry and significance of condensed tannins. *New York Plenum Press,* 553.

Deepti, K., Umadevil, P., Vijayalakshmi, G. and Vinodpolarao, B. 2012. Antimicrobial Activity and Phytochemical Analysis of *Morindatinctoria* Roxb. Leaf Extracts. *Asi. Paci. J. Trop. Biomed.,* 28:1440-1442.

Dhale, D.A. and Birari, A.R. 2010. Preliminary screening of Antimicrobial and phytochemical studies of *Jatropha gossypifolia* Linn. *Rec. Res. Sci. Tech.* 2(7):24-28.

Dipankar Choudhury, Arun, K., Pathak, Murugan Sevanan 2011. Phytochemical screening and antimicrobial activity of extracts from leaves and stem of *Ecboliumlinnean. Bang. J. Pharm.,* 6 (2):781-190.

Duthie, G.G. and Brown, K.M. 1994. Reducing the risk of cardiovascular disease, In Goldberg (1st Ed), Functional foods - designer foods, *pharmafoods, nutraceuticals.* Chapman and Hall, New York, 19-38.

Elampariti, D. and Boominathan, M, 2011. Comparative evalution of crude extract and fractions of Camellia sinensis leaves aginsthuman pathogenic bacterial strains *Streptococcuspnemoniae*, and 8 mm zone against *staphylococcus aureus. Inter. J. Uni. Pharm. Life Sci.,* 1(2):2249-6793.

Espin, J.C., Garcia-Conesa, M.T. and Tomas-Barberan, F.A., 2007. Nutraceuticals: Facts and fiction. *Phytochemistry;* 68:2986-3008.

Gauravkumar, L., Karthik. and Bhaskara Rao, KV. 2010. Phytochemical composition and invitro Antimicrobial activity of *Bauhinia racemosa* Lamk (CAESALPINIACEAE). *Inter. J. Pharma. Sci. Res.*, 1(11):51-58.

Gautam, S.S., Navneet, Kumar, S. and Prabhat, 2012. Screening of antibacterial activity of *Nepetaciliaris* benth against respiratory tract pathogen, *Kath. Univer. J. Sci. Tech.*, 8(1):100-103.

German, J.B. and Dillard, C.J. 1998. Phytochemicals and targets of chronic disease. In: Bidlack WRS et al. (Ed), Phytochemicals – a new paradigm. Technomic Publishing Company.

Harbrone, J.B., 1998. Phytochemical Method: A Guide To Modern Techniques Of Plant Analysis. *3rd Ed. New York.*, 45:356-389.

Hausteen, B. 1983. Flavonoids, a class of natural products of high pharmacological potency. *Biochem. Pharm.* 32:1141-1148.

Hostettmann, K. and Marston, A., eds. 1995. Pharmacologic consequences of cholesterol absorption inhibition: alteration in cholesterol metabolism and reduction in plasma cholesterol concentration induced by the synthetic saponin β-tigogenincellobioside (CP-88818; tiqueside). In: Saponins: *Cambridge University Press Cambridge, UK* 267-268.

Hussain, I., Ullah, R., Khurram, M., Ullah, N., Baseer, A., Khan, FA., Khattak, M.R., Zahoor, M., Khan, J. and Khan, N. 2011. Phytochemical analysis of selected medicinal plants, *Arf. J. Biotechnol.*, 10:7487- 7492.

Igbinosa, O.O., Igbinosa, E.O. and Aiyegoro, O.A. 2009. Antimicrobial activity and phytochemical screening of stem bark extracts from *Jatrophacurcas* (Linn), *Afr. J. Pharm. Pharmacol.*, 3: 58-62.

Inderjit Kaur, Chauhan P.K., Munish Jaryal., Shubhamsaxena., and Kanishak, 2012. Antioxidant and antimicrobial activity of leaf extract of Adhatodavasica against the bacteria isolated from the sputum samples of asthmatic patients: *Int. J Drug Res. Tech.* 2(3):273-278.

Iqbal Ahmad, I. and Aqil, F. 2007. In vitro efficacy of bioactive of 15 medicinal plants against ESBL-producing multidrug-resistant enteric bacteria. *Microbio. Res.* 162:264 -275.

Johnson, M.A., Jalaja, S.A., Solomon, J., Selvamony, S. and Babu, A. 2012. Preliminary phytochemical studies on the methanolic flower extracts of some selected medicinal plants from India. *Asi. Pac. J. Trop. Biomed.*, 79 - 82.

Kakar, S.A., Rasoolbakhshtareen., Azam Kakar, M., Hameedajabeen., Saced-ur-rehmankakar., Yasser, M.S.A., al-Kahraman., Muhammad Shafee, 2012. Screening of antibacterial activity of fout medicinal palnts of Banchistan-pakistan. *J. Bat.* 44;245-250.

Kareru, P.G., Keriko, J.M., Gachanja, A.N. and Kenji, G.M. 2008. Direct Detection of Triterpenoid Saponins in Medicinal Plants. *Afr. J. Tradit. Complement Altern. Med.* 5(1):56–60.

Kaur, K., Bedi, G., Kaur, M., Anil, V. and Kaur, I. 2008. Lipid Peroxidation and the Levels of Antioxidant Enzymes in Coronary Artery Disease, *Ind. J. Clin. Biochem.*, 23(1):33-37.

Killeen, G., Madigan, C., Connolly, C., Walsh, G., Clark, C., Hynes, M., Timmins, B., James, P., Headon, D. and Power R. (1998). Antimicrobial saponins of Yucca schidigera and the implications of their in vitro properties for their in vivo impact. *J. Agric. Food Chem.*, 46(8):3178-3186.

Li, H., Wang, Z. and Liu, Y., 2003. Review in the studies on tannins activity of cancer prevention and anticancer. Zhong-Yai-cai 26(6):444-448.

Liu, J. and Henkel, T. 2002. Traditional chinesmedicine are polyphenols and saponins the key ingredients Triggering biological activities. *Curr. Med. Chem.* 9(15):1483-1485.

McChesney, J.D., Venkataraman, S.K. and Henri, J.T. 2007. Plant natural products. Back to the future or into extinction? *Phytochem.* 68:2015-2022.

Mithraja, M.J., Johnson, M., Mahesh, M., Paul, Z.M. and Jeeva, S. 2011. Phytochemical studies on *Azolla pinnate* R.BR., Marsilea *minuta L.* and *Salviniamolesta Mitch. Asian Pac. J. Trop Biomed* 1: $S_{26} - S_{29}$.

Mitschar, L.A., 1974. Division of Natural products chemistry. The ohiostate University, Columbus, Ohio in recent advances in phytochemistry, 9:189-243.

Murugananthum, S., Anbalagan, G. and Ramamoorthi, N. 2009. FT-IR and SEM-EDS Comparative Analysis of Medicinal Plants, *Ecliptaalba*Hassk and *Ecliptaprostratea* Linn. *Roman. J. Biophy.*, 19(4):285-294.

Musa, K.Y., Ahmen, A., Ibrahim, H., Arowsiaye, G. and Olanioloa, O.S. 2000. Phytochemical and Antibacterial studies of leaves of *Acalypharacemosa.* Nige. *J. Nat. Prod.& Med.* 4: 67-69.

Nickavar, B., Kamalinejad, M. and Izadpanah, H. 2007. *In vitro* free radical scavenging activity of five *Salvia* species, *Pak. J. Pharm. Sci.*, 20: 291-294.

Niveditapatel, Pinal Pate, dharapatel, Sharavdesai and Dhananjaymeshram, 2014. Phytochemical analysis and antibacterial activity of *moringa Oleifera. Inter. J. Med. Pharm. Sci.*, 2250-0049.

Nobori, T., Miurak, K., Wu, D.J., Takabayashik, L.A. and Carson, D.A. 1994. Deletion of cyclin-dependent kinase-4 inhibitor gene in multiple human cancers. Nature, 46: 753-756

Nwokocha, A., Blessing, I.O., Agbagwa and Okoli, O.E. 2011. Comparative phytochemical screening of *Jatropha L.* species in Niger Delta. *Res. J. phytochem.*, 5: 107-114

Oliver-Bever, B. 1986. Medicinal plants in tropical West Africa, Cambridge University press, London. 245-267.

Omulokoli, B., Khan, S.C. and Chhabra, 1997. Antiplasmodial activity of four Kenyan medicinal plants, *J. Ethnopharmacol.* 56:133-137.

Parag, A., Pednekar. and Bhanu Raman, 2013. Antimicrobial and Antioxidant Potential with FTIR analysis of *Ampelocissus latifolia* (roxp.) Planch. Leaves. *Asi. J. Pharmaceu. Clin. Res.*, 6(1):67-73.

Pollak, O.J., 1953. Reduction of blood cholesterol in man.Circulation, 7:702-706

Poonkothai, M., Hemaiswarya, S. and Kavitha, D. 2005. Antibacterial activity of *Gymnemasylvestre. J. Ecotoxicol. Environ. Monit.* 15(1): 33-36.

Prema, R., Paulsamy, S., Thambiraj, J., and Saradha, M. 2004. The antibacterial activity in eleven medicinal plants. *Inter. J. Pharma., Chem. Biol. Sci.*, 3(4):1001-1005.

Ragavendran, P., Sophia, D., Arul Raj, C. and Gopalakrishnan, V.K. 2011. Functional group analysis of various extracts of *Aervalanata* (L.,) by FTIR spectrum. *Pharmacology online*. 1:358-364.

Rajore, S. and Batra, A. 2004. *Jatropha curcus L.* A valuable multipurpose plant species. Agrobias newsletter, 2(12):46-48.

Ramamurthy, N. and Kennan, S. 2007. Fourier transform infrared spectroscopic analysis of a plant (*Calotropis gigantean* Linn) from an Industrial village, CuddaloreDt, Tamilnadu, India. *Roman. J. Biophy.*, 17(4):269-276.

Ravindranath, N. 2003. Jatrophane, a novel macrocyclic bioactive diterpene from *Jatropha gossypifolia L. Chem. pharm. Bull.* [Tokyo], 51[7]: 870-1

Saeed, U.I., Hassan, S., Khalil, U.T., Rehman, M., Niaz, U., Saeed, M.A., Hussain , K., Iao, S.A. and Ahmed, I. 2013. Isolation and characterization of irritant components of *Euphorbiapilulifera*. L. *Pak. J. Pharmsci.*, 26(1):31-37.

Sakarkar, D.M. and Deshmukh, V.N. (2011). Ethno- pharmacological Review of Traditional Medicinal Plants for Anticancer Activity. *Inter. J. Pharm. Tech. Res.*, 3(1):298-308.

Sandeep, B.P., Nilofar, S.N. and Chandrakant, S.M. 2009. Review on Phytochemistry and Pharmacological Reasearch and health care 1(1):113-133.

Singhal, G., Bhavesh, R., Kasariya, K., Sharma, A.R. and Singh, R.P. 2011. Biosynthesis of silver nanoparticles using *Ocimum sanctum* (Tulsi) leaf extract and screening its antimicrobial activity. *J. Nanopart Res.* 13: 2981-2988.

Souad Akroum, and Korrichi Lalaoui, 2012. Antimicrobial activity of some alimentary and medicinal plants. *African Journal of Microbiology Research.* 6(8):1860-1864.

Steinmetz, K.A. and Potter, J.D., 1991. Vegetables, fruit and cancer. *I. Epidemiology. Cancer Causes Control.*, 2:325-357.

Sukumaran, S., Kiruba, S., Mahesh, M., Nisha, S.R., Miller Paul, Z., Ben, C.P. and Jeeva, S. 2011. Phytochemical constituents and antibacterial efficacy of the flowers of peltophorumpterocarpum. *Asi. Paci. J. Trop. Med.*, 4(9):735-738.

Sulaiman, G.M., Hussien, N.N., Marzoog, T.R. and Abood, H. 2013. Phenolic Content, Antioxidant, Antimicrobial and Cytotoxic activities of Ethanolic extract of Salix Alba Awad. *Amer. J. Biochem. Biotech.*, 9(1):41-46.

Thangarajan Starlin, Paramasivam Ragavendran, Chinthamony Arul Raj., Palanisamy Chellaperumal. and Velliyurkanniappan Gopalakrishnan, 2012. Element and functional group Analysis of *Ichnocarpous frutescens* R.Br (Apocynaceae). *Inter. J. Pharm. Pharmaceut. Sci.*, 4(5):343-345.

Vinoth, S., Kanna, P.R., Gurusaravanan, P. and Jayabalan, N. 2011. Evaluation of phytochemical, antimicrobial and GC-MS analysis of extracts of *Indigoferatriata L.F. Int. J. Agri. Res.*, 6:358- 367.

Woyengo, T.A., Ramprasath, V.R. and Jones, P.J.H., 2009. Anticancer effects of phytosterols. *European Journal of Clinical Nutrition*, 63(7):813-20.

Yasukazu, Y. and Etsuo, N., 2003. Antioxidant Effects of Phytosterol and Its Components, *J. Nutr. Sci. Vitamin.*, 49:277-280.

Yusha, U.M., Taura, D.W., Bello, B. and Abdullah, A. 2011; Screening of Annonasaqumosa extracts for antibacterial activity against some respiratory tract isolates; *Inter. Res. Pharm. Pharmacol.,* 1(9):237-241.

STUDIES ON MACROSCOPIC, MICROSCOPIC AND ANTIBACTERIAL ACTIVITY OF TWO SPECIES OF *SIDA*

A short term project work submitted to

St. Mary's College (Autonomous)

Re accreditation with A Grade by NAAC affiliated to

MANONMANIAM SUNDARANAR UNIVERSITY

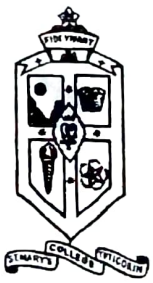
in partial fulfilment of the requirement for the

Degree of Bachelor of Science in Botany,

St.Mary's College (Autonomous), Thoothukudi.

By

J. ANTONY DAISY	-	**16AUBO07**
A. ANTONY LORTHU JERCY	-	**16AUBO08**
G. ASLIN BEMISHA	-	**16AUBO10**
K. CHITHRA DEVI	-	**16AUBO12**
M. DIVYA	-	**16AUBO13**
G. GOWSALYA	-	**16AUBO14**



DEPARTMENT OF BOTANY

ST. MARYS COLLEGE (AUTONOMOUS)

THOOTHUKUDI – 628 001

APRIL -2018-2019

CERTIFICATE

It is certified that this short term project work **"STUDIES ON MACROSCOPIC, MICROSCOPIC AND ANTIBACTERIAL ACTIVITY OF TWO SPECIES OF *SIDA*"** submitted to St. Mary's college (Autonomous) affiliated to **MANONMANIAM SUNDARANAR UNIVERSITY** in partial fulfilment of the requirements for the degree of Bachelor 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 2018 – 2019 by the following students.

By

J. ANTONY DAISY	-	**16AUBO07**
A. ANTONY LORTHU JERCY	-	**16AUBO08**
G. ASLIN BEMISHA	-	**16AUBO10**
K. CHITHRA DEVI	-	**16AUBO12**
M. DIVYA	-	**16AUBO13**
G. GOWSALYA	-	**16AUBO14**

GUIDE

29/3/19

HEAD OF THE DEPARTMENT

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

EXAMINER

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

ACKNOWLEDGEMENT

We offer our praise and sincere thanks to the Almighty God, for his avalanche of graces and bountiest blessings, enabling us 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 wish to express my deep sence of gratitude to **Dr. A. Jacintha Tamil Malar M.Sc., M.Phil., Ph.D.** Assistant Professor of Botany for suggesting this research problem, her constant encouragement and inspiring guidance throughout this project work.

I also thank the non-teaching staff members of Department of Botany St. Mary's College (Autonomous) for their help in various stages of my work.

CONTENTS

CHAPTER-I

INTRODUCTION

Plants are the backbone of all life on Earth and an essential resource for human well-being. Plant is an important source of medicine and plays a key role in world health. Herbal medicines proved to be the major remedy in traditional system of medicine. They have been used extensively in medical practices since ancient times. This prompts the development in the practices of medicinal plants. The reasons are because of their biomedical benefits as well as place in cultural beliefs in many parts of world in the development of potent therapeutic agents. During 1950-1970, approximately 100 plants based new drugs were introduced in the USA drug market including deserpidine, reseinnamine and vincristine which are derived from higher plants.

Modern medicine depends on biological materials as an incomparable source of molecular diversity. One-quarter of all prescription drugs come directly from or are derivatives of plants. Recently however, attention is turning back to natural products as drug sources, since they have been so successful in the past. These days the term "Alternative Medicine" became very common in western culture, it focus on the idea of using the plants for medicinal purpose.

Medicinal herbs or plants have been known to be an important potential source of therapeutics or curative aids. The use of medicinal plants has attained a commanding role in health system all over the world. Medicinal plants have provided mankind a large variety of potent drugs to alleviate or eradicate infections and suffering from diseases in spite of advancement in synthetic drugs, some of the plant-derived drugs still retained their importance and relevance. This involves the use of

medicinal plants not only for the treatment of diseases but also as potential material for maintaining good health and conditions. Many countries in the world, that is, two-third of the world's population depends on herbal medicine for primary health care. The reasons for this is because of their better cultural acceptability, better compatibility and adaptability with the human body and pose lesser side effects. From records, most of the used drugs contain plant extracts. Some contain active ingredients (bioactive components or substances) obtained from plants. Through recent researches, plant-derived drugs were discovered from the study of curative, therapeutic, traditional cures and most especially the folk knowledge of indigenous people and some of these claims and believe of people are irreplaceable despite the recent advancement in science and technology.

The use of plant-based drugs all over world is increasing. There have been records of advances made in the modern (synthetic) medicine there are still a large number of ailments or infection (diseases) for which suitable drugs are yet to be found. These have brought an urgent need to develop safer drugs (both for man and his environment) for the treatment of inflammatory disorders, diabetes, liver diseases, and gastrointestinal disorder. Through recent researches on herbal plants or medicine, there have been great developments in the pharmacological evaluation of various plants used in traditional systems of medicine. Consequently, plants can be described as a major source of medicines, not only as isolated active principles to be dispensed in standardized dosage form but also as crude drugs for the population.

The term of medicinal plants include a various types of plants used in herbalism and some of these plants have a medicinal activities. These medicinal plants consider as a rich resources of ingredients which can be used in drug development and synthesis. Medicinal plants frequently used as raw materials for

extraction of active ingredients which used in the synthesis of different drugs. Besides that these plants play a critical role in the development of human cultures around the whole world.

Herbal drugs from ethno medicinal plants have gained considerable importance in the recent past not only in India but also around the world (Farnsworth, 1990). Traditional medicinal knowledge in India has passed from one generation to the next, within specific geographical locations or tribal groups (Dey *et al.*, 2017). This traditional knowledge finds its root in Indian traditional systems of medicine i.e., Ayurveda and Siddha which is now gaining popularity in western world too. Herbal medicines are much in demand as they are affordable and have much less side effects (Modak *et al.*, 2015). Recently WHO has also recognized the importance of traditional medicine in the healthcare sector. In Ayurveda and Siddha systems, formulations from appropriate parts of plants are made and used for treatment of various ailments. For almost past three decades, many ethno medicinal plants mentioned in Ayurveda and Siddha systems of medicines are being scientifically evaluated (Sharma and Patki, 2010). Scientific evaluation of ethno medicinal plants provides evidence-based alternative medicines which form the basis of herbal drug industry and discovery of drug targets in the pharmaceutical industry (Patwardhan, 2005). It may be emphasized here that usage of ethno medicinal plants for traditional medical treatment or for use in manufacture of Ayurvedic medicines or other herbal drugs, when supported by scientific evidences can ensure safe and more effective utilization of natural product drugs universally. Medicinal plants have a promising future because there are about half million plants around the world, and most of them their medical activities have not investigate yet, and their medical activities could be decisive in the treatment of present or future studies.

The family Malvaceae is one of the largest flowering plants and is commonly known as "Mallow family". It has 82 genera and 1500 species distributed widely in tropical and subtropical regions of the world. In India, the family is represented by 22 genera and 93 species many of which have ethno medicinal value. *Sida acuta* and *Sida cordifolia* are ethnomedicinal plants of Malvaceae, commonly used in Indian traditional system of medicines. Traditionally these plants were used in the form of extracts, powder, paste by tribal populations of India for treating common ailments like cough and cold, fever, stomach, kidney and liver disorders, pains, inflammations, wounds, etc.

In view of these fact, the present investigation is undertaken.

- To elucidate the macroscopic characteristic of *Sida acuta* and *Sida cordifolia*
- To study the microscopic characteristic of root, stem and leaves of *Sida acuta* and *Sida cordifolia*
- To evaluate the antimicrobial activity of *Sida acuta* and *Sida cordifolia*

CHAPTER-II

REVIEW OF LITERATURE

Plants have been shown to have genuine utility and about 80% of the rural population depends on them as primary health care (Akinyemi, 2000). In developing countries, notably in West Africa, new drugs are not often affordable. Thus, up to 80% of the population uses medicinal plants as remedies (Kirby, 1996).

The ethnomedicinal usage of *Sida acuta* (Sanskrit name: Balapatta) has been reported from among the ethnic tribes from many parts of India. The tribal population from north eastern and southern parts of India have been extensively using different parts of the plant for treatment of dandruff, rheumatism, liver problems, kidney stones and nervous disorders (Gairola *et al.*, 2013). Juice of fresh leaves are used as anti-helminthic, anti-vomitting and gastric disorders (Akilandeswari *et al.*, 2010). Paste of roots in lemon juice is applied on boils and abscises (Shivanna and Rajakumar, 2010). Decoction of roots is used to treat rheumatism and breathing disorders. Hot water extract of whole plant is used as diuretic (Nadkarni, 1976).

Plant is also used by tribes of Tamil Nadu (Southern India) for treating bronchitis dysentery, diarrhoea and skin diseases (Ignacimuthu, 2006). Besides India, other Asian (Sri Lanka, Taiwan); Central and South American (Mexico, Venezuela, Colombia, Cuba, Nicaragua, Guatemala) and African countries (Nigeria, Togo, Ivory Coast, Kenya) also use this ethnomedicinal plant for treating dysentery, hemorrhoids, malaria, venereal diseases, ulcers, renal inflammations, fever and asthma (Dinda *et al.*, 2015). Root sour and sweet, removes tridosha, digestive and diuretic, useful in fever, burning of the body and urinary discharges (Kirtikar and Basu, 1994).

Bala (*Sida cordifolia* Linn.) that is also known as "Indian Ephedra" is a plant drug, which is used in the various medicines in Ayurveda, Unani and Siddha system of medicine since ages. It has good medicinal value and useful to treat diseases like fever, weightloss, asthma, chronic bowel complaints and nervous system disease and acts as analgesic, anti-inflammatory, hypoglycemic activities etc. (Sharma, 2013). Due to ephedrine, various Ayurvedic preparation of *Sida cordifolia* are used in asthma, fat lose, increase energy, chronic dysentery and gonorrhea in the Indian subcontinent (Anonymous, 1988). It has folklore use as a general tonic, anti-inflammatory agent, and blood coagulant. It has also been used in some gynecological practices, sexual inadequacies, and Parkinson's disease (Puri, 1993).

Kanth and Diwan, 1999 reported that *Sida cordifolia* possess analgesic, anti-inflammatory, anticancer, diuretic, laxative, hypoglycemic, antispasmodic, antiamoebic, antiurinary filariasis, antiasthmatic, antihypertensive and hepatoprotective activities. Medeiros *et al.* (2006) showed that aqueous fraction of hydroalcoholic extract of leaves induces vasorelaxation and hypotension. The plant have weight loss and wound healing, thyroregulatory, adaptogenic, antibacterial, antiplaque, and antifungal activities (Muauza *et al.*, 1994). Studies of Rastogi and Malhotra (1985) showed that the roots possessed diuretic and tonic properties and administered for nervous disorders such as hemiplegia and facial paralysis.

ANATOMICAL STUDIES

Anatomy along with plant morphology always treated as the backbone of plant taxonomy and systematists elucidated the plant diversity, phylogeny and evolution following these traits (Endress *et al.*, 2000). Morphological and microscopic studies of leaves act as a reliable aid for detecting adulteration. These simple but reliable

standards will be useful to a lay person in using the drug as a home remedy (Khandelwal, 2007). Microscopy is an important tool for authentification of crude drugs and study of powdered drugs (Ponnudurai *et al.*, 2011). Morphological and anatomic characters of leaf are used as taxonomic markers to assist in the correct identification of the plant species. Some particular groups of plants or taxa seem to be characterized by specific type of cross sectional anatomy, epidermal features, which are the epidermis, stomata, gland and trichomes (Park, 1994).

Anatomical data are applied to improve classification schemes and it is often used for identification. Wide range of anatomical data is used by systematists including anatomy from stem, leaf, petiole, stipule, node, flower, fruit, seed etc. Often these anatomical features are correlated with environmental factors (Naskar, 2016). Establishment of the pharmacognostic, morphological and microscopical characters of leaves and bark of the plant will assist in standardization, which can guarantee quality, purity and identification of samples (Karthikeyan, 2012).

Cutler *et al.* (2007) reported that in systematic anatomy has a long history since the invention of microscope. Taxonomists found anatomical similarities among related plant groups. The leaf epidermis is generally a valuable character for the classification and delimitation of species and genera, and/or for the discussion of relevant phylogenetic problems (Jones, 1986). It is important to interpret morphological and anatomical descriptions of crude drugs as well as characteristic features of drugs and adulterants of commercial significance (Dharmesh *et al.*, 2010).

Ozkan and Uzunhisarcikli (2009) provided detailed comparative anatomical information for the genus *Althaea*, which is hitherto unavailable among the Turkish species. Anatomical studies of the root of four *Sida* species, *S. rhombifolia*,

S. almifolia, *S. cabrida* and *S. rhomboidea* coming under *Sida rhombifolia* complex (Malvaceae) were carried out and compared (Navas *et al.*, 2013). Garcia *et al.* (2014) investigated the leaf anatomy of *T. grandiflorum*, *T. speciosum* and *T. subincanum* in order to contribute to the biological knowledge of species of Theobroma and provide support for the biotechnological studies of native fruit plants of the Amazon.

Micro morphological investigations of the foliar epidermal anatomy, particularly the diversity and distribution of glandular and eglandular trichomes on leaves of *Sida alba* L., *S. alii*, *S. Abedin* var. *alii*, *S. cordata*, *S. mysorensis*, *S. ovata*, *S. spinosa* L and *S. yunnanensis* have been carried out by Shaheen *et al.* (2009) to assess the systematic relevance of epidermal features and trichome diversity within the genus *Sida* L.

Nurhanim *et al.* (2014) carried out leaf anatomy and micromorphology study on five selected taxa in the genus *Schoutenia* Korth of Malvaceae subfam. Brownlowioideae to investigate the taxonomic value of leaf anatomical and micromorphological characteristics of the genus *Schoutenia*. Ltahir *et al.* (2017) compared the macro and micro morphology of the leaves of *Abutilon figarianum* and *Abutilon pannosum* (Malvaceae) to assist as a relevant source of information and contribute towards the standards to dispose the quality and identity of these plants to avoid adulterations.

ANTIBACTERIAL ACTIVITY

Microbial infections pose a health problem all over the world, and plants are a potential source of antimicrobial infections pose a health problem all over the world and plants are a potencial source of antimicrobial agents (Burapadaja and Bunchoo, 1995). Medicinal plants have been a valuable source of natural active constitiuents

that products for maintain human health and treatment of many human disease (Stary and Hans, 1998). From ancient times, humans have utilized plants for the treatment or prevention of diseases, leading to the dawn of traditional medicine (Audu *et al.*, 2007). Effective antimicrobials have been developed over the past years, several reports development of antibiotic resistance of human pathogens to available antibiotics (Martino *et al.*, 2002).

Due to the cost effectiveness, safety, increasing failure of chemotherapy and antibiotic resistance, search for plant resources has been increased for their potential antimicrobial activity (Hammer *et al.*, 1999). Antibiotic resistance has become a global concern (Westh *et al.*, 2004). There has been an increasing incidence of multiple resistances in human pathogenic microorganisms in recent years, largely due to indiscriminate use of commercial antimicrobial drugs commonly employed in the treatment of infectious diseases. Recent work revealed the potential of several herbs as sources of drugs (Iwu, 2002). The screening of plant extracts and plant products for antimicrobial activity has shown that higher plants represent a potential source of novel antibiotic prototypes (Afolayan, 2003).

The emergence of resistant bacteria, especially those causing infections on wounds, has become a health care problem that has caused serious concern to medical practice (Arias and Murray, 2008). Drug resistant bacteria render many synthetic antibiotics ineffective or useless (Venkatesan *et al.*, 2009). Antibacterial drugs have been in used since several years ago to handle these infections. However, in the recent decade, bacterial resistance to these drugs is being reported (Steven *et al.*, 2015). Extreme interest in plants with microbial activity has revived as result of current problems such as resistance associated with the use of antibiotics obtained from microorganisms (Koday *et al.*, 2010).

Hoffman *et al.* (2004) analysed the antibacterial activity of *Sida acuta* against the gram positive microorganism *Staphylococcus aureus*. Ekpo and Etim (2009) reported the antibacterial property of *Sida acuta* (ethanolic and water extract) on isolated microbes from human skin infection. *Staphylococcus aureus*, *Bacillus subtilis*, *P. aeruginosa* and *E. coli*. Ethanol extract revealed a higher significant inhibition against *S. aureus* and *B. subtilis*. A similar antibacterial property of *S. acuta* was also reported by Karou *et al.* (2006).

Prabahar *et al.* (2009) carried out the antibacterial activity of the plant *A. indicum* (L.) Sweet, which is a cosmopolitan genus belonging to the family of Malvaceae, to determine the antibacterial activity of different extracts of the leaves on gram positive and gram negative micro organisms against penicillin potassium (20 units/ml) and streptomycin sulphate (25 µg/ ml). Kumar *et al.* (2009) formulated new, cost effective antimicrobial combination for multidrug resistant diseases based on the synergistic activity of oxytetracycline with methanolic extract of *Thespesia populnea* (Malvaceae) a medicinal plant common in South India.

Sowmya *et al.* (2018) investigated the potential presence of naturally occurring antimicrobials in petals of flowers of *Hibiscus sabdariffa* L., (Malvaceae) against isolated eye pathogens. Owing to the usage of these flowers in common folklore medicine, the extracts of petals were screened for antibacterial activity against pathogenic microbes isolated from the eyes of eye infected persons. Abdul *et al.* (2010) conducted an investigation with crude methanolic extract of leaf of *Abutilon indicum* for its cytotoxic and antimicrobial activity against various Gram-positive, Gram-negative bacteria and fungi using disk diffusion technique. Junior *et al.* (2015) investigated the antifungal effect of ethanol extract from different parts of *Luehea paniculata* Mart & Zucc., a medicinal tree of multiple effects, individually

and in combination with a commercial drug, against clinical isolates of multidrug-resistant strains of *Candida*.

Woldeyes *et al.* (2012) isolated compounds from roots of *Sida rhombifolia* and subsequently evaluated their antibacterial activities on four different pathogenic bacterial strains (*Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa* and *Salmonella typhimurium)* using agar disc diffusion technique. The antimicrobial activity of the 90% ethanol extract of the aerial parts of *Sida acuta* Burm. F. (Malvaceae) was investigated in order to verify its claimed ethno medicinal use in the treatment of microbial infections. (Oboh *et al.*, 2007). Jacob *et al.* (2018) validated the folklore use of *Sida corymbosa* on wound healing and reported that *Sida corymbosa* leaves are good source of antibacterial agents that possess wound healing property in line with its ethno-medicinal use.

Debalke *et al.* (2018) assessed the antibacterial activity of the aqueous-methanol extract of the *Sida rhombifolia's* aerial part on five pathogenic bacteria species using agar well diffusion method at different concentrations of plant extracts and screened phytochemical constituents of the plant. Ruban and Gajalakshmi (2012) determined the role of flower in *Hibiscus rosa-sinensis* extract in the *in-vitro* antibacterial activity against human pathogens *viz.*, Gram positive bacteria *Staphylococcus aureus*, *Streptococcus*, *Bacillus subtillis* and Gram negative bacteria *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella*.

CHAPTER-III

MATERIALS AND METHODS

Collection and identification of plant materials

The fresh plant materials of *Sida acuta* and *Sida cordifolia* are collected from the State bank colony and Suntharavalepuram in the month of January 2019. The plants are identified with the help of local floras. The collected plants are preserved as per the standard procedure (Jain and Rao, 1977). Voucher specimens of all the selected taxa are deposited and preserved in the St. Mary's College Herbarium (SMCH), Research Centre for Plant Sciences, St. Mary's College, Thoothukudi, Tamil Nadu, India.

Macroscopic studies

Macroscopic evaluation is the method of qualitative evaluation established on the study of morphological and sensory profiles of whole plant. Fresh, full-grown and healthy plant of both species are collected and washed in pure water to remove all the impurities. The samples are subjected to macroscopic evaluation by observation with naked eyes. A magnifying lens with a dissecting microscope is used for a better evaluation of surface characters

Morphology

Study of plant morphology is the first step in the medicinal plant research and is useful in the identification of plants. The morphological parameters like plant height, leaf size and shape, phyllotaxy, inflorescence type, flower colour, flower structure, fruit, seed characters and other important features of all plants are noted.

Organoleptic characters (Khandelwal, 2003)

Organoleptic evaluation can be done by means of organs of sense which includes the parameters like colour, shape, odour, taste, surface characteristics and texture and there by define some specific characteristics of the material which can be considered as the first step towards establishment of identity and degree of purity of the drug.

Microscopic studies

The microscopic evaluation is used for studying the anatomical features of transverse section of root, stem and leaf of *Sida acuta* and *Sida cordifolia*. Linn. Enough number of sections are taken by hand using razor blade. The sections are carefully transferred to a petridish containing water and few thin sections that floated in water are selected. Then selected sections are stained in saffranin. A stained section is carefully transferred on a clean glass microslide using thin brush. With the help of a forceps and a needle a clean cover slip is placed gently over the section. With the help of a blotting paper excess glycerine is removed and the slide is observed under a digital microscope.

Photomicrographs

Microscopic descriptions of tissues are supplemented with micrographs wherever necessary. Photomicrographs of different magnifications are taken with Nikon Laphot 2 microscopic unit. For normal observations bright field is used. Magnifications of figures are indicated by scale bars. Descriptive terms of anatomy features are as given in the standard anatomy books (Esau, 1964).

Antibacterial studies

Preparation of plant powder

Fresh leaves of *Sida acuta* and *Sida cordifolia* are washed 2-3 times with tap water and subjected to drying at room temperature. The dried plant materials are powdered using a clean mixer grinder and filled in air tight container and stored in a dry place on room temperature till analysis.

Preparation of plant extract

The powdered plant samples (20 g) are extracted with methanol and ethanol using soxhlet apparatus. Solvent was evaporated under vacuum and concentrates are used for antibacterial assay.

Collection of microorganisms

Stock cultures of bacteria such as, *Escherichia coli* and *Bacillus subtilis* are obtained from the Department of Microbiology, St Mary's College (Autonomous), Thoothukudi.

E.coli - *Escherichia coli,* also known as *E. coli*, is a Gram-negative, facultative anaerobic, rod- shaped, coliform bacterium of the genus *Escherichia* that is commonly found in the lower intestine of warm-blooded organisms.

Bacillus - *Bacillus* is a genus of Gram-positive, rod-Shaped bacteria, a member of the phylum Firmicutes, with 266 named species. The term is also used to describe the shape of certain bacteria; and the plural *Bacilli* is the name of the class of bacteria to which this genus belongs.

Preparation of media

The growth media employed in the present study included Nutrient agar and Nutrient broth. Nutrient agar is composed of

Beef extract	-	3.0 g
Peptone	-	5.0 g
Agar	-	15.0 g
Distilled water	-	1000 ml

Nutrient broth is composd of

Beef extract	-	3.0 g
Peptone	-	5.0 g
Distilled water	-	1000 ml

The medium was adjusted to pH 7.4 and sterilized by autoclaving at 15 lb pressur (121°C) for 15 min.

Sub culturing of microorganisms

The pure culture of microorganisms was maintained on nutrients agar slants by frequent sub culturing. The culture was stored at 4°C.

Preparation of inoculum

Each organism was recovered for testing by sub culturing on fresh media. A loopful inoculum of each bacterium was suspended in 5 ml of nutrient broth and

inculum of each bacterium was suspended in 5 ml of nutrients both and incubated overnight at 37°C. These overnight cultures were used as inoculums.

Antimicrobial activity

Antimicrobial activity is demonstrated by modification of the method described by Barry and Thornsberry (1985). 0.1 ml of the diluted microbial culture is spread on sterile nutrient agar plate. The pre-soaked and dried discs of 6 mm diameter of Whatman No.1 filter paper are then placed on the seeded plates and gently pressed down to ensure contact. At the same time standard antibiotic of tetracycline is used as reference or positive control. Respective solvents without plant extracts served as negative control. The plates are incubated at 37°C for 24 hrs. After the incubation period, the diameter of the inhibition zone around the plant extract saturated discs are measured and also compared with the diameter of inhibition zone of commercial standard antibiotic discs. The inhibition zone around the discs are measured and recorded as the difference in diameter between the disc (6 mm) and growth free zone

CHAPTER-IV

RESULT AND DISCUSSION

Indigenous knowledge had a role to play in the understanding coexistence of man with fauna and flora over centuries. In spite of great importance biological diversity and the amount of attention is currently being given to traditional medicine at both national and international levels. Many of these biological diversities are used in local traditional medicine and have been reputed through experience inherited from one generation to the other useful medicinal activity (Ibrahim *et al*., 2007). In the present investigation, *Sida acuta* and *Sida cordifolia* belongs to family Malvaceae have been subjected to macroscopic and microscopic analysis. The antibacterial potential of the leaves are studied.

Systematic position

In Bentham Hooker's System of classification. the Systematic position of *Sida* is as follows.

Kingdom	-	Plantae
Class	-	Dicotyledons
Sub class	-	Polypetalae
Order	-	Malvales
Family	-	Malvaceae
Genus	-	*Sida*

Macroscopic Characters

***Sida acuta* Burm. F**

Annual, erect herbs, 0.5 - 2 m high; stems pubescent with simple minute stellate hairs; Leaves 1 - 9 x 0.5 - 2.5 cm, lanceolate to linear, elliptic-lanceolate, acute at apex, mostly serrate, 3-nerved at base, hairs simple; petioles 2 - 6 mm long Flowers axillary, solitary, buttercup like in shape, with overlapping petals; Calyx 5 - 6 mm across, campanulate; Corolla light yellow, 8 - 10 mm across; petals as long as or slightly exceeding calyx lobes; Mericarps 6 - 10, apically 2 awned of equal length; 1-seeded; Seeds dark brown.(Plate-1)

Flowering and Fruiting : July to March

Distribution : Along roadsides, in wastelands, both shady and open places

Vernacular name : Common wireweed, Morning mallow,Palambasi

***Sida cordifolia* L.,**

Erect under shrubs, up to 1 m high; stems branched with minute stellate hairs mixed with simple hairs. Leaves 6 x 5 cm, ovate to oblong or sub-orbicular, shallowly cordate at base, crenate-serrate, 5 - 7-nerved at base, densely velutinous with minute stellate hairs on both surfaces; petioles 4 - 5 mm long, densely stellate-hairs mixed with some simple hairs; Flowers axillary, solitary; pedicels 2 - 10 mm long, accrescent up to 2 cm, jointed towards apex. Calyx 5 - 9 mm across, campanulate, somewhat accrescent; lobes triangular acute to acuminate, densely tomentose with stellate and simple hairs. Corolla dark yellow, 15 mm across; petals obliquely obovate, Mericarps 8 - 10, apex of mericarp with a pair of awns with unequal length; Seeds grayish black. (Plate 2)

Plate 1 : *Sida acuta*

Plate 2 : *Sida cordifolia*

Flowering and Fruiting : Throughout the year.

Distribution : In dry waste places.

Vernacular name : Arivalmanaippundu, Nilatutti, Paniyara tutti

Organoleptic characters

The macroscopic characters such as size, shape, margin, apex, surface, colour, odour, taste, nature, texture are studied for morphological investigation. Organoleptic characters of *Sida acuta* and *Sida cordifolia* are examined and presented in Table 1 and 2.

Microscopic characters

The microscopic evaluation of transverse section of root, stem and leaf of *Sida acuta* and *Sida cordifolia* are carried out.

Sida acuta

Root

Transverse section of root is circular in outline. Bark is thin with cork consisting of 4 to 7 rows of thin walled tangentially elongated cells. Cortex is narrow, comprising of 3 to 4 layers. Calcium oxalate crystals and starch grains are frequent in cortical cells. Bast fibre is seen in tangential bands of 3 to 6 rows alternating with thin walled phloem elements. The vessels are larger in size and more in number. Xylem parenchyma cells that surround the vessel contain starch grains. Medullary rays are uniseriate or biseriate with deposition of calcium oxalate crystals and starch. Pith is prominent. (Plate 3a)

Table 1 : Organoleptic character of *Sida acuta*

Colour	Green,upper side darker than lower side
Shape	Elongated
Odour	Slight odour
Texture	Slightly rough
Taste	Bitter
Apex	Acuta the apex
Base	Obtuse at the base
Petiole	Short 2 to 6
Leaf arrangment	Simple and alternate
Margin	Toothed margin
Venation	Reticulate venation

Table 2: Organoleptic character of *Sida cordifolia*

Colour	Greenish grey in colour
Shape	Cordate
Odour	No any specific odour
Texture	Felty
Taste	Tasteless
Apex	Subacute
Base	Obtuse at the base
Petiole	Hairy and shining brightly
Leaf arrangement	Simple, alternate, breadly ovate
Margin	Dentate, serrate
Venation	Reticulate

Stem

Transverse section of stem is oval in outline. Epidermis is made up of single layer of rectangular thin walled cells. The cortex consists of outer two layered chlorenchyma and middle 3-4 layered collenchyma cells and inner 3-4 cells deep rotund to oval parenchyma cells, some of the parenchyma cells contain druses of calcium oxalate crystals, Pericyclic fibres in groups occur as a ring, external to the phloem. Vascular bundles are closely arranged forming a continuous ring. Pith is made up of thin walled parenchyma cells, Mucilage cells are present in the cortex and pith. Most of the cells are filled with starch grains. Vascular bundles are closely arranged forming a continuous ring. Pith is made up of thin walled parenchyma cells, Mucilage cells are present in the cortex and pith. (Plate 3b)

Leaf

A transverse section of the leaf of *Sida acuta* through the mid rib shows a dorsiventral structure. The epidermal cells have wavy walls and a straight cuticle. The epidermis possesses numerous unicellular non-glandular trichomes. The midrib is partly surrounded by an arc of pericyclic fibres, above and below which, is a considerable amount of collenchyma. The xylem fibres occur in an arc arrangement The cells are round and spirally arranged. The phloem also forms a continuous arc below the xylem fibres. The palisade cells are cylindrical in shape and present on the upper epidermis only. The spongy mesophyll shows thin walled irregular parenchymatous cells. The transverse section of the leaf of *Sida acuta* through the lamina shows a thin walled cuticle, cylindrical palisade cells and a spongy mesophyll with thin walled irregular parenchymatous cells. (Plate 3c)

Plate 3a : T.S. of root of *Sida acuta*

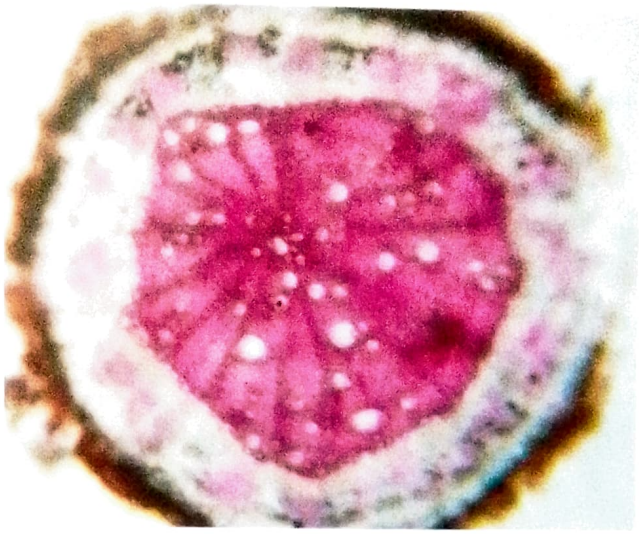


Plate 3b : T.S. of stem of *Sida acuta*

Plate 3c : T.S. Leaf of *Sida acuta*

Sida cordifolia

Root

The transverse section of root has 4-6 rows of thin walled tangentially elongated cells formed the cork. The outermost 2 layers were slightly ruptured and light brown in colour. Inner to the cork was the Phellogen consisting of a single layer of narrow, thin walled tangentially elongated cells. Cortex: The cortex is made up of 3-4 group of parenchymatous cells. Vessels were many, occuring solitary or in scattered groups of 2 to 4. (Plate 4a)

Stem

Transverse section of stem is circular in outline with stellate trichomes on epidermis followed by conspicuous zone of collenchyma, parenchyma, conducting elements and central pith. Epidermis is composed of oval to oblong, radially elongated, thin-walled cells covered by a thin cuticle. Trichomes are stellate or glandular. Epidermis followed by 1-2 layers of chlorenchyma followed by 4-6 layers of collenchyma consisting of round to oval cells. (Plate 4b)

Leaf

Transverse section of the leaf shows very thin cuticle with stellate and glandular trichomes on upper and lower epidermis. Stomata are anisocytic, Single layered upper epidermis consists of oval to oblong cells followed by compactly arranged, rectangular elongated palisade cells, spongy parenchyma oval to round, and loosely arranged. (Plate 4c)

Plate 4a : T.S. of root of *Sida cordifolia*

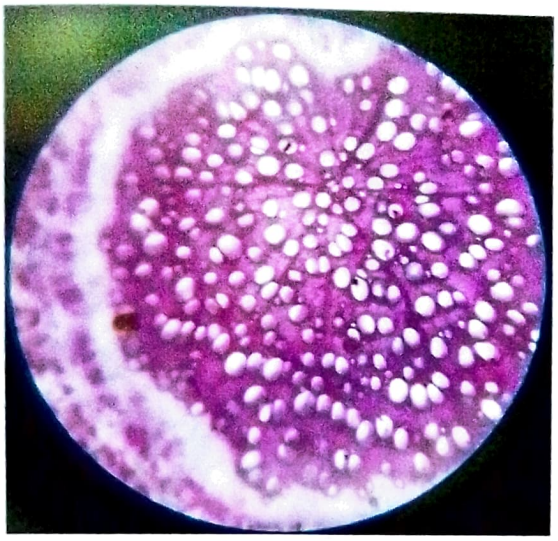
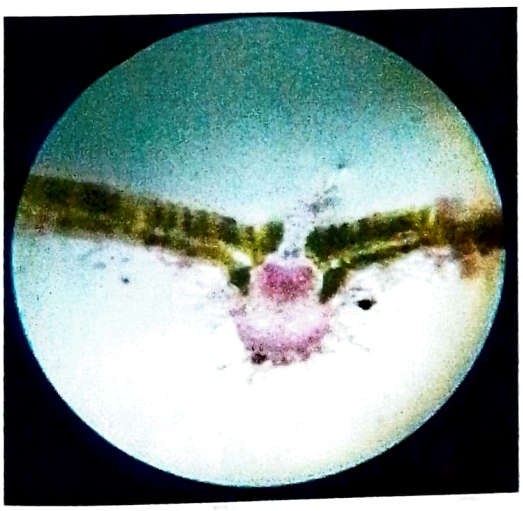


Plate 4b : T.S. of stem of *Sida cordifolia*

Plate 4c : T.S. of leaf of *Sida cordifolia*



Antibacterial activity

Antimicrobial properties of medicinal plants are being increasingly reported from different parts of the world. The World Health Organization estimates that plant extracts or their active constituents are used as folk medicine in traditional therapies of 80% of the world's population (Shaik *et al.*, 1994). In this study we analysed the antibacterial activity of ethanol and methanolic extracts of *Sida acuta* and *Sida cordifolia* against *E. coli* and *B. subtilis.*

The methanol extract of *Sida aucta* showed excellent zone of inhibition 10 mm against *Escherichia coli* and 6 mm against *Bacillus subtilis* (Fig.1). The ethanol extract of *S. acuta* showed a zone of inhibition (7 mm) against *Escherichia coli* followed by *B. subtilis*. 4 mm (Plate 5 and 6). The antibacterial activity of *Sida cordifoila* (Methanolic extract) showed different results on *E. coli* and *B. subtilis*, but the maximum zone of inhibition was found on *E. coli*, that is 11 mm. (Fig. 2) on the some bacteria the positive control Streptomycin gave 13 mm zone of inhibition. The ethanol extract of *S. cordifolia* also showed zone of inhibition as 6mm and 7mm against *E. coli* and *B. subtilis* respectively (Plate 7 and 8). Among the two plants, *S. cordifolia* extracts displayed potential activity against bacterial pathogens.

Some of the extracts had 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 high activity against other Gram–negative bacteria compared to Gram-positive bacteria. This may mean that the activity is not related to the differences in cell wall structure. Because there is such a wide range of MICs for different strains of the same bacterial species (Elisha *et al.*, 2017).

Plate 5 : Antibacterial activity of *Sida acuta* against *Escherichia coli*

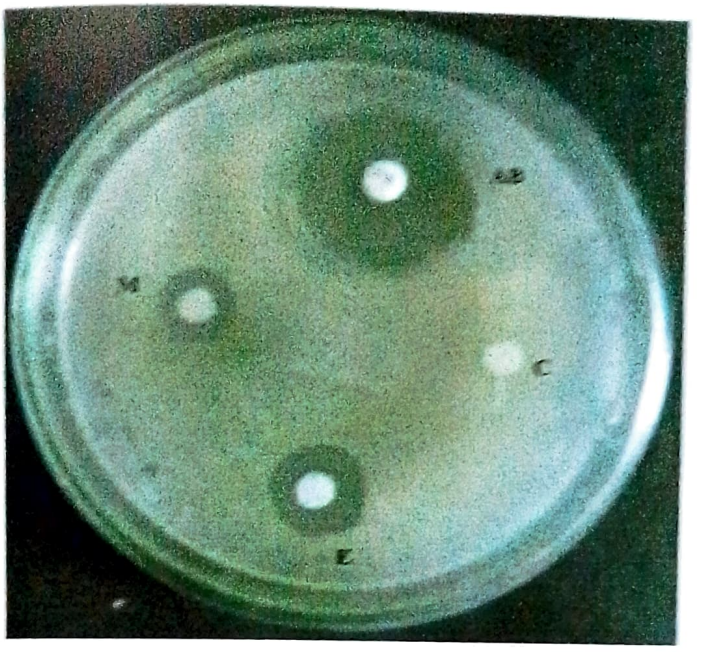


Plate 6 : Antibacterial activity of *Sida acuta* against *Bacillus subtilis*

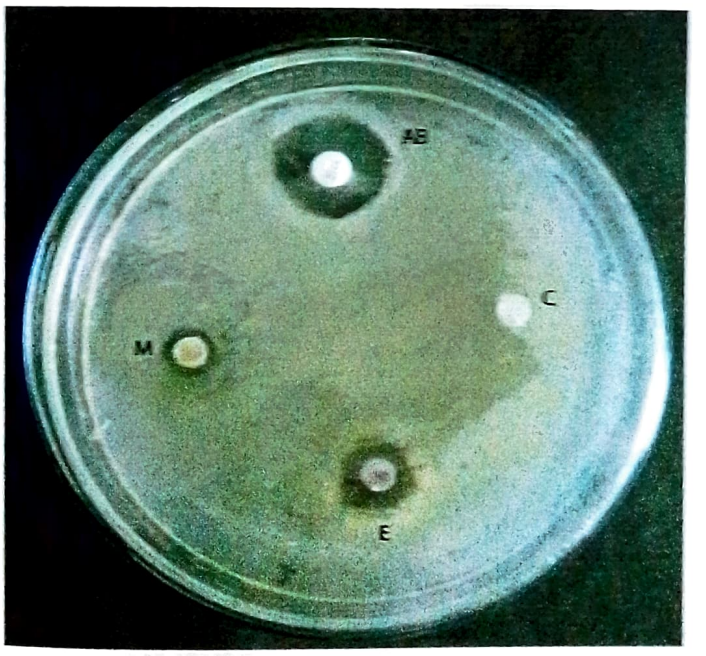


Plate 7: **Antibacterial activity of *Sida cordifolia* against *Escherichia coli***

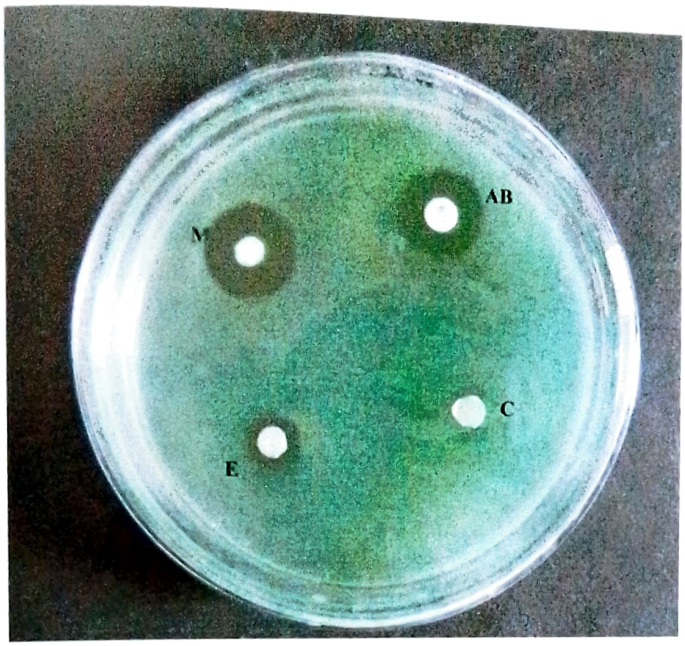
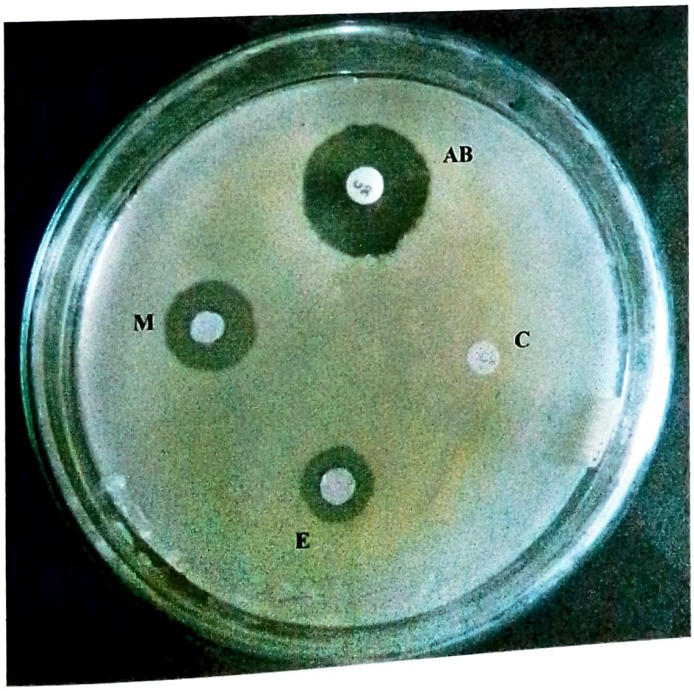


Plate 8 : Antibacterial activity of *Sida cordifolia* against *Bacillus subtilis*



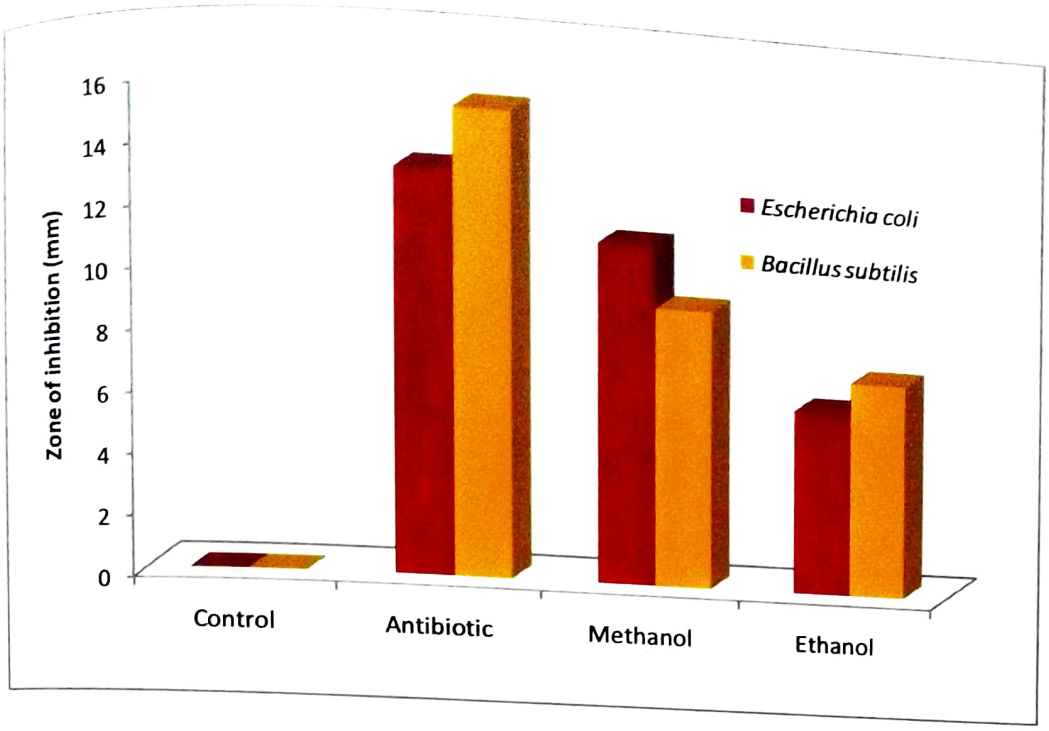


Fig. 1 : Antibacterial activity of *Sida acuta*

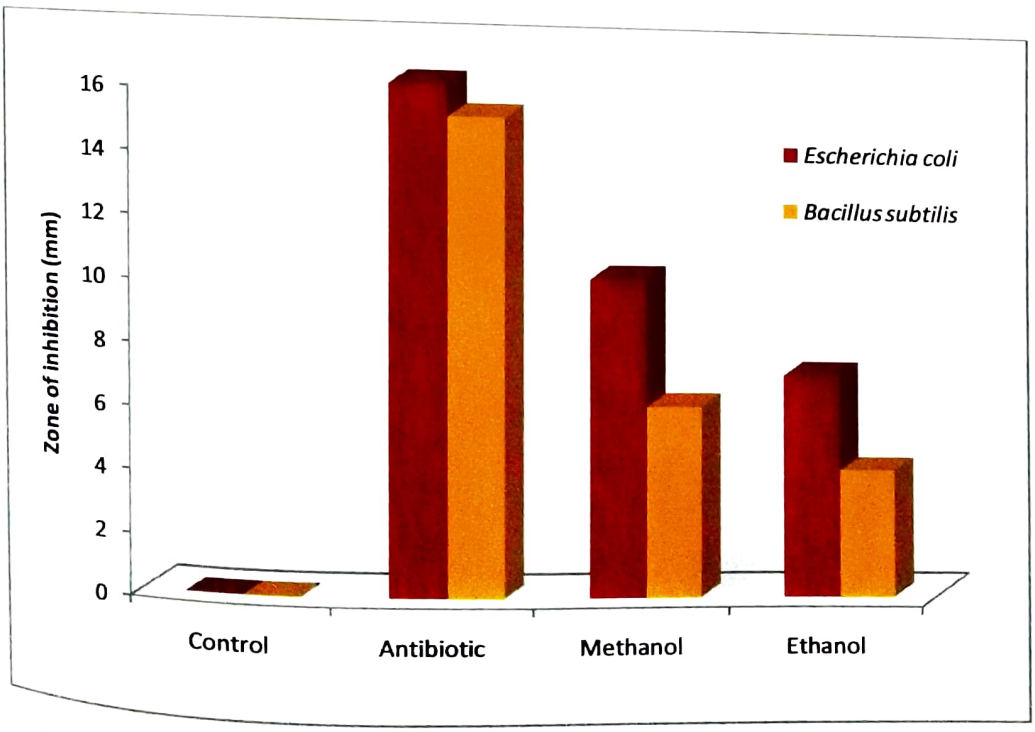


Fig. 2 : Antibacterial activity of *Sida cordifolia*

CHAPTER-V

SUMMARY AND CONCLUSION

The thesis entitled "Studies on macroscopic, microscopic and antibacterial activity of two species of *Sida*" deals with a systematic evaluation of macroscopic, microscopic and antibacterial activity of leaves of *Sida acuta* and *Sida cordifolia* belongs to family Malvaceae.

The present work is focused on the following aspects of the two selected medicinal plants.

- Macroscopical and microscopical characters of *Sida acuta* and *Sida cordifolia*
- Antibacterial potential of *Sida acuta* and *Sida cordifolia* 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. In pharmacognostical studies the organoleptic characters, macroscopic and microscopic are carried out. Morphological study had provided a characteristic identity of leaf of both taxa.

The various distinguishing features of two selected taxa observed through morpho anatomical study are

- *Sida acuta* differs from *S. cordifolia* by having leaves with acute tip
- The stem is pubescent with simple stellate hairs.

- In root the cortex is narrow, comprising of 3 to 4 layers in *S. acuta* and 5 to 6 in *S. cordifolia*.
- In *S. acuta* the vessels are larger in size and more in number. In *S. cordifolia* the vessels are smaller in size, lesser in number
- Among the two plants, *S. cordifolia* extracts displayed potential activity against bacterial pathogens.

The pharmacognostic standardization of the present study can be used as a standard in future research work to identify the two species of *Sida*. Further phytochemical analysis of these plants will be helpful for elucidation of lead molecules.

REFERENCE

Abdul. Sarker, A.A., Islam, M.D., Saiful, Muniruddin, A. 2010. Cytotoxic and Antimicrobial Activity of the Crude Extract of Abutilon Indicum Muhit Md. *Int. J. Pharm. Phytochem. Res.*, 2(1):1-4.

Akilandeswari, S., Senthamarai, R. and Valarmathi, R. 2010. Screening of gastric antiulcer activity of *Sida acuta* burm. *Int. J. Pharmtech. Res.* 2:1644-1648.

Anonymous, 1988. The Wealth of India. A dictionary of Indian raw material and Industrial products, New Delhi, Publication and Information Directorate CSIR., 323-324.

Arias, C.A. and Murray, B.E. 2008. Emergence and management of drug-resistant entero coccal infections. Expert Review of Anti-infective Therapy, 6(5):637-55

Audu, S.A., Mohammed, I. and Kaita, H.A. 2007. Phytochemical screening of the leaves of Lophira lanceolata (Ochanaceae). *Life Science Journal*, 4(4):75-79.

Burapadaja, S. and Bunchoo A. 1995. Antimicrobial activity of tannins from Terminalia citrine, *Planta Medica*. 61:365-366.

Cutler, D.F., Botha, C.E. and Stevenson, D.W. 2007. Plant Anatomy: *An Applied Approach*, (Blackwell Publishing Ltd., Oxford, U.K.)

Debalke, D., Birhan, M., Kinubeh, A. and Yaye, M. 2018. Assessments of Antibacterial Effects of Aqueous-Ethanolic Extracts of *Sida rhombifolia*'s Aerial Part. *Sci. World J.*, 8429809.

Dey, A., Gorai, P., Mukherjee, A. and Dhan, R. 2017. Ethnobiological treatments of neurological conditions in the Chota Nagpur Plateau, India. *J. Ethnopharmacol.*, 198:33-44.

Dharmesh, K., Golwala, Laxman, D. Patel, Santosh, K., Vaidya, Sunil, B., Bothara, Mani, M. and Patel, P. 2010. Anticonvulsant activity of *Abutilon indicum* leaf. *Inter. J. Pharm. Pharmaceu. Sci.*, (2)1: 251-25.

Dinda, B., Das, N., Dinda, S. and Dinda M. 2015. The genus *Sida* L.—A traditional medicine: Its ethnopharmacological, phytochemical and pharmacological data for commercial exploitation in herbal drugs industry. *J. Ethnopharmacol.*, 176:135-176.

Elisha, I.L., Botha, F.S., McGaw, L.J. and Eloff, J.N. 2017. The antibacterial activity of extracts of nine plant species with good activity against *Escherichia coli* against five other bacteria and cytotoxicity of extracts. *BMC Complement Altern Med.*, 17:133.

Endress, P.K., Baas, P. and Gregory, M. 2000. Systematic plant morphology and anatomy-50 years of progress. *Taxon.* 49:401-434.

Esau, K. 1964. Plant anatomy. John Wiley and sons, New York.

Farnsworth, N.R. 1990. Ciba Foundation Symposium 154-Bioactive Compounds from Plants. John Wiley & Sons, Ltd.; Chichester, UK. *The role of ethnopharmacology in drug development,* 2-21.

Gairola, S., Sharma, J., Gaur, R.D., Siddiqi, T.O., Painuli, R.M. 2013. Plants used for treatment of dysentery and diarrhoea by the Bhoxa community of district Dehradun, Uttarakhand, India. *J. Ethnopharmacol.* 150:989-1006.

Garcia, T.B., Potiguara, R.C.V., Kikuchi, T.Y.S., Demarco, D. and Aguiar-Dias, A.C.A. 2014. Leaf Anatomical Features of three Theobroma Species (Malvaceae s.l.) native to the Brazilian Amazon. *Acta amazonica*, 44(3):291-300.

Hammer, K.A., Carson, C.F. and Riley T.V. 1999. Antimicrobial activity of essential oils and other plant extract. *J. App. Microbio.*, 86: 985-990.

Ignacimuthu, S. and Ayyanar, M. 2006. Ethnobotanical investigations among tribes in Madurai district of Tamil Nadu (India). *J. Ethnobiol. Ethnomed.*, 2:25.

Jacob, A.G. Tijjani, A. and Imam, I.I. 2018. Antibacterial studies of the leaves extracts of *Sida corymbosa* (Malvaceae), *FUDMA Journal of Sciences (FJS)*., 2(2):169-175.

Jain, S.K. and Rao, R. 1977. *A hand book of field and herbarium methods*. Today and Tomorrow, New Delhi.

Jones, J.H. 1986. Evolution of the Fagaceae: the implications of foliar features. *Ann. Missouri Bot. Garden.*, 73:228-275.

Júnior, C., Selene, M., Morais, Clécio. G., Martins, Larissa, G., Vieira, Flaviana. M.B., Morais-Braga, Joara, N.P., Carneiro, Antonio, J.P., Machado, Irwin. R.A., Menezes, Saulo, R., Tintino and Henrique D.M. 2015. Phytochemical Analysis and Modulation of Antibiotic Activity by *Luehea paniculata* Mart. & Zucc. (Malvaceae) in Multiresistant Clinical Isolates of *Candida* Spp. *Bio. Med. Res. Int.*, Article ID 807670.

Kanth, V.R. and Diwan, P.V. 1999. Analgesic, antiinflammatory and hypoglycaemic activities of Sida cordifolia. *Phytother Res.*, 13(1):75-7.

Karthikeyan, R., P., and Chandrasekhar, N. 2012. Morpho anatomical studies of leaves of *Abutilon indicum* (linn.) sweet. *Asi. Paci. J. Trop. Biomed.*, 464-469.

Khandelwal, K.R. 2003. Practical pharmacognosy. 18th ed. Pune. Nirali publication, 10-14.

Kirtikar, K.R. and Basu, B.S. 1984. Indian Medicinal Plants. 2ndEdition. Revised by Blatter E., Caius J.F. and Mahaskar K.S. Published by Mohan Basu Lalit, Allhabhad. 1:308.

Koday, N.K., Rangaiah, G.S., Bobbarala, V. and Cherukuri, S. 2010. Bactericidal activities of different medicinal plants extracts against ocular pathogen viz Corynebacterium macginleyi. *Drug Invent Today*, 2(1):5.

Kumar, A.S., Venkateshwaran, K., Vanitha, J., Saravanan, V.S., Ganesh, M., Vasudevan, M. and Sivakumar, T. 2009. Synergistic activity of methanolic extract of *Thespesia populnea* (Malvaceae) flowers with oxytetracycline. *Bangladesh J Pharmacol.*, 4: 13-16.

Ltahir, M.E, Kamali, H. and Ahlam Sali, S. 2017. Comparative morph-anatomical studies on selected Sudanese medicinal plants: part iv. abutilon figarianum and abutilon pannosum leaves. *Int. J. Sci. World*, 5(2):168-171.

Martino, P.D., Gagniere, H., Berry, H. and Bret, L. 2002. Effective antimicrobials have been developed over the past years, several reports development of antibiotic resistance of human pathogens to available antibiotics. *Microbes and infection*. 613- 620.

Medeiros, I.A., Santos, M.R., Nascimento, N.M. and Duarte, J.C. 2006. Cardiovascular effects of *Sida cordifolia* leaves extract in rats. *Fitoterapia*. 77(1):19-27.

Modak, B., Gorai, P., Dhan, R. and Mukherjee, A. 2015. Tradition in treating taboo: Folkloric medicinal wisdom of the aboriginals of Purulia district, West Bengal, India against sexual, gynaecological and related disorders. *J. Ethnopharmacol.* 169:370-386.

Muauza, D.N., Kim, B.W., Euler, K.I. and Williams, L. 1994. Pharmacognostic studies of *Sida cordifolia*. *Int. J. Pharmacog.*, 32:334.

Naskar, S. 2016. 7 Anatomical studies of some common members of malvaceae s.s. from West Bengal. *Ind. J. Plant Sci.*, 5(1):1.

Navas, Mytheenkunju, Dan, Mathew, Latha and Panikamparambil Gopalakrishnan. 2013. Comparative root anatomy of the species under *Sida rhombifolia* complex (Malvaceae). *Pharmacog. J.*, 5(6):269-274.

Nurhanim, M.N. Talip, N., Chung, R.C.K., Nurul-Aini, C.A.C. and Ruzi, A.R. 2014. Taxonomic value of leaf anatomical characterisctics in Schoutenia Korth. (Malvaceae subfam. Brownlowioideae). *Sains Malaysiana*. 43(3):331-338.

Oboh, I.E., Akerele, J.O. and Obasuyi, O. 2007. Antimicrobial activity of the ethanol extract of the aerial parts of *sida acuta* burm.f. (Malvaceae). *Trop. J. Pharma. Res.*, 6 (4):809-813

Olatunji, O.A. and Bakare, O.A. 1993. Folial anatomy of the species *Sida* L. (Malvaceae) in Nigeria. 104:27-34.

Ozkan, A.M.G. and Uzunhisarcikli, E.M. 2009. Stem and Leaf Anatomy of Althaea L. (Malvaceae) Species Growing in Turkey. *Journal of the Faculty of Pharmacy*, 28(2):133-148.

Park, K.W. 1994. A taxonomy study of the Magnoliaceae [J]. *Res. For. Res. Inst.*, 50:173-190.

Patwardhan, B. 2005. Ethnopharmacology and drug discovery. *J. Ethnopharmacol.*, 100:50-52.

Ponnudurai, K., Prabhu, K., Jebasingh, D. and Prabu, D. 2011. Evaluation of anti-ulcer activity of ethanolic extract of Abutilon Indicum (Linn.) sweet leaves. *Der Pharmacia Sinica.*, 2(4): 148-158

Prabahar, A.E., Thangabalan, B., Chavala, A., Kumar, S.S., Kathiravan, M. and Karthikeyan, R. 2009. Antibacterial activity of various extracts of Abutilon indicum (L.) Sweet leaves. *J Pharm. Res.*, 2(8):1324-1325.

Puri, H.S.. 2003. 'Rasayana'-Ayurvedic Herbs for Longevity and Rejuvenation. *London: Taylor and Francis.*, 8:64-70.

Ruban, P. and Gajalakshmi, K. 2012. *In vitro* antibacterial activity of *Hibiscus rosa-sinensis* flower extract against human pathogens. *Asian Pac J Trop Biomed.*, 2(5):399-403.

Shaheen, N., Khan, M.A., Yasmin, G., Ahmad, M., Mahmood, T., Hayat, M.Q. and Zafar, M. 2009. Foliar epidermal anatomy and its systematic implication within the genus *Sida* L. (malvaceae). *Afri. J. Biotech.*, 8(20):5328-5336.

Shaik, D., Malika, F.A., Rafi, S.M. and Naqui, B. 1994. Studies of antibacterial activity of ethanolic extract from *Nerium indicum* and *Hibiscus rosa sinensis*. *J Islamic Acad Sci.*,7:167-168.

Sharma, A.K., 2013. Medicinal Properties of Bala (*Sida Cordifolia* Linn. and its Species). *Int. J. Ayur. Pharma Research,* 1(2): 1-9.

Sharma, R. and Patki, P. 2010. Double-blind, placebo-controlled clinical evaluation of an Ayurvedic formulation (Glucocare capsules) in non-insulin dependent diabetes mellitus. *J. Ayurveda Integr.*, 1:45-51.

Shivanna, M. and Rajakumar, N. 2010. Ethno-medico-botanical knowledge of rural folk in Bhadravathi taluk of Shimoga district, Karnataka. *Indian J. Nat. Prod. Resour.*,1:102-108.

Sowmya1, T., Mounika1, G., Chowdary, C., Ch., Reddy, G.T., Unissa, R. and Dhanraj, P. 2018. Antimicrobial Activity of Hibiscus sabdariffa against Ocular Pathogens. *The Saudi J. Life Sci.*, 3(6):489-492.

Stary, F. and Hans, S.1998.The National guides to medical herbs and plants Tiger Books, Int. Plc. UK.

Steven, L.P., Louise, S., Claudia, V. and Gianfranco, D. 2015. Healthcare-associated infections, medicaldevices and biofilms: risk, tolerance and control. *J. Med. Microbio.*, 64:323-334.

Venkatesan, D., Karan, K. and Kumar, S.S. 2009. Studies on phytochemical constituents, functional group identification and antimicrobial activity of *Solanum nigrum. Ethnobotanical Leaflet,* 13:1485-1503

Woldeyes, S., Adane, L., Tariku, Y., Muleta, D. and Begashaw, T. 2012. Evaluation of Antibacterial Activities of Compounds Isolated From *Sida rhombifolia* Linn. (Malvaceae). *Nat. Prod. Chem. Res.* 1:101.

Green synthesis of silver nanoparticles from some weeds of St. Mary's College (Autonomous), Thoothukudi, Tamilnadu, India.

A Short – term project submitted to

ST.MARY'S COLLEGE (AUTONOMOUS)

Affiliated to

MANONMANIAM SUNDARANAR UNIVERSITY

in partial fulfilment of the requirements for the degree of

BACHELOR OF SCIENCE IN BOTANY

By

R. IMMACULATE	**- 16AUBO15**
D. JEEVA SATHYA PITHA	**-16AUBO16**
A. JOSPHINE NIVETHA	**-16AUBO17**
P. JOTHILAKSHMI	**-16AUBO18**
M. KARTHIKA	**-16AUBO19**
A. KILPIYA	**-16AUBO20**

DEPARTMENT OF BOTANY

ST.MARY'S COLLEGE (AUTONOMOUS)

THOOTHUKUDI - 628 001.

APRIL -2018-2019

CERTIFICATE

It is certified that this short term project work entitled **"Green synthesis of silver nanoparticles from some weeds of St. Mary's College (Autonomous), Thoothukudi, Tamilnadu, India."** submitted to **St. Mary's College (Autonomous)** affiliated to **Manonmaniam Sundaranar University** in partial fulfilment of the requirements for the degree of Bachelor 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 2018-2019 by the following students.

R. IMMACULATE	**- 16AUBO15**
D. JEEVA SATHYA PITHA	**-16AUBO16**
A. JOSPHINE NIVETHA	**-16AUBO17**
P. JOTHILAKSHMI	**-16AUBO18**
M. KARTHIKA	**-16AUBO19**
A. KILPIYA	**-16AUBO20**

24/3/2019

GUIDE

29/3/19

HEAD OF THE DEPARTMENT

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

5/4/19

EXAMINAR

Lucia Rose

PRINCIPAL

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

ACKNOWLEDEGEMENT

We offer our praise and sincere thanks to the **Almighty God**, for his avalanche of graces and bountiest blessings, enabling us to complete this project.

We consider it a privilege to express our gratitude to **Dr. Sr. A. S. J. Lucia Rose M.Sc., M. Phil., PGDCA., 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. Mrs. M. Glory, M.Sc., M. Phil., Ph. D.,** Head of the Department of Botany, for her constant encouragement and support.

We wish to express my deep sense of gratitude to **Dr. G. Flora, M.Sc., M. Phil., Ph. D.,** Assistant Professor in Botany for suggesting this research problem, her constant encouragement and inspiring guidance throughout this project work.

CONTENTS

INTRODUCTION

Introduction

The rapid expanding of works carrying the theme of nanomaterials in the past several years is widely evidenced in the literature (Syafiuddin *et. al.*, (2017)). Since it is well-recognized that nanomaterials possess extraordinary features in terms of physical, chemical and biological compared to their larger form, they have been hugely explored for characterization and application. Up to the present time, nanoparticle production remains as one of the central issues in the nanomaterial research. To name a few, widely examined nanoparticle types include iron, copper, silicon, gold and silver (Desireddy (2013), Huang (2015), Fu, (2013), Kreyling (2015), Zanganeh (2015). Among these, silver nanoparticles (AgNPs) have been extensively investigated because of their appealing properties (Syafiuddin *et. al.*, (2017)). Therefore, AgNPs have been utilized for various applications such as electronics, sensors and recently widely explored for medical devices. The exploration of AgNPs for medical fields particularly as an antibacterial agent has been extensively carried out. To explore their application, the antibacterial capability of AgNPs against Nanomaterials several bacteria has been ratified (Abalkhil *et. al.*, (2017)). The role of the antibacterial capability of AgNPs in terms of the surface science has been well reviewed by Le Ouay and Stellacci Trujillo *et. al.*, (1986). The specific effect of the nanoparticulate objects on these nanoparticles such as adsorption at bacterial surfaces can significantly improve their antibacterial capability. In addition, the role of Ag+ release has been found possible. In general, the antibacterial capability of AgNPs is crucially affected by their surface physical and chemical properties as well as bacteria types. Therefore, investigation for effects on new potential bacteria is urgently needed. Since the physical and chemical approaches were ratified to have numerous constraints in terms of time consumption, cost and

Synthesis methods for AgNPs production by means of roots, seeds, fruits and leaves are well-studied. Accessibility of roots and seeds is relatively more difficult compared to leaves and fruits. Fruits and leaves are still recognized as the vitamin and vegetable sources for developing countries including Indonesia and Malaysia. To overcome these matters, an alternative natural resource that is abundantly available is urgently needed. In this respect, the application of weeds seems strategic for the explorative purpose. In addition, a huge usage of weeds may also support the enhancement of the environmental quality since they are well-known as plants with a negative impact on agriculture particularly by monopolizing resources. The intensive use of the plant extracts of weeds is due to their internal biomolecule such as protein, terpenoids and flavonoids, which offer potential as a bioreductant to reduce metal ions to form AgNPs. (Dher (2017), Catteau (2013). Therefore the present work aims to synthesize and characterize silver nanoparticles from some weeds collected from St. Mary's College campus.

SCOPE AND OBJECTIVE

Scope and Objectives

The nanoparticle synthesis is one of the important areas of modern nanotechnology. The current research is aimed for synthesize of silver nanoparticles in different weeds (*Boerhaavia diffusa*, *Corchorus tridens*, *Gomphrena procumbens*, *Hybanthus enneaspermus*, *Indigofera linnaei* and *Merremia tridentata*) collected from St. Mary's College (Autonomous) Campus and Characterization of the biosynthesized silver nanoparticles by UV-Vis spectrophotometer.

LITERATURE REVEW

Literature Review

The word "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 Nanobiotechnology represents and economic alternative for chemical and physical methods 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 the zinc oxide) there is growing interest in inorganic nanoparticles i.e of noble metal nanoparticles as they provide superior material properties with functional 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.

Silver nano particle

The use of Silver nanoparticles dates back to the 16th Century, for both medical and staining purposes. There is growing need to develop environmentally friendly processes through green synthesis and other biological approaches.

In nanotechnology, nanoparticles synthesis 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, silver nanoparticals have a great bactericidal effect on a several range of micro organisms, its bactericidal effect depends on the size and shape of the particle. Recently there are, reports that algae are being used as a biofactory for synthesis of metallic nanoparticles.

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

Production of silver nanoparticles from plant extract and 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 provides vital information in terms of application of these nanoparticles. For instance, it provides answers to know wherter 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 method suited to probe nanomaterials is , however,a continuous process (Poole and Owens, 2003).

Torresday *et. al.*, (2002) reported that the preparation and study of quantum dots and quantum wires play a very important role in nanotechnology. In this particular study, the report on the uptake of silver by living alfalfa plants. X-ray absorption spectroscopy and Transmission Electron Microscopy (TEM) studies corroborated silver metal uptake by Alfalfa plants from a silver-rich solid medium and the subsequent formation of silver nanoparticles. Silver nanoparticle alignment, structure, and coalescence were observed using TEM with anatomic resolution analysis. Dark field image TEM showed the connection of silver nanoparticles of different sizes by possibly nanocrystalline silver atomic wires.

Nagendragandhi *et. al.*, (2013) reported that the green synthesis of silver nanoparticles. The bioreduction performance of different plant leaf remove such as *Helianthus annus* (Asteraceae), Sorghum bicolour, Basella alba (Basellaceae) in the synthesis of silver nanoparticles were examined utilizing UV-Vis, XRD and SEM, Helianthus annus was found to exhibit strong potenion for rapid reduction of silver ions it was observed that there is no association forever between the colour growth and the arangment in absorbance displayed by the nanomaterial synthesized.

Shanmugavadiru *et. al.*, (2014) reported that the synthesis of first extract mediated silver nanoparticles and its antibacterial activity nanoparticles were characterized using UV-Vis absorption spectroscopy, FTIR, XRD and SEM, XRD and SEM analysis showed the average particle size of 15nm as well as revealed their cubic structure. Further these biologically synthesis nanoparticles were found to be highly toxic against different multi drug resistant pathogens (E. coli and Pseudomonas aeruginosa). This is for the first time that any plant fruit extract was used for the synthesis of silver nanoparticle.

Mohamed *et. al.*, (2014) reported that the antioxidant and antibacterial activity of silver nanoparticles biosynthesized using Chemopodium murale leaf extract. The current study revealed that silver nanoparticles can be synthesized in a simple method using C.murale leaf extract. The TEM analysis showed that the size of the synthesized silver nanoparticle ranged from 30 to 50nm. The essential oil of plant-silver nanoparticle was formed mainly of X-Terpinene, *Ascaridole* and *cis-Ascaridole*. The total phenolic compounds and total flavonoids were higher in plant – silver nanoparticle compared to the plant extract alone. Peanut – silver nanoparticle showed on higher antioxidant and antimicrobial activity compared to C. murale leaf extract alone or silver nitrate.

Krishnaraj *et. al.*, (2010) reported that the biosynthesis of silver nanoparticles and its activity on water borne bacterial pathogens were investigated. Silver nanoparticles were rapidly synthesized using leaf extract of *Acalypha indica* and the formation of nanoparticles was observed within 30 min. The results recorded from UV–vis spectrum, scanning electron microscopy (SEM), X-ray diffraction (XRD) and energy dispersivespectroscopy (EDS) support the biosynthesis and characterization of silver nanoparticles. From high resolution transmission electron microscopy (HRTEM) analysis, the size of the silver nanoparticles was measured 20–30 nm. Further, the antibacterial activity of synthesized silver nanoparticles showed effective inhibitory activity against water borne pathogens Viz, Escherichia coli and Vibrio cholerae. Silver nanoparticles 10g/ml were recorded as the minimal inhibitory concentration (MIC) against E. coli and V. cholerae. Alteration in membrane permeability and respiration of the silver nanoparticle treated bacterial cells were evident from the activity of silver nanoparticles.

Virender *et. al.*, (2009) reported that the silver nanoparticles ($AgNP_s$) preparation by green synthesis approaches that have advantages over conventional methods involving

mixed-valence polyoxometallates, polysaccharide, Tollens, irradiation, and biol

mixed-valence polyoxometallates method was carried out in water, an enviro

friendly solvent. Solutions of $AgNO_3$ containing glucose and starch in water g

protected AgNPs, which could be integrated into medical applications. Tollen

involves the reduction of $Ag(NH3)^{2}$ + by saccharides forming AgNP films with parti

from 50–200 nm. Ag hydrosols with particles in the order of 20–50 nm, and Ag

particles of different shapes. The reduction of $Ag(NH3)^{2}$ + by HTAB (n-hexade

ethylammonium bromide) gave AgNPs of different morphologies: cubes, triangles,

and aligned wires. AgNPs synthesis by irradiation of Ag+ ions does not involve a red

agent and is an appealing procedure. Eco-friendly bio-organisms in plant extracts co

proteins, which act as both reducing and capping agents forming stable and shape-contro

AgNPs. The synthetic procedures of polymer-Ag and TiO2–AgNPs are also given. Both

NPs and AgNPs modified by surfactants or polymers showed high antimicrobial activi

against Gram-positive and Gram-negative bacteria. The mechanism of the Ag NP bactericid

activity is discussed in terms of AgNP interaction with the cell membranes of bacteria

Silver-containing filters are shown to have antibacterial properties in water and air

purification. Finally, human and environmental implications of AgNPs to the ecology of

aquatic environment are briefly discussed.

Priya Banerjee *et. al.*, (2014) reported that the Green synthesis of silver

nanoparticles (AgNPs) has gained much interest from chemists and researchers. In this

concern, Indian flora has yet to divulge innumerable sources of cost-effective non-hazardous

reducing and stabilizing compounds utilized in preparing AgNPs. This

efficient and

mixed-valence polyoxometallates, polysaccharide, Tollens, irradiation, and biological. The mixed-valence polyoxometallates method was carried out in water, an environmentally-friendly solvent. Solutions of $AgNO_3$ containing glucose and starch in water gave starch protected AgNPs, which could be integrated into medical applications. Tollens process involves the reduction of $Ag(NH3)^2$ + by saccharides forming AgNP films with particle sizes from 50–200 nm, Ag hydrosols with particles in the order of 20–50 nm, and Ag colloid particles of different shapes. The reduction of $Ag(NH3)^2$ + by HTAB (n-hexadecyltrim ethylammonium bromide) gave AgNPs of different morphologies: cubes, triangles, wires, and aligned wires. AgNPs synthesis by irradiation of Ag+ ions does not involve a reducing agent and is an appealing procedure. Eco-friendly bio-organisms in plant extracts contain proteins, which act as both reducing and capping agents forming stable and shape-controlled AgNPs. The synthetic procedures of polymer-Ag and TiO2–AgNPs are also given. Both Ag NPs and AgNPs modified by surfactants or polymers showed high antimicrobial activity against Gram-positive and Gram-negative bacteria. The mechanism of the Ag NP bactericidal activity is discussed in terms of AgNP interaction with the cell membranes of bacteria. Silver-containing filters are shown to have antibacterial properties in water and air purification. Finally, human and environmental implications of AgNPs to the ecology of aquatic environment are briefly discussed.

Priya Banerjee *et. al.,* (2014) reported that the Green synthesis of silver nanoparticles (AgNPs) has gained much interest from chemists and researchers. In this concern, Indian flora has yet to divulge innumerable sources of cost-effective non-hazardous reducing and stabilizing compounds utilized in preparing AgNPs. This study investigates an efficient and sustainable route of AgNP preparation from 1 mM aqueous $AgNO_3$ using leaf extracts of three plants, *Musa balbisiana* (banana), *Azadirachta indica* (neem) and *Ocimum tenuiflorum* (black tulsi)

Mody *et. al.*, reported that bio-molecules from various plant components and microbial species have been used as potential agents for the synthesis of silver nanoparticles (AgNPs). In spite of a wide range of bio-molecules assisting in the process, synthesizing stable and widely applicable AgNPs by many researchers still poses a considerable challenge to the researchers. The biological agents for synthesizing AgNPs cover compounds produced naturally in microbes and plants. More than 100 different biological sources for synthesizing AgNPs are reported in the past decade by various authors. Reaction parameters under which the AgNPs were being synthesized hold prominent impact on their size, shape and application. Available published information on AgNPs synthesis, effects of various parameters, characterization techniques, properties and their application are summarized and critically discussed in this review.

Mohammed Rafi Shaik *et. al.*, (2018) reported that the plant-mediated green synthesis of nanomaterials has been increasingly gaining popularity due to its eco-friendly nature and cost-effectiveness. In the present study, we synthesized silver nanoparticles (AgNPs) by using an aqueous solution of Saudi *Origanum vulgare* L. plant extract as a bioreducing agent. The as-synthesized AgNPs were characterized using various microscopic and spectroscopic techniques. The results indicated the formation of crystalline face-centered cubic (fcc) AgNPs. Additionally, FT-IR study confirmed that the O. vulgare L. extract not only functioned as a bioreductant but also stabilized the surface of the AgNPs by acting as a capping agent. Moreover, the effect of the amount of the plant extract on the size and the antimicrobial activity of the NPs was also assessed. It was found that with increasing amounts of plant extract, the size of the NPs was decreased. Moreover, as-synthesized AgNPs as well as *O. vulgare* L. plant extract were separately tested to examine their antimicrobial activities. The activities were tested against various bacterial and fungal microorganisms including *Shigella sonnei*, *Micrococcus luteus*, *Escherichia coli*, *Aspergillus flavus*,

Alternaria alternate, *Paecilomyces variotii*, *Phialophora alba*, and so on. These results evidently show that the inclusion of *O. vulgare* L. extracts improves the solubility of AgNPs, which led to a significant enhancement in the toxicity of the NPs against the assessed microorganisms.

Ana-Alexandra Sorescu., *et. al.*,(2016) reported that the nanoparticles, compared to bulk materials, exhibit improved characteristics due to their size, distribution and morphology and are widely used in numerous scientific fields. Among metallic nanoparticles, silver nanoparticles (AgNPs) are very important especially due to their physiochemical and antimicrobial properties which help in therapies, molecular diagnostics and in devices used for medical procedures. A major drawback of the chemical synthesis is that it involves the use of hazardous chemicals and toxic by-products are obtained. Therefore, there is a constant need for economic and eco-friendly methods to synthesize them and the use of aqueous or alcoholic plant extracts is rapidly expanding and gaining importance. Numerous plant extracts are used for the green synthesis of AgNPs and ten of them are presented in this article: *Abutilon Indicum*, *Bergenia Ciliata*, *Clitoria Ternatea*, *Cochlospermum Religiosum*, *Dianthus Caryophyllus*, *Garcinia Mangostana*, *Hyacinthus Orientalis*, *Pinus Eldarica*, *Rumex Hymenosepalus* and *Saraca Indica*. This review provides a useful and comprehensive presentation regarding the synthesis of silver nanoparticles using these plant extracts, describing their main physical-chemical properties and some of their medical application

Arpita Roy (2017) reported that the nano-biotechnology holds a great potential in various fields of life sciences. Nanotechnology involves the use of materials with components that have dimensions less than 100nm. The demand for green synthesis of nanoparticles increases day by day due to the drawbacks of chemical synthesis. Application of materials is an emerging area of nanotechnology. Among various metal nanoparticles,

silver nanoparticles gain special interest due to its remarkable properties. Silver nanoparticles are usually ranging from 1-1000nm size. They have unique electrical, optical and thermal properties and which can be incorporated into industrial. Application of electronics catalysis and photonics. Silver nanoparticles exhibit a broad spectrum of anti-bacterial and anti-fungicial acitivities malving them extremely popular in a diverse range of consumer products, including plastics, soaps, pastes, food. And textiles thus increasing their market value. The presence of soluble organics in the plant extracts was mainly responsible for the silver ions reduction to nano-sized silve particles. The present review provides information on silver nanoparticles synthesis from various medicinal plants various methods of characterization and its biological application.

Pratima Chauhan *et. al.*, (2017) reported that the silver nanoparticles are being used in numerous technologies and incorporated into a wide array of consumer products that take advantage of their desirable optical, conductive, and antibacterial properties. Silver nanoparticles have attained a special focus due to its antimicrobial property Conventionally silver nanoparticles are synthesized by chemical method using chemicals as reducing agents which later on become accountable for various biological risks due to their general toxicity, engendering the serious concern to develop environment friendly processes Thus, to solve the objective, principles of green chemistry have now become a torch for chemical technologist, biotechnologist and nanotechnologist worldwide in developing less hazardous chemicals. The present review explores the synthesis of silver nanoparticles through a natural and single step protocol preparatory method using the different plant products of different texa belonging to different families with green principles over the conventional ones.

Abhishek Mathur *et. al.*, 2018 reported that the research in the field of metal nanoparticles has become an important branch of nanotechnology and

there is an increasing commercial demand for nanoparticles due to their wide applications. In the present study, we report an eco-friendly and economical way for the synthesis of silver nanoparticles using leaf extract *Azadirachta indica*. The plant properly known as 'neem' belongs to the family Meliaceae. For the synthesis of silver nanoparticles (SNPs) using the leaf extract of *Azadirachta indica* as a reducing agent from 1 mM silver nitrate ($AgNO_3$) has been investigated. The resulting SNPs are characterized using UV–Vis, TEM. Silver nanoparticles were synthesized within 24 hours of incubation period and synthesized SNPs showed an absorption peak at around 400 nm in the UV-visible spectrum. The morphological study of Silver nanoparticles using TEM suggests that the nanoparticles are spherical in shape with a diameter around 50-nm. This route is rapid, simple without any hazardous chemicals as reducing or stabilizing agents and economical to synthesized SNPs.

Jae Yong Song *et. al.*, (2008) reported that the five plant leaf extracts (*Pine*, *Persimmon*, *Ginkgo*, *Magnolia* and *Platanus*) were used and compared for their extracellular synthesis of metallic silver nanoparticles. Stable silver nanoparticles were formed by treating aqueous solution of $AgNO_3$ with the plant leaf extracts as reducing agent of Ag+ to Ag0. UV-visible spectroscopy was used to monitor the quantitative formation of silver nanoparticles. Magnolia leaf broth was the best reducing agent in terms of synthesis rate and conversion to silver nanoparticles. Only 11 min was required for more than 90% conversion at the reaction temperature of 95^0 C using Magnolia leaf broth. The synthesized silver nanoparticles were characterized with inductively coupled plasma spectrometry (ICP), energy dispersive X-ray spectroscopy (EDS), scanning electron microscopy (SEM), transmission electron microscopy (TEM), and particle analyzer. The average particle size ranged from 15 to 500 nm. The particle size could be controlled by changing the reaction temperature, leaf broth concentration and $AgNO_3$ concentration. This environmentally friendly method of biological silver nanoparticles production provides rates of synthesis faster or comparable to those of

chemical methods and can potentially be used in various human contacting areas such as cosmetics, foods and medical applications.

MATERIALS AND METHODS

Materials and Methods

Systematic position

Class	Dicotyledon
Order	Malvales
Family	Teliaceae
Common name	Horn fruited jute
Botanical name	*Corchorus tridens* L.

Description;

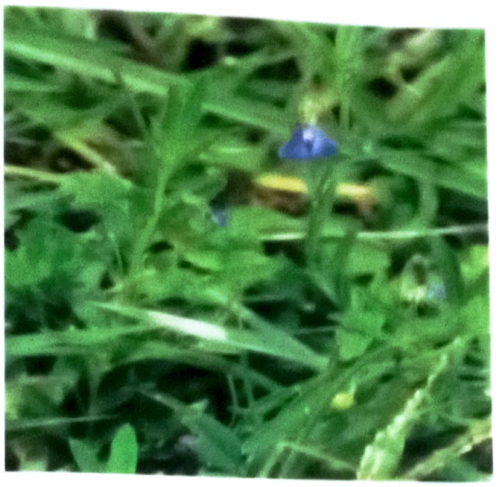
The plants are tall, usually annual herbs, reaching a height of 2–4 m, unbranched or with only a few side branches. The leaves are alternate, simple, lanceolate, 5–15 cm long, with an acuminate tip and a finely serrated or lobed margin. The flowers are small (2–3 cm diameter) and yellow, with five petals; the fruit is a many-seeded capsule.

Systematic position

Class	Dicotyledons
Order	Solanales
Family	Convolvulaceae
Common name	Arrow leaf morning glory
Botanical name	*Merremia tridentata* (L.) Hallier f

Description

A slender, perennial, prostrate herb with angular stems. Leaves are various in shape and size, but deeply emarginated and 3-toothed at the apex, cordate at base, the basal lobes clasping the stem, petioles very short . Inflorescence is axillary solitary. Calyx infundibuliform, outer sepals shorter than inner. Corolla pale yellow. Pale brown, glabrous, seeds trigonous, glabrous. Flowering and Fruiting Time is During monsoon season. The roots and seeds are used with the medicines which is given in the treatment of rheumatism. Leaves are applied on wounds and the whole plant is boiled in oil and the oil is applied on blotches.



Systematic position

Class	Dicotyledons
Order	Malpighiales
Family	Violaceae
Commen name	Spade flawer
Botanical name	*Hybanthus enneaspermus* (L.) F.Muell.

Description

Annual herbs to 30 cm high; stem woody at base, scabrous. Leaves subsessile, 0.6-3 x 0.3-1 cm, linear-lanceolate or elliptic-lanceolate, base attenuate, margins distantly crenate, crenations scabrous hairy, apex acute, hirsute; stipules 1-2 mm long, linear-lanceolate. Flowers axillary, solitary; pedicel 0.6-1.5 cm long, slender. Sepals 5, subequal, 2-3 x 1 mm, lanceolate, acute, ciliate. Petals 5, pinkish, unequal; lower one 0.8-1.5 cm long, suborbicular, clawed, other 4 smaller, 3-5 mm long, elliptic or triangular-oblong. Stamens 5, c. 2 mm long. Ovary ovoid, 1-celled; ovules many; style clavate; stigma oblique. Capsules 5-8 mm long, ovoid. Seeds many, ovoid.

Systematic position

Class Dicotyledons

Order Cayophyllales

Family Amaranthaceae

Commen name Globe Amaranth

Botanical name *Gomphrena procumbens* Jacq.

Description

An annual erect herb, much-branched, stems and branches thickened, stout, young parts grooved, geniculate, pubescent and often tinged with red; older parts terete and glabrous, greenish-red or yellowish-brown. Shortly petioled, opposite, oblong or oblong-obovate, acute, subacute or obtuse at apex, subcordate, rounded or tapering at base, entire along the margins, thinly hairy on both surfaces. Small, yellowish-white or purplish, in solitary or terminal or fascicled spicate heads, globose, large peduncled, bracts 2, foliaceous, below the heads. Perianth of 5 sepals, unequal, lanceolate. Stamens 5, filaments united at the base to form a tube, which has 5 teeth, anthers 1 celled. Ovary 1 celled, 1 ovuled, stigma 2, lobes, ovoid. Seeds swollen, reniform.

Systematic position

Class	Dicotyledons
Order	Fabales
Family	Fabaceae
Commen name	true indigo
Botanical name	*Indigofera linnaei* L.

Description

Indigofera linnaei is a spreading, usually prostrate woody herb, 15–50 cm high with a long taproot, which forms a flat mat up to 1.5 m across, and up to 45 cm high. The compound leaves are up to 3 cm long, with (generally) 7 or 9 obovate, alternate leaflets which have a mucronate apex and are about 8–15 mm long and 2–5 mm wide. The stipules are lanceolate (shaped like a lance-head) and about 5 mm long with broad, dry margins. The inflorescences are dense and up to 2 cm long. The calyx is covered with spreading, white hairs. The petals are red. The standard slightly exceeds the calyx, and the wings and keel are shorter. The pod is oblong and silky, about 3–7 mm long, pointed at apex, and usually contains two seeds.

Systematic position

Class	Dicotyledons
Order	Caryophyllales
Family	Nyctaginaceae
Commen name	Punarnava
Botanical name	*Boerhaavia diffusa L. nom. cons*

Description

Boerhavia diffusa is a procumbent to erect, glabrous. Slender, jointed, more or less fleshy and woody below. It is greenish or sometimes purplish, low branching, glabrous and not sticky from glandular hairs. The leaves are borne on stalks of very varied length and are 3-5 cm long × 2-4 cm wide, broadly ovate in cm long × 2-4 cm wide, broadly ovate in shape and blunt at the tip, with a somewhat wavy margin. The under surface of the leaves is paler than the upper. Leafless and the umbels, terminating the branches are mostly 2-4 flowered. Diffuse much-branched, leafless cyme, 2-4 crimson or deep purple flowers, about 3 mm long in terminal clusters and, usually 3 stamens. 1-seeded sticky capsule with ribs, and about 3 mm long.

Collection of plants

Corchorus tridens (leaf and bud) , *Merremia tridentate* (leaf, stem and flower), *Hybanthus enneaspermus* (leaf), *Gomphrena procumbens* (leaf and flower), *Indicofera titingtoria* (leaf and stem) and *Boerhaaria diffusa* (leaf, stem and flower) were collected from Campus of St. Mary's college, Thoothukudi. They were washed, dried and powdered. They were dissolved in (100 ml) of distilled water and ethanol and the extracts formed were filtered through a muslin cloth. Then they filtrate in further experiments.

Biosynthesis of silver nanoparticles

For all experiments, the source of silver will be silver nitrate ($AgNo_3$) in distilled water. Typical reaction mixtures contained 1 ml of plant extract of aqueous and ethanol in 9 ml of silver nitrate solution (1mM).

Characterization of silver nanoparticles

UV- Visible spectroscopy analysis

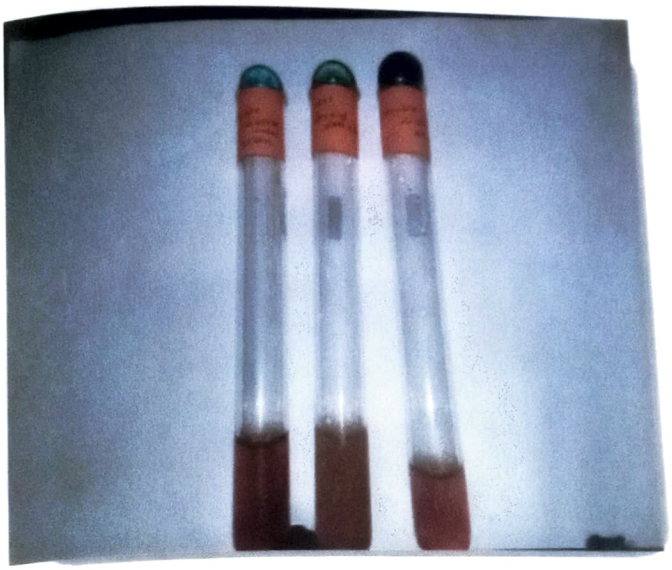
The bioreduction of silver ion in solution was monitored using UV-spectrometer in 300-650 nm length range. It was observed that upon addition of the plant extracts into the test tube containing the aqueous silver nitrate solution, the color of the medium changed to brown within 2 minutes. This indicated the formation of silver nanoparticles.

RESULTS AND DISCUSSION

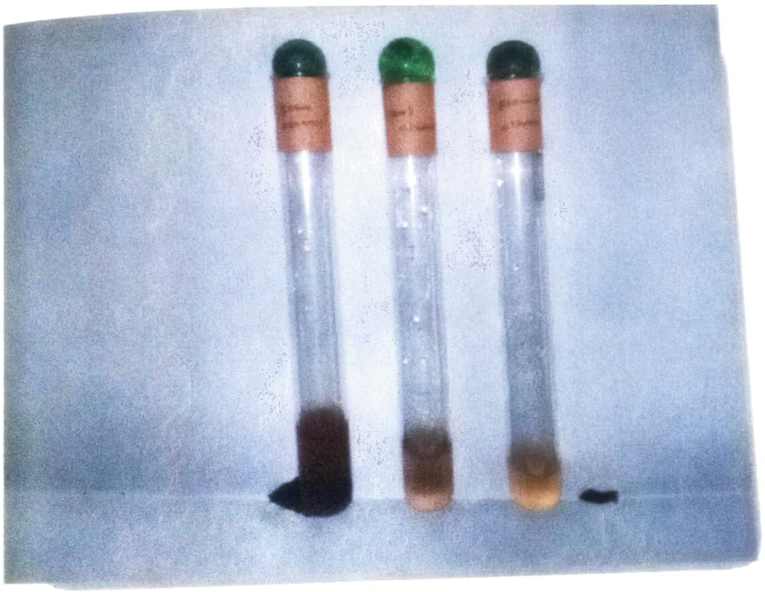
Result and Discussion

Research based on advanced nanomaterials of noble metals like silver has conquered a lot of interest among scientists during the past decades for its physiochemical properties such as size, distribution and morphology, they have been studied for catalytic activity, optical properties, electronic properties, antibacterial properties and magnetic properties (Song (2009), Sontos (2012), Degaetano (2005), Carbtree (2003), Krolikowska (2003)) and its application in various field such as biomaterial production, biochemistry, medical and pharmaceutical products, toothpastes, optical receptors, biosensing, etc. (Awwad (2013), Benerjee (2014), Navaladian (2007), Rajasekharreddy (2010)) Chemical, physical, and biological methods have been developed to synthesis nanoparticles but chemical and physical methods are involved in the production of toxic byproducts which are hazardous moreover the methods are very expensive (Phanjom (2015), Vinod (2011)). To synthesis stable metal nanoparticles with controlled size and shape, there has been search for inexpensive, safe, and reliable and "green" approach. The novel methods so called green/biosynthesis have been recently developed by a variety of plant extract such as *Ocimum Sanctum* (Singhul *et al.*, 2011), *Petroselinum crispum* (Ahmad *et. al.*, (2010). *Murraya koenigii*, *Coriandrum sativum* for the synthesis of metal nanoparticles. Nature has devised various processes for the synthesis of nano and micro length scaled inorganic materials which have contributed in the development of relatively new and largely unexplored area of research based on the biosynthesis of the nanomaterials. Synthesis using bio-organisms is compatible with the green chemistry principles. "Green synthesis" of nanoparticles makes use of environmental friendly, non-toxic and safe reagents. Nanoparticles synthesized using biological techniques or green technologies have diverse natures, with greater stability and appropriate dimensions [illegible] are synthesized using a one-step procedure. Plants provide a better platform for

Plate 1



(a)

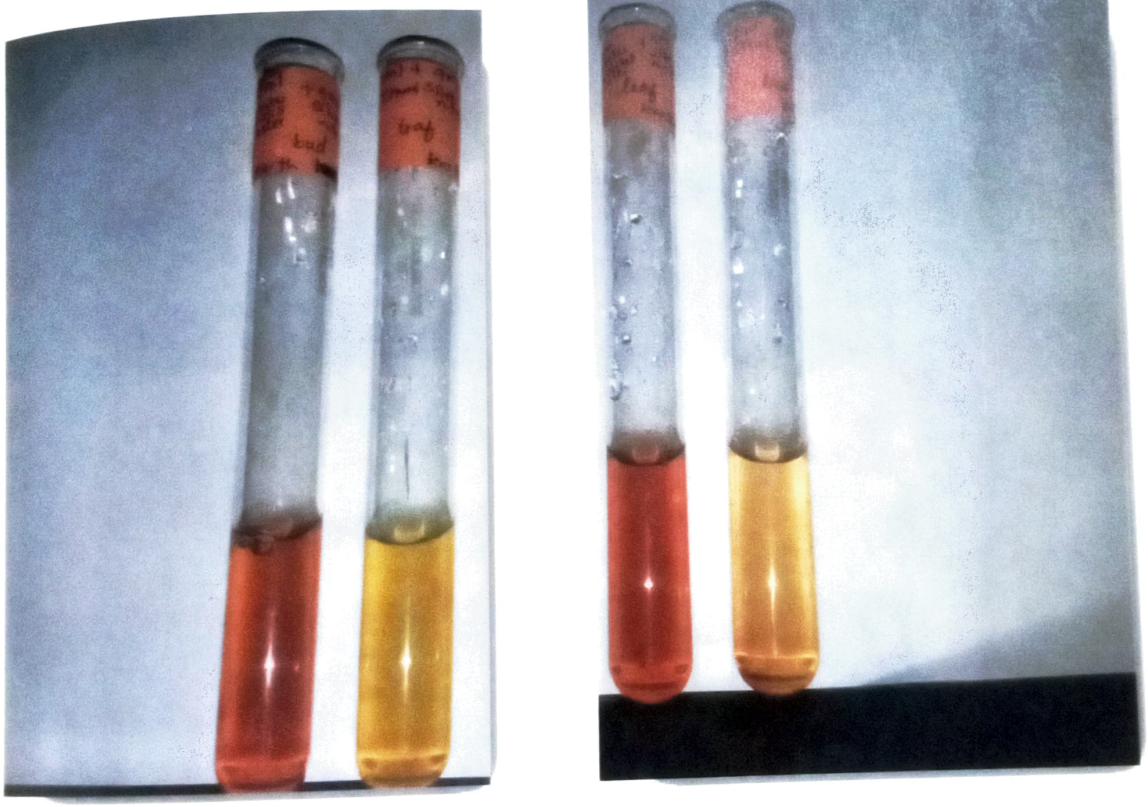


(b)

(a) Aqueous extract (b) Ethanol extract

Photograph of synthesized AgNPs using *Boerhaavia diffusa* leaf , stem and flower extracts

Plate 2

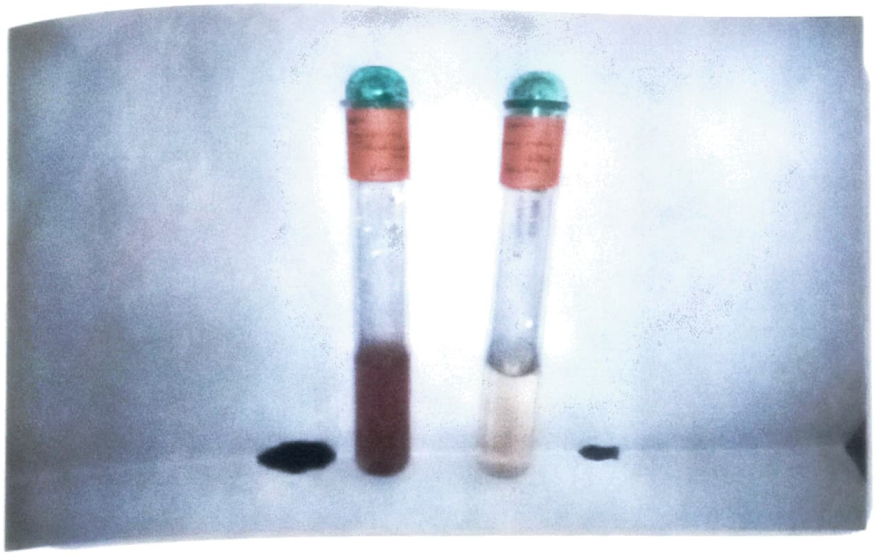


(a) Aqueous extract

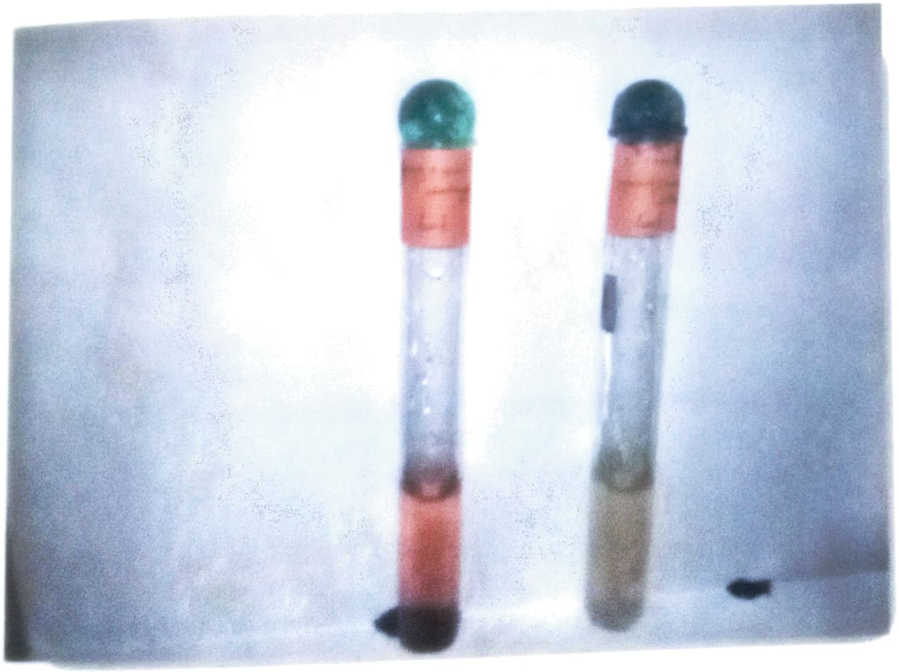
(b) Ethanol extract

Photograph of synthesized AgNPs using *Corchorus tridens* leaf and bud extracts

Plate 3



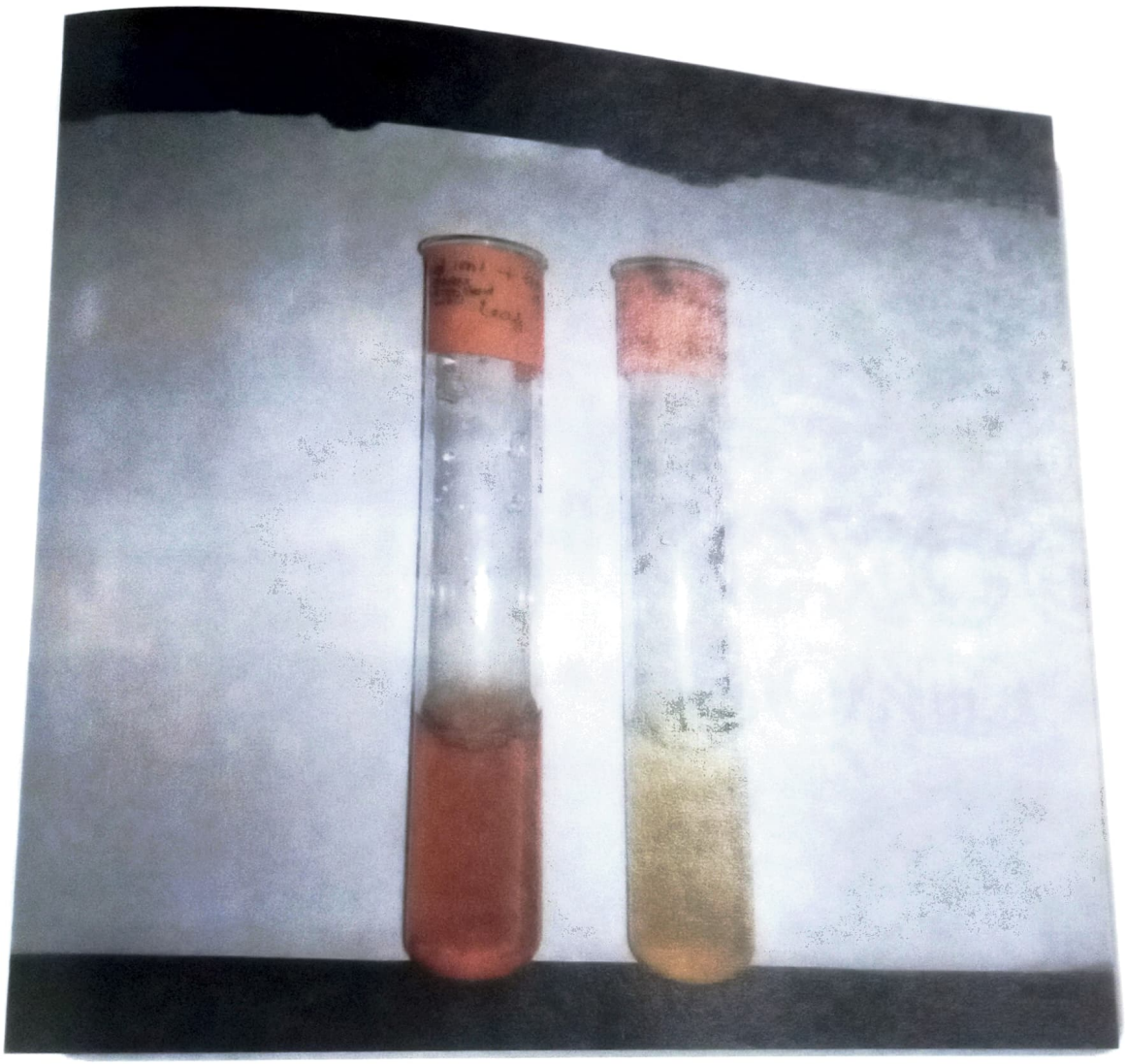
(a) Aqueous extracts



(b) Ethanol extracts

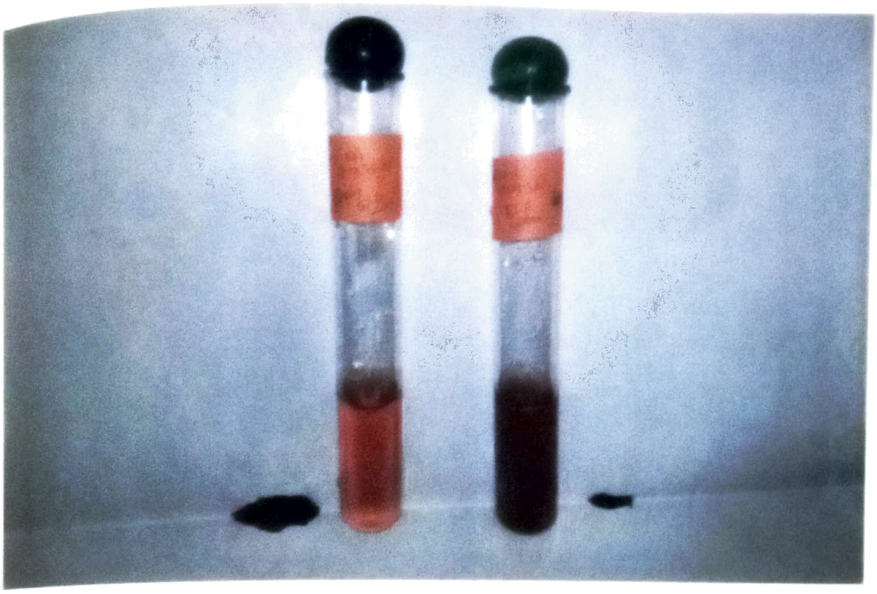
Photograph of synthesized AgNPs using *Gomphrena procumbens* **leaf and flower extract**

Plate 4

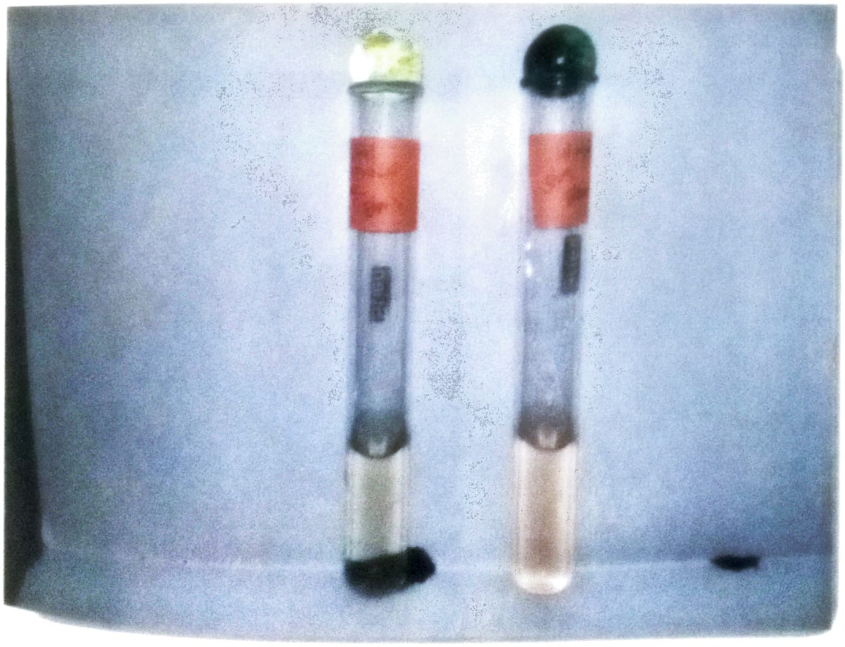


Photograph of synthesized AgNPs using aqueous and ethanol *Hybanthus enneaspermus* leaf extracts

Plate 5



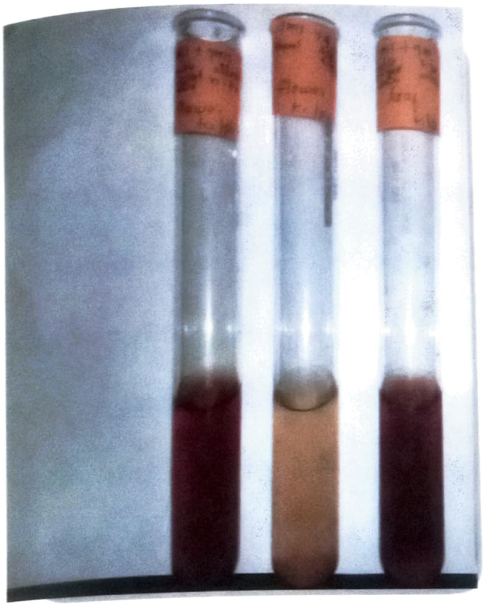
(a) Aqueous extracts



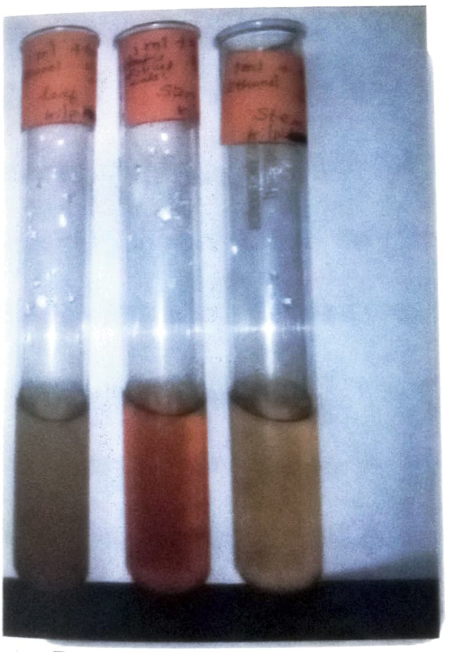
(b) Ethanol extracts

Photograph of synthesized AgNPs using *Indigofora linnaei* leaf and stem extracts

Plate 6



(a) Aqueous extracts



(c) Ethanol extracts

Photograph of synthesized AgNPs using *Merremia tridentata* leaf , stem and flower extracts

nanoparticles synthesis as they are free from toxic chemicals as well as contain natural capping agents Singh *et. al.*,(2011). Among various plants, we have chosen *Boerhaavia diffusa, Corchorus tridens, Gomphrena procumbens, Hybanthus enneaspermus, Indigofera linnaei,* and *Merremia tridentata*

UV-vis spectroscopy monitors the absorption in the ultraviolet-visible spectral region and is commonly used to characterize plasmonic properties of synthesized nanoparticles. Studies have demonstrated that AgNPs absorb the spectrum in the visible region of around 400 nm due to the excitation of the localized surface Plasmon resonance (Bastus (2014), Gomezgrana (2013, Chan (2013), Dhand (2016). Since this property is highly affected by nanoparticle size and the surrounding media, it is also possible that AgNPs have the Plasmon band of around 500 nm or slightly higher (Zong *et. al.,*(2014)). Several researchers have evaluated the correlation between the UV-vis spectrum and nanoparticle properties. For instance, uniform spherical AgNPs can be associated with a single peak in the UV-vis spectrum while AgNPs in the irregular shapes have two or more peaks depending on their symmetry (Shervani *et. al.,*(2007)). In addition, increase in nanoparticles size can be identified by their maximum absorbance located at the higher wavelength range (Link *et. al.,*(1999). Moreover, increasing the absorbance spectra indicates a higher production of AgNPs (Tan *et. al.*,(2013). Plate 1 to 6 shows the UV-vis spectra of AgNPs synthesized using currently plant extracts. It has been found that AgNPs synthesized using aqueous extracts of *Boerhaavia diffusa* leaf, *Corchorus tridens* leaf and bud, *Gomphrena procumbens* flower, *Indigofera linnaei* stem and *Merremia tridentate* flower have single maximum peaks at 450, suggesting that they are spherical in shapes. On the other hand, there is an absence of a clear maximum peak for AgNPs synthesized using *Boerhaavia diffusa* stem and flower, *Hybanthus enneasperus* leaf, *Indigofera linnaei* leaf and *Merimia tridentata*

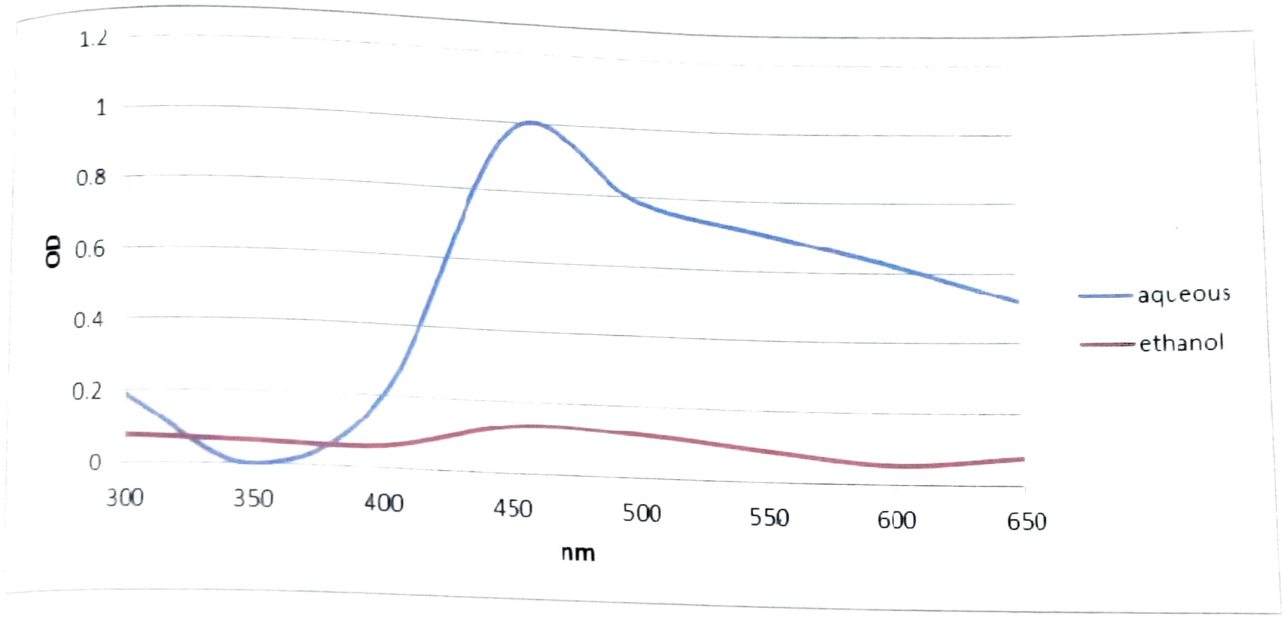


Fig. 1: Uv-vis spectra recorded as a function of reaction time of $AgNo_3$ solution with *Boerhaavia diffusa* leaf extracts

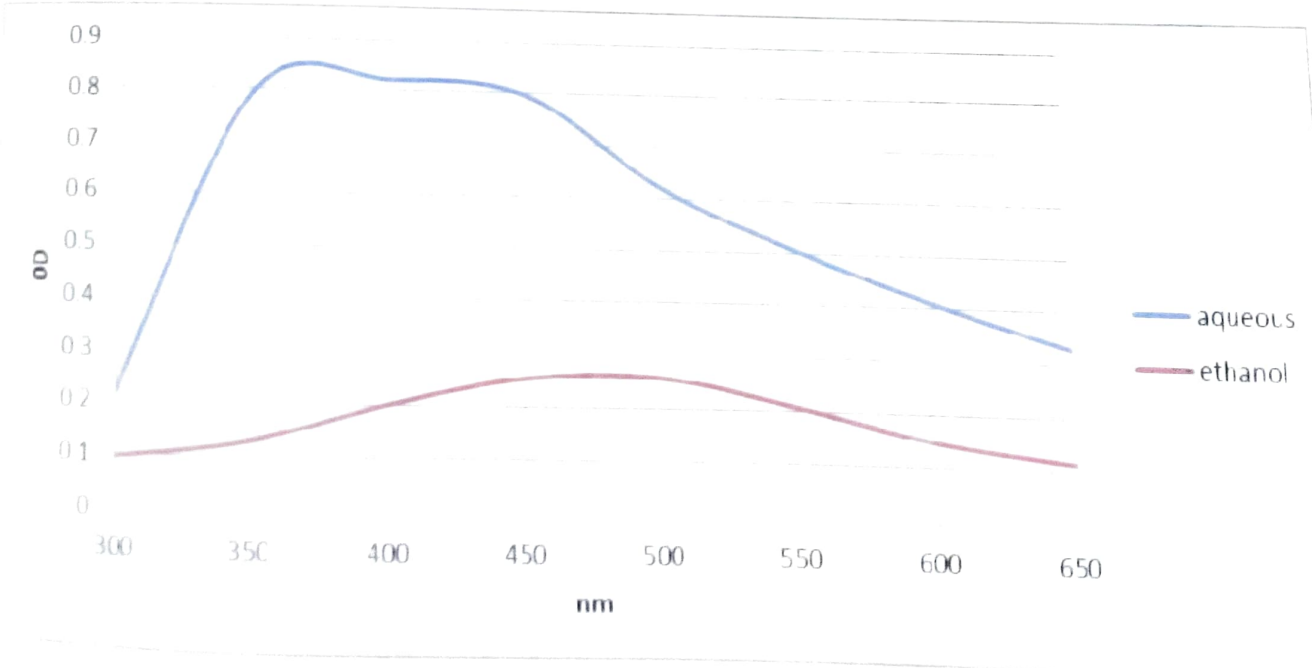


Fig. 2: Uv-vis spectra recorded as a function of reaction time of $AgNo_3$ solution with *Boerhaavia diffusa* stem extracts

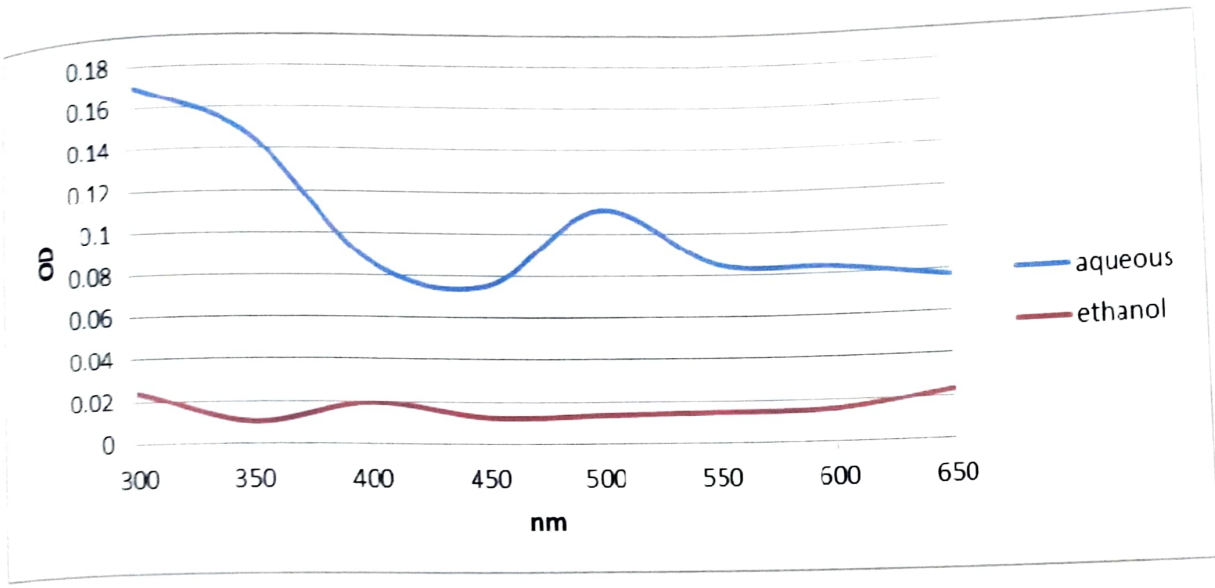


Fig.3 .Uv-vis specra recorded as a function of reaction time of $AgNo_3$ solution with *Boerhaavia diffusa* flower extracts

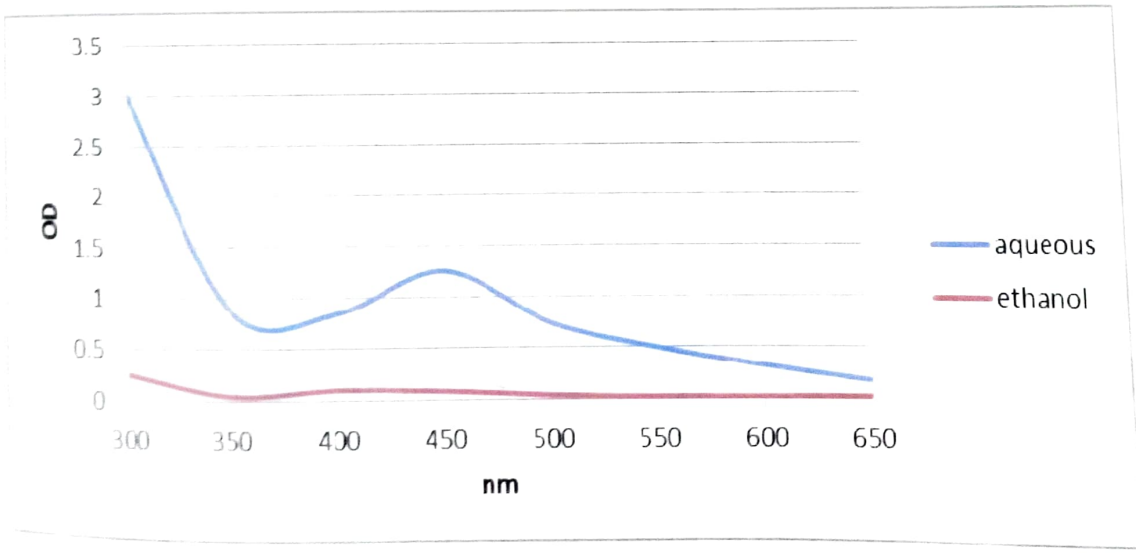


Fig.4.Uv-vis spectra recorded as a function of reaction time of $AgNo_3$ solution with *Corchorus tridens* leaf extracts

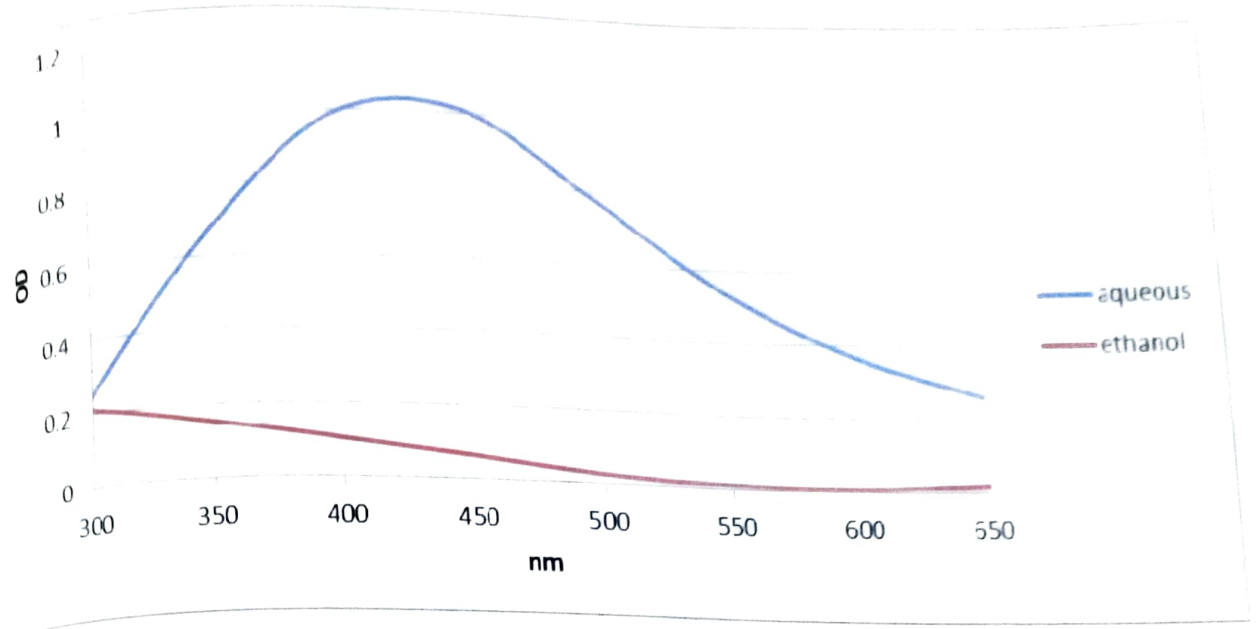


Fig.5.Uv-vis spectra recorded as a function of reaction time of $AgNo_3$ solution with *Corchorus tridens* bud extracts

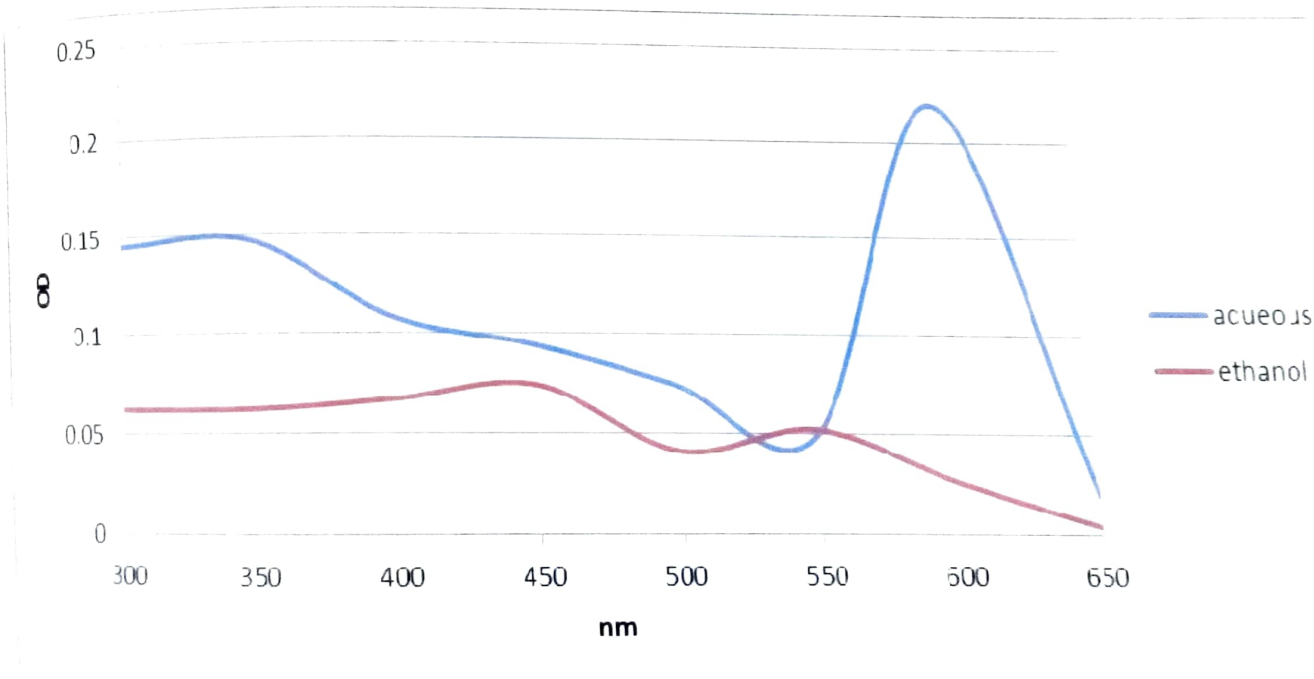


Fig. 6.Uv-vis spectra recorded as a function of reaction time of $AgNo_3$ solution with *Gomphrena procumbers* leaf bud extracts

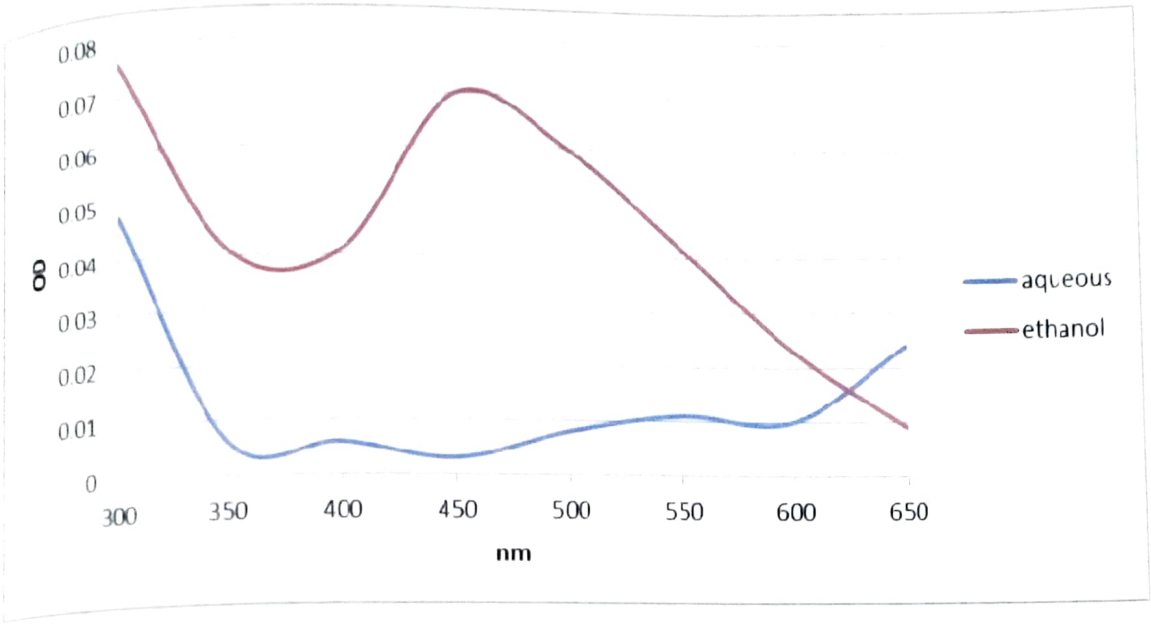


Fig.7.Uv-vis spectra recorded as a function of reaction time of $AgNo_3$ solution with *Gomphrena procumbers* flower extracts

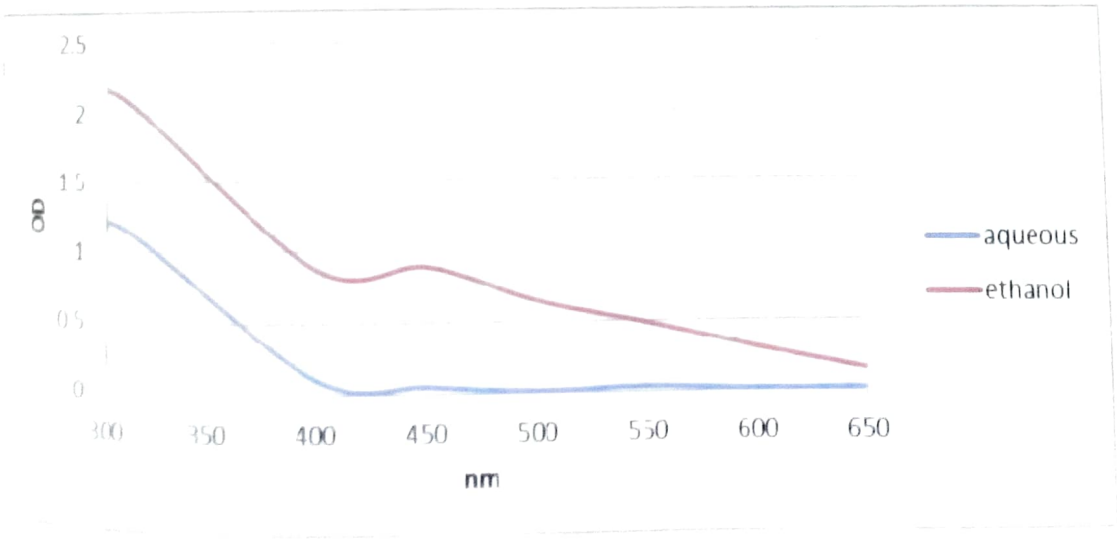


Fig.8.Uv-vis spectra recorded as a function of reaction time of $AgNo_3$ solution with *Hybanthus enneaspermus* leaf extracts

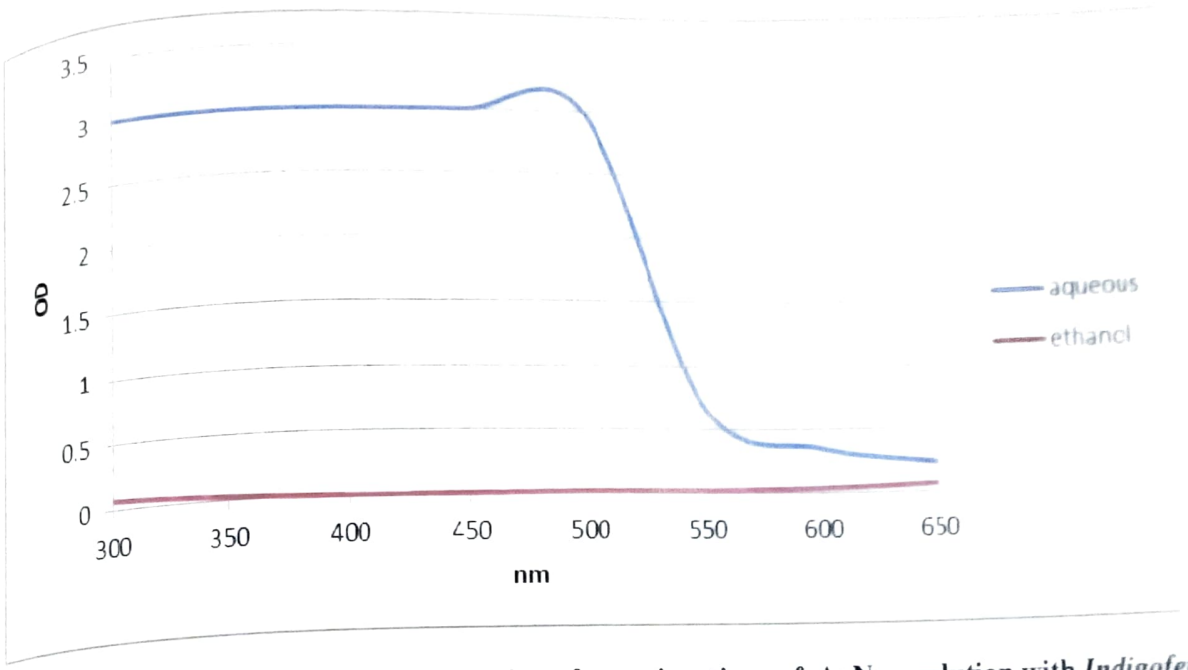


Fig.9.Uv-vis spectra recorded as a function of reaction time of $AgNo_3$ solution with *Indigofera linnaei leaf* extracts

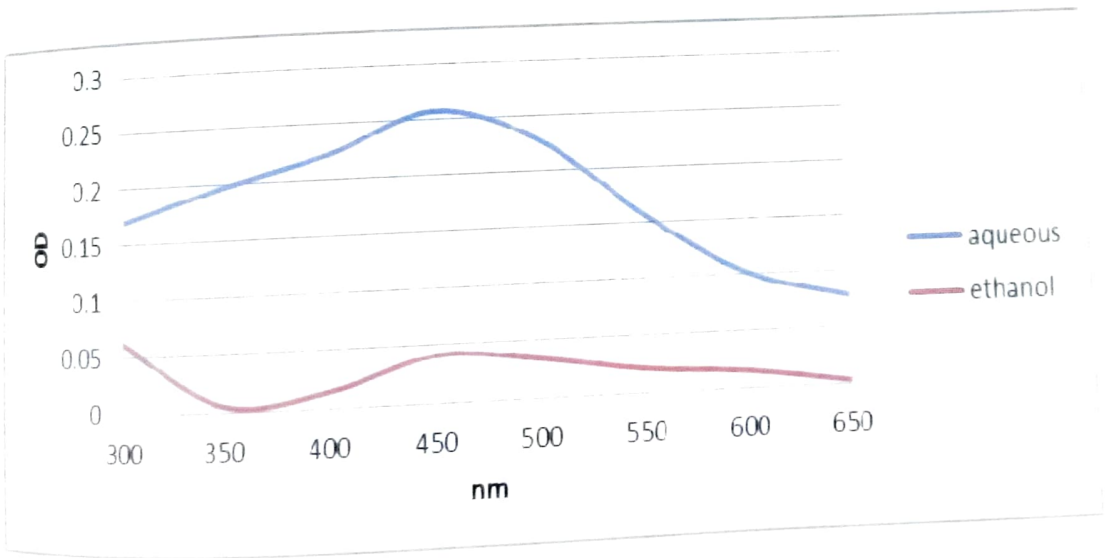


Fig.10. Uv-vis spectra recorded as a function of reaction time of $AgNo_3$ solution with *Indigofera linnaei* stem extracts

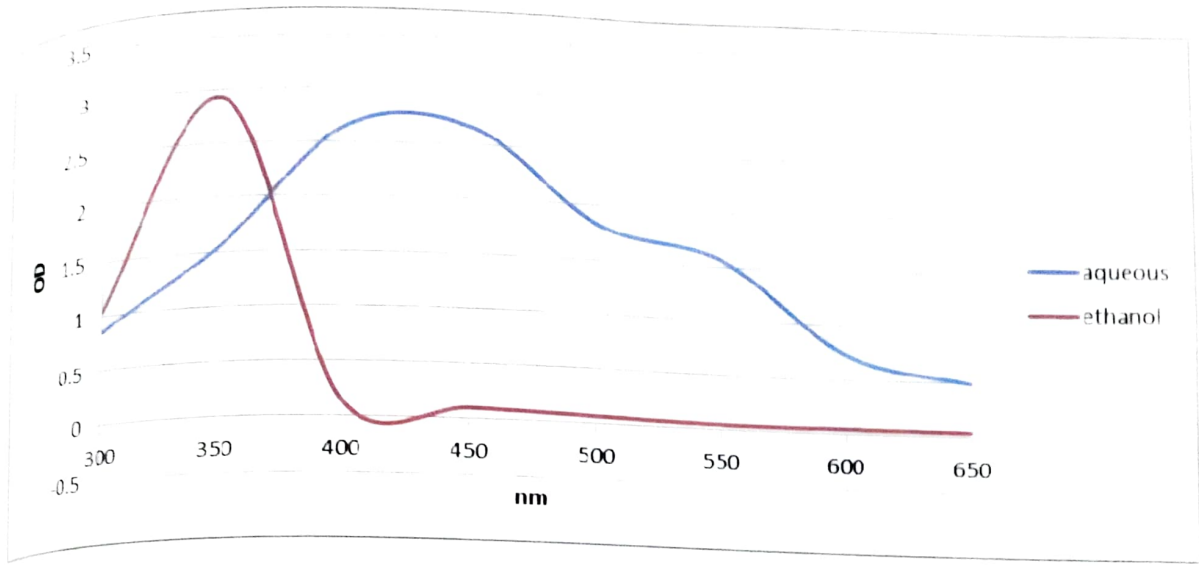


Fig.11. Uv-vis spectra recorded as a function of reaction time of $AgNo_3$ solution with *Merremia tridentata* leaf extracts

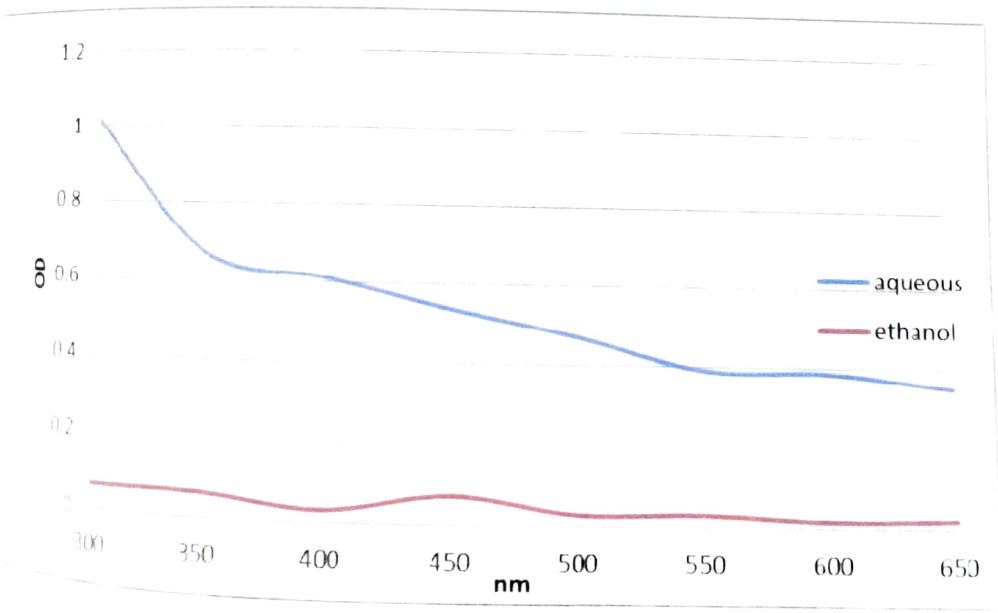


Fig.12. Uv-vis spectra recorded as a function of reaction time of $AgNo_3$ solution with *Merremia tridentata* stem extracts

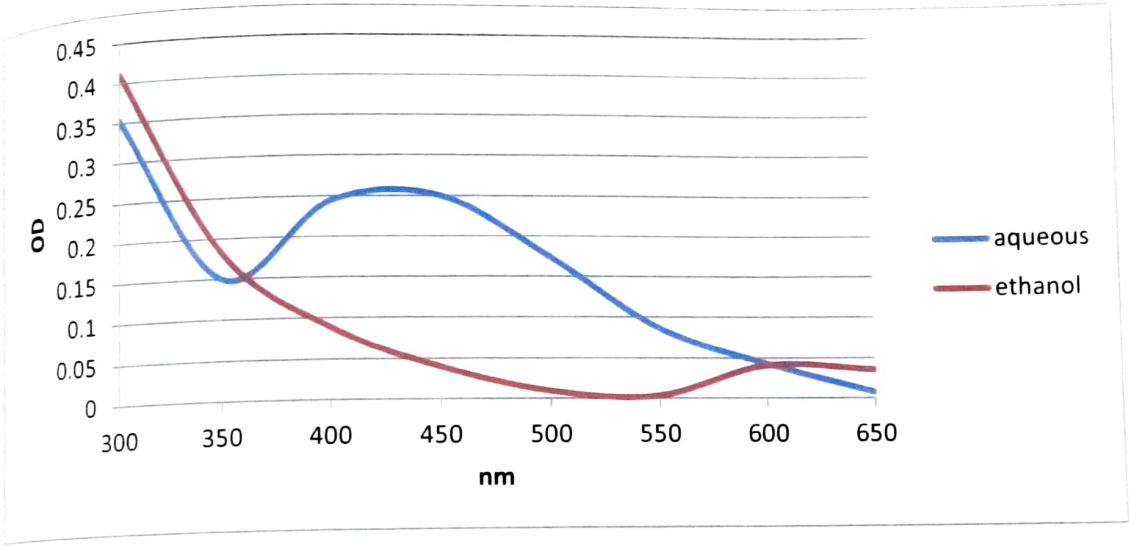


Fig.13.Uv-vis spectra recorded as a function of reaction time of AgNo solution with Merremia tridentata flower extracts

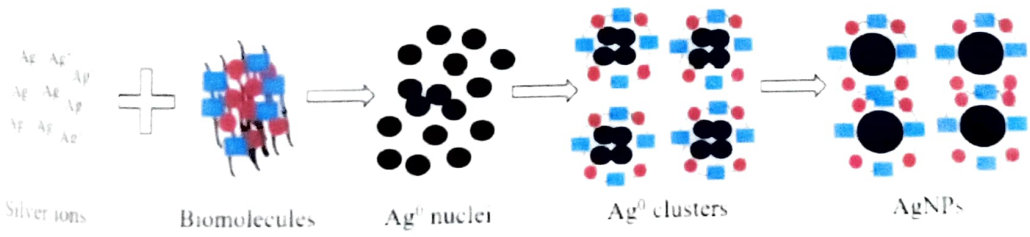


Fig.14. A Schematic presentation of AgNPs synthesized using biomolecules extracted from plants

o the formation of the nanoparticle using biochemicals present within all presently used plant

xtracts is shown in Figure 14.

Summary and conclusion

The salient findings of the present study on green synthesis of AgNPs using six weeds collected from St. Mary's College (Autonomous), Thoothukudi *viz., Boerhaavia diffusa, Corchorus tridens, Gomphrena procumbens, Hybanthus enneaspermus, Indigofera linnaei, Merremia tridentata* . The intensive use of the plant extracts of weeds is due to their internal biomolecule such as protein, terpenoids and flavonoids, which offer potential as a bioreductant to reduce metal ions to form AgNPs. The weeds synthesized AgNPs characterized by Uv-vis spectroscopical analysis and Ag elemental composition and ability of plant biomolecules to act as reducing, capping and stabilizing agent of AgNPs were compared to identify the most desirable plant sources. Accordingly the aqueous extract of *Boerhaavia diffusa* leaf, *Corchorus tridens* leaf and bud, *Gomphrena procumbens* flower, *Indigofera linnaei* stem and *Merremia tridentata* flower have single maximum peaks at 450 was rated as most desirable plants for the green synthesis of AgNPs compared to other plant extracts. The spherical nanoparticle, it is found that AgNPs synthesized using methanol extract of *Merremia tridentata* leaf had the maximum absorbance located at the lower wavelength range 350 nm.

Further studies need to be carried out in order to validate the effects of physio-chemical parameters to synthesize AgNPs using the presently studied plant extracts. In addition, the cytotoxicity investigations are also crucial for further antibacterial characterization in the future work.

REFERENCES

Reference

Abalkhil, T.A., Alharbi, S.A., Salmen, S.H., Wainwright, M...2017. Bactericidal activity of biosynthesized silver nanoparticles against human pathogenic bacteria. Biotechnol. Biotechnol. *Equip*. 31, 411–417.

Abhishek Mathur., Akhilesh Kushwaha, Vandana Dalakoti, Garima Dalakoti and Deep Shikha Singh.,2014. Green synthesis of silver nanoparticles using medicinal plant and its characterization Pelagia Research Library. Der Pharmacia Sinica, 2014, 5(5):118-122

Ahmad, N.,Sharma, S.,Alam, M.K.,Singh, V.N., Shamsi, S.F., Mehta, B.R.., Fatma, A.2010. Rapid synthesis of silver nanoparticles using dried medicinal plant of basil. Colloids and Surfaces B: Biointerfaces 81, (1), 81-86.

Akomas, S.,Ijioma, S., Emelike, C.,2015 Effect of euphorbia hirta on haematological and biochemical indices in albino rats. Appl. *J. Hyg*. 4, 1–5.

Ana-Alexandra Sorescu., Alexandrina Nu ă, Rodica- Mariana Ion, Suică-Bunghez Ioana-Raluca.,2016. Green synthesis of silver nanoparticles using plant extracts. DOI: 10.18638/scieconf.4.1.386.

Arpita Roy., 2017. Synthesis of silver nanoparticles from medicinal plants and its biological application: A review. Biosci.;12(4):138.

Pratima Chauhan.2017 . Green Synthesis of Silver Nanoparticles Involving Extract of Plants of Different Taxonomic Groups. UP. 211002.

Awwad, A.M., Salem, N.M., and Abdeen, A.O. 2013. Green synthesis of silver nanoparticles using carob leaf extract and its antibacterial activity. International Journal of Industrial Chemistry, 4(1), 1-6.

Balaprasad, A., Chinmay D., Absar A., Murali S. 2005. Biosynthesis of Gold and Silver Nanoparticles Using Emblica Officinalis Fruit Extract, Their Phase Transfer and Transmetallation in an Organic Solution, Journal of Nanoscience and Nanotechnology, **5** (10): 1665-1671.

Banerjee, P., Satapathy, M., Mukhopahayay, A., and Das, P.2014. Leaf extract mediated green synthesis of silver nanoparticles from widely available Indian plants: synthesis, characterization, antimicrobial property and toxicity analysis. Bioresources and Bioprocessing, 1(1), 1-10.

Bastús, N.G., Merkoçi, F., Piella, J., Puntes, V.2014. Synthesis of highly monodisperse citrate-stabilized silver nanoparticles of up to 200 nm: Kinetic control and catalytic properties. *Chem. Mater.* , 26, 2836–2846.

Latteau, L., Lautié, E., Koné, O., Coppée, M., Hell, K., Pomalegni, C.B.,Quetin-Leclercq, J.2013. Degradation of rotenone in yam bean seeds (Pachyrhizus sp.) through food processing. J. *Agric. Food Chem.* 61, 11173–11179.

Chan, Y.S., Mat Don, M..2013. Biosynthesis and structural characterization of Ag nanoparticles from white rot. 282-8. doi: 10.1016.

Crabtree, J.H., Burchette, R.J., Siddiqi, R.A., Huen, I.T., Hadnott, L.L., and Fishman, A., 2003. The efficacy of silver-ion implanted catheters in reducing peritoneal dialysis-related infections. Peritoneal Dialysis International, 23(4), 368-374.

De Gaetano, F., Ambrosio, L., Raucci, M.G., Marotta, A., and Catauro, M. (2005). Sol-gel processing of drug delivery materials and release kinetics.16: 261–8.

Desireddy, A., Conn, B.E., Guo, J., Yoon, B., Barnett, R.N., Monahan, B.M., Kirschbaum, K., Griffith, W.P., Whetten, R.L., Landman, U.,2013. Ultrastable silver nanoparticles. Nature 501, 399–402.

Dhand, V., Soumya, L., Bharadwaj, S., Chakra, S., Bhatt, D., Sreedhar, B,(2016). Green synthesis of silver nanoparticles using Coffea arabica seed extract and its antibacterial activity. Mater. *Sci. Eng. C Mater. Biol.*, 58, 36–43.

Dhar, P., Dhar, D.G., Rawat, A.K.S.,,2017.Srivastava, S. Medicinal chemistry and biological potential of Cyperus rotundus Linn.: An overview to discover elite chemotype(s) for industrial use. *Ind. Crops Prod.* 108, 232–247.

Fayaz, A.M., Balaji, K.,Girilal, M., Yadav, R., Kalaichelvan, P.T., Venketesan, R.,2010. Biogenic synthesis of silver nanoparticles and their synergistic effect with antibiotics: A study against gram-positive and gram-negative bacteria. *Nanomedicine* 6, 103–109.

Fu, Y.H.., Kuznetsov, A.I., Miroshnichenko, A.E.,Yu, Y.F., Luk'yanchuk, B.,2013. Directional visible light scattering by silicon nanoparticles. *Nat. Commun.* , 4, 1527. fungi. Mater. *Sci. Eng. C Mater. Biol.* 33, 282–288.

Gómez-Graña, S., Perez-Ameneiro, M., Vecino, X.,Pastoriza-Santos, I.,Perez-Juste, J.,Cruz, J.; Moldes, A 2017. Biogenic synthesis of metal nanoparticles using a biosurfactant extracted from corn and their antimicrobial properties. *Nanomaterials* 7, 139.

Han, X.Y., Han, F.S., Segal, J.2008. Chromobacterium Haemolyticum sp. nov., a strongly haemolytic species. *Int. J. Syst. Evol. Microbiol.* 58, 1398–1403.

Huang, C., Liu, Q., Fan, W.,2015. Boron nitride encapsulated copper nanoparticles: A facile one-step synthesis and their effect on thermal decomposition of ammonium perchlorate. Sci Rep. 5, 16736.

Jae Yong Song., Beom Soo Kim,2009. Rapid biological synthesis of silver nanoparticles using plant leaf extracts. Chungbuk 361 763, 32:79–84.

Jyoti, K.,Baunthiyal, M.,2016. A Characterization of silver nanoparticles synthesized using Urtica dioica Linn. leaves and their synergistic effects with antibiotics. J. Radiat. Res. Appl. Sci. 9, 217–227.

Kreyling, W.G., Abdelmonem, A.M.., Ali, Z.,Alves, F., Geiser, M.,Haberl, N., Hartmann, R., Hirn, S., de Aberasturi, D.J., Kantner, K.,2015. In vivo integrity of polymer-coated gold nanoparticles. *Nat. Nanotechnol.* , 10, 619–623.

Krishnaraj.C., E.G. Jagan, S. Rajasekar, P. Selvakumar, P.T. Kalaichelvan, N. Mohan., 2010. Synthesis of silver nanoparticles using *Acalypha indica* leaf extracts and itsantibacterial activity against water borne pathogens. Biointerfaces 76 (2010) 50–56

Królikowska, A., Kudelski, A., Michota, A., and Bukowska, J. (2003). SERS studies on the structure of thioglycolic acid monolayers on silver and gold. Surface science, 532, 227-232.

Le Ouay, B., Stellacci, F.,2015. Leaf extract mediated green synthesis of silver nanoparticles from widely available Indian plants: synthesis, characterization, antimicrobial property and toxicity analysis. Nano Today , 10, 339–354.

Link, S., El-Sayed, M.A.,1999. Size and temperature dependence of the plasmon absorption of colloidal gold Materials in Medicine, 16(3), 261-265.

Meng, F.B., Wang, L., Xu, H.; Liu, C.C., Hu, P.C., Lan, W., Song, J.X., Chen, L.S.,2016. Biosynthesis of silver nanoparticles using oriental medicinal herb Gynostemma pentaphyllum Makino extract and their antibacterial activity against aquatic pathogen. Mater. *Technol.* 31, 181–186.

Mie, R., Samsudin, M.W.,Din, L.B., Ahmad, A., Ibrahim, N., Adnan, S.N.A.,2014. Synthesis of silver nanoparticles with antibacterial activity using the lichen Parmotrema praesorediosum. *Int. J. Nanomed.* , 9, 121–127.

Mody., V.V., Siwale, R., Singh, A. and Mody H.R., 2010. Introduction to Metallic Nanoparticles. Journal of Pharmacy and Bioallied Sciences, 2, 282-289.

Mohamad, M.F, Kamarudin, Faithlah N.F.N and M.M Salleh. 2014. The effect of sodium chloride in the formation of size and shape of Gold and Silver nanoparticles by microwave polyol method for mercury adsorption. *World Acad.* Sci.Eng. Technol., **74:**691-695.

Mohammed Rafi Shaik., Mujeeb Khan , Mufsir Kuniyil , Abdulrahman Al-Warthan ,Hamad Z. Alkhathlan , Mohammed Rafiq H. Siddiqui , Jilani P. Shaik , Anis Ahamed Adeem Mahmood , Merajuddin Khan, and Syed Farooq Adil .,2014. Plant-Extract-Assisted Green Synthesis of Silver Nanoparticles Using *Origanum vulgare.* nanoparticles. *J. Phys. Chem. B* 103, 4212–4217.

Natarajan, K., Subbalaxmi, SV., RamchandraM. 2010. Microbial production of silver nanoparticles. Dig J Nanomat Bios;**5** (1):135-140.

Navaladian, S., Viswanathan, B., Viswanath, R.P., and Varadarajan, T.K. 2007. Thermal decomposition as route for silver nanoparticles using dried medicinal plant of basil. Nanoscale research letters, 2(1), 44-48.

Phanjom, P., and Ahmed, G. 2015. Biosynthesis of silver nanoparticles by Aspergillus oryzae (MTCC No. 1846) and its characterizations. Nanoscience and Nanotechnology, 5(1), 23-29. Phys. 16, 4236–4241.

Priya Banerjee., Mantosh Satapathy, Aniruddha Mukhopahayay and Papita Das., 2014. doi:10.1186/s40643-014-0003-y.

Rajasekharreddy, P., Rani, P.U.,and Sreedhar, B. 2010. Qualitative assessment of silver and gold nanoparticle synthesis in various plants: a photobiological approach. Journal of Nanoparticle Research, 12(5), 1711-1721. reduction route. *J. Nanopart.* , 15, 1537

Santos, K.D.O., Elias, W.C., Signori, A.M..,Giacomelli, F.C., Yang, H., and Domingos, J.B. 2012. Synthesis and catalytic properties of silver nanoparticle–linear polyethylene imine colloidal systems. The Journal of Physical Chemistry C, 116(7), 4594-4604.

Shervani, Z.,Ikushima, Y., Sato, M.., Kawanami, H..,Hakuta, Y., Yokoyama, T..Nagase, T., Kuneida, H., Aramaki, K. 2007. Morphology and size-controlled synthesis of silver nanoparticles in aqueous surfactant polymer. *Sci.*, **286**, 403–410.

Singh, P., Kim, Y.J., Singh, H.,Wang, C., Hwang, K.H..,Farh, M.E.-A.,2015. . Biosynthesis, characterization and antimicrobial applications of silver nanoparticles. *Int. J. Nanomed.* 10, 2567–2577.

Singhal, G., Bhavesh, R.., Kasariya, K.., Sharma, A.R..,and Singh, R.P. 2011. Biosynthesis of silver nanoparticles using Ocimum sanctum (Tulsi) leaf extract and screening its antimicrobial activity. Journal of Nanoparticle Research, 13(7), 2981-2988.

dations. *Colloid Polym. Sci.*, 286, 403–410.

Song, J.Y., and Kim, B.S. 2009. Rapid biological synthesis of silver nanoparticles using plant leaf extracts. Bioprocess and Biosystems Engineering, 32(1), 79-84.

Syafiuddin, A., Salmiati., Salim, M.R.., Kueh, A.B.H.., Hadibarata, T .,,Nur, H., 2017. A review of silver nanoparticles: Research trends, global consumption, synthesis, properties and future challenges. *J. Chin. Chem. Soc.* , 64, 732–756.

Syafiuddin, A., Salmiati; Hadibarata, T., Salim, M.R., Kueh, A.B.H., Sari, A.A.,2017. A purely green synthesis of silver nanoparticles using Carica papaya, Manihot esculenta and Morinda citrifolia: Synthesis and antibacterial evaluations. Bioprocess *Biosyst. Eng.* 40, 1 349–1361.

Tan, K.S..2013. Cheong, K.Y. Advances of Ag, Cu and Ag–Cu alloy nanoparticles synthesized via chemical. ISBN: 978-0-323-47720-8.

Torresdey, J.L.G., Gomez E., Videa J.RP., Parsons J.G., Troiani H., Yacaman M.J., 2002. Alfalfa Sprouts: A Natural Source for the Synthesis of Silver Nanoparticles. Langmuir, **19**: 1357-1361.

Trujillo, E.E..Latterell, F., Rossi, A.,1986. Colletotrichum gloeosporioides, a possible biological control agent for Clidemia hirta in Hawaiian forests. Plant Dis. 70, 974–976.

Vinod, V.T.P., Saravanan, P., Sreedhar, B., Devi, D.K..and Sashidhar, R.B. 2011. A facile synthesis and characterization of Ag, Au and Pt nanoparticles using a natural hydrocolloid

gum kondagogu (Cochlospermum gossypium). Colloids and Surfaces B: Biointerfaces. 83(2). 291-298.

Virender K., Sharma , Ria A. Yngard, Yekaterina Lin., 2009. Silver nanoparticles: Green synthesis and their antimicrobial activities. 145 (2009) 83–96.

Zanganeh, S., Hutter, G., Spitler, R.., Lenkov, O., Mahmoudi, M.., Shaw, A.. Pajarinen. J.S.,Nejadnik, H.,Goodman, S., Moseley.,2016. M. Iron oxide nanoparticles inhibit tumour growth by inducing pro-inflammatory acrophage polarization in tumour tissues. *Nat. Nanotechnol.* 11, 986–994.

Zong, R.,;Wang, X., Shi, S.,Zhu, Y.,2014. Kinetically controlled seed-mediated growth of narrow dispersed silver nanoparticles up to 120 nm: Secondary nucleation, size focusing and Ostwald ripening. Phys. Chem. 4236-41. doi: 10.1039/c3cp54846e.

GROWTH AND YIELD PERFORMANCE OF OYSTER MUSHROOM (PLEUROTUS SPP.) ON DIFFERENT SUBSTRATES AND ITS BIOCHEMICAL AND NUTRIENT ANALYSIS

A Short Term Project Work Submitted to St. Mary's college (Autonomous) affiliated to Manonmaniam Sundaranar University in Partial Fulfillment for the Degree of

BACHELOR OF SCIENCE IN BOTANY

By

K. KISHANIYA	**16AUBO21**
T. LOURDU SANGEETHA	**16AUBO23**
A. LURTHU VINOTHA	**16AUBO24**
P. MANIMEGALAI	**16AUBO25**
C. MARIA ABISHA	**16AUBO26**
A. MARIA BENISTA	**16AUBO27**

DEPARTMENT OF BOTANY

ST.MARY'S COLLEGE (AUTONOMOUS)

THOOTHUKUDI-628001

2018-2019

CERTIFICATE

It is certified that this short term project work entitled **"GROWTH AND YIELD PERFORMANCE OF OYSTER MUSHROOM (PLEUROTUS SPP.) ON DIFFERENT SUBSTRATES AND ITS BIOCHEMICAL AND NUTRIENT ANALYSIS"** submitted to **St. Mary's College (Autonomous)** affiliated to **Manonmaniam Sundaranar University** in partial fulfillment of the requirements for the degree of **Bachelor 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 2018-2019 by the following students.

K. KISHANIYA	**16AUBO21**
T. LOURDU SANGEETHA	**16AUBO23**
A. LURTHU VINOTHA	**16AUBO24**
P. MANIMEGALAI	**16AUBO25**
C. MARIA ABISHA	**16AUBO26**
A. MARIA BENISTA	**16AUBO27**

G. Arunsiya 29/3/19
GUIDE

M. 29/3/19
HEAD OF THE DEPARTMENT

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

EXAMINER

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

ACKNOWLEDGMENT

First of all,we thank Lord Almighty for giving us the strength to complete our project successfully.

We express our cordial thanks and deep sense of gratitude to our guide **G. Anusiya**, M.Sc., M.Phil., Assistant Professor of Botany, St. Mary's College (Autonomous), Thoothukudi for her inspiring guidance, infinitive help, valuable ideas, critical comments, fruitful discussions and genuine friendliness which led us to the successful completion of our project.

We are greatly indebted to **Dr. Sr. A. S. J. Lucia Rose** M.Sc., PGDCA, M.Phill., Ph.D., Principal and the management of St. Mary's College for allowing us to do the course in St. Mary's College (Autonomous), Thoothukudi.

We are immensely grateful to **Dr. M. Glory** M.Sc., M.Phil., Ph.D., Head of the Department of Botany for providing us the laboratory facilities throughout our project. Thanks are also extended to all the staff members and the laboratory assistants of the Department of Botany and to our friends for their generous help.

Last but not least, we thank our parents for their lovable care, encouragement and constant support during the course of study.

CONTENT

INTRODUCTION

INTRODUCTION

Mushrooms are the fruiting bodies of a variety species of fungi belonging to the class of Basidiomycetes or Ascomycetes. They are the "natural scavengers" usually grows on wet, moist surfaces such as lawns, dead tree trunks, fences and wooded areas. Worldwide approximately about 1.5 million varieties of mushrooms are identified. Of which 5,000 species are edible (Ramanathan *et al.*, 2013). In India mushrooms like *Agaricus bisporous* (white buttom mushroom), *Calocybe indica* (milky mushroom), *Volvariella spp.* (paddy straw mushroom) and *Pleurotus spp.* (Oyster mushroom or dhingri) are cultivatable. Among that *Pleurotus spp.* (oyster mushroom) is the second largest commercially produced and edible mushroom in the world market due to its excellent flavor and taste. (Kang, 2004).

Mushrooms are rich with proteins, fat, sugars, mycocellulose, minerals like potassium, phosphorus, calcium, sodium and also rich in vitamins such as niacin, riboflavin, vitamin D, C, B1, B5 and B6. (Stanley, 2011; Ahmed *et al.*, 2009). Mushrooms also synthesize a variety of secondary metabolites that possess anti-tumoral, antiviral, anti-inflammatory (Carvalho *et al.*, 2007), antibacterial, antifungal (Owaid *et al.*, 2015a) and anti-yeast activities (Owaid *et al.*, 2015b).

Cultivation of edible mushrooms has being increasingly practice. Oyster mushrooms are the broadly cultivated variety because of its low cost of production and grow within a temperature range from 15-25°C and also *Pleurotus spp.* grown on a wide range of lignocellulosic residues such as paddy straw, wheat straw, cotton wastes, sugarcane bagasse, banana leaf, sugar cane leaves, saw dust, maize stover, sorghum

stalks and leaves etc. (Beetz and Kustida, 2004; Lourdes *et al.*, 2008; Mathews *et al.*, 1996; Sangwan and Saini, 1995).

A huge mass of lingo-cellulosic agro industrial wastes which are rich in organic compounds deposited daily in the soils. Around 200 billion tons per year of organic wastes were produced in our planet (Zhang, 2008). Among various bioconversion processes of agro-industrial wastes, mushroom cultivation is an appropriate technology (Dundar *et al.*, 2009). It is also helpful to overcome problems related to nutritional deficiency among low and middle income countries (Imtiaj *et al.*, 2008). Hence, nowadays, mushroom cultivation technology is being increasingly practice by farmers in and around world. The selected substrates for our study include paddy straw, banana leaf and sugarcane bagasse are readily available and are the rich sources of lignin and cellulose. Taking in to account the bioconversion of agro-industrial wastes, the present work was aimed to evaluate the effect of different substrates on growth, productivity and nutrient analysis of *Pleurotus spp.*

LITERATURE REVIEW

Soniya *et.al.*, 2013 studied the cultivation of on different substrates such as rice straw, rice straw + wheat straw, rice straw+ paper, sugarcane bagasse and sawdust. The results shows that among all aspects, rice straw (control) was found as a best substrate with yield (381.85 gm) and BE (95.46%) %) followed by rice plus wheat straw, rice straw plus paper waste for the production of mushroom.

Mondal, *et al.*, 2010 analysed the growth and yield performance of oyster mushroom (*Pleurotus florida*) on different substrates. Highest mycelium running rate was found in banana leaves and rice straw (1:1) but the lowest in control. Completion of mycelium running time was lowest in banana leaves and rice straw (1:3 and 3:1).

Senthilraja, K., 2014 studied the cultivation of mushroom in *Pleurotus euos* on paddy straw. The pressmud fibre mixed with the paddy straw at 1:3 ratio recorded relatively highly yield of oyster mushroom *Pleurotus euos* (apki) when compared to there treatment.

Momiro and Mamiro, 2011 studied the yield and mushroom size of *Pleurotus ostreatus* grow on rice straw basal substrate mixed and supplemented various crop residues. Yields ranged as low as 50g from rice bran to as high as 1040g from substrate mixture of 50% banana leaves and 50% rice straw.

Oyster mushroom is consumed all over the world due to its taste, flavour, high nutritional Value and some medicinal properteies. Many species of this genus are rich in proteins with essential amino acids, polysaccharides, essential amino acidacid, dietary fibers, important minerals and some vitamins.Because of these nutritional composition and presence of bioactive molecules oyster mushromm have been reported to have anticancer, antihypertensive, anti diabetic and antioxidant.The high

nutritional value and potent medicinal uses suggests that pleurotus mushrooms are important functional foods or nutraceuticals (Savita and Anjana,2017).

Mushrooms have been used as food supplement from time immemorial not only for their flavor , aroma and nutritive values , and but also for their medicinal properties . In the present day world they are known for culinary values due to their high -quality proteins , vitamins , fibers and many medicinal properties . The chemical nature of the bioactive compounds present in the mushroom includes : polysaccharides lipopolysaccharides , proteins , peptides , glycoprotein , nucleoside , lectins ,lipid and their derivatives. Mushrooms are used in folk medicine throughout the world since ancient times as the ultimate health food. (Yashvant *et.al*, 2012)

Anita, 2010 evaluate the yield performance of paddy straw mushrooms (*Volvariella spp.*) on various lignocellulosic wastes. Two species namely *Volvariella volvaceae* and *Volvariella diplasia* were experimentally evaluated on untreated organic wastes including rice bran, wheat bran, rice straw, sawdust, banana leaf and sugarcane baggage supplemented with wheat. The highest yield of *V. volvaceae* (1360g) and *V. diplasia* was obtained from wheat grain with rice bran.

Ritika and Ishita, 2017 studied the cultivation of mushroom in agricultural wastes such as rice straw, wheat straw, cotton straw, tea leave and banana leaves. Banana stalk and bahia grass possess a biological efficiency of 74.4% and 74.12% respectively but there is a low yield when they are supplemented with other components.

Randive, 2012 analysed the cultivation of oyster mushroom on paddy straw and wheat straw. The paddy straw and wheat straw gives very high yield as well as

the nutritional contain like carbohydrate, protein ash, calcium, magnesium, crude fibers and lipid were checked.

Patil, 2012 analysed the cultivation of mushroom (*pleurotus sajor-caju*) on soybeans straw, paddy straw, wheat straw, groundnut straw, sunflower stalk and pigeon pea stalk. Maximum fat and ash content of pleurotus was recorded on groundnut straw.

Belewu and belewn, 2005 studied the cultivation of mushroom (*volvariella volvacea*) on banana leaves. The result shows that full colonization of the substrate was observed in 15 day and the told weight the fruits was 2.5 kg.

Silva *et al.*, 2007 stuided the cultivation of *Pleurotus sajor-caju* on banana stalk and bahia grass based. Substrates the various treatment conditions showed colonization times that varied from 24 to 35 day the banana stalks and bahia grass were both more efficient in the production of the mushroom P*leurotus sajor-caju* when utilized without the addition of other substrates with biological efficiencies of 74.41% and 74.12% respectively.

Vanathi *et al.*, 2016 studied cultivation of mushroom on three different substrates like paddy straw, sugarcane trash, and sorghum stem. Among the three substrates paddy straw was gave highest yield 83.4% of biological efficiency compared with sorghum stem 50.3% and sugarcane trash 44.7%. The biochemical analysis confirms that the protein, carbohydrates, lipids and amino acids in *Pleurotus florida*.

Dlamini *et al.*, 2012 evaluated the gowth and yield of *Pleurotus ostreatus* using four replicate bags of sugarcane tops, maize stover, maize stover and cobs and banana leaves as substrates. The highest yield was obtained from maize stover and cobs followed in decreasing order by banana leaves, sugarcane tops and lastly maize

stover gave the least yield. The maize stover and cobs substrate gave the highest yield which was 221.7, 189.2 and 107.9 g in the first, second and third flashes, respectively.

Hossain, 2017, studied the effect of different substrates such as paddy straw, wheat straw, banana leaves, sugarcane bagasse, sugarcane leaves, newspapers and maize stalks and leaves on spawn running time, primordial initiation time, fruiting body formation time, yield performance and biological efficiency of oyster mushroom (*Pleurotus sajorcaju*). Lowest time required for spawn running, primordial initiation and fruiting body formation was recorded in sugarcane bagasse followed by newspapers, paddy straw, banana leaves and wheat straw. Amongst the substrates, paddy straw showed highest yield and biological efficiency followed by banana leaves, wheat straw, sugarcane bagasse, newspapers and sugarcane leaves. Least yield of mushroom was obtained in maize stalks and leaves.

Edet *et al.*, 2016 studied the phytochemical screening of ethanolic and aqueous extracts showed the presence of secondary metabolites such as alkaloid, glycosides, saponin, tannin, flavonoid, reducing compound, polyphenol, but not phlobatannin, anthraquinone and hydroxymethyl anthraquninone.

Mago *et al.*, 2014 studied the commercial production of *P. sajorcaju* and *P.florida* using different substrates such as paddy straw, leaf litter and saw dust. The highest yield as well as number of sporophores of was achieved paddy straw followed by paddy straw + leaf litter and paddy straw + sawdust. Biological efficiency of *P. sajor-caju* was recorded to be 82.84% on paddy straw, 61.06% on leaf litter and 55.26% on sawdust containing substrate *P. florida* gave bioefficiency of 75.7%, 73.56% and 51.42% on paddy straw, leaf litter and sawdust containing substrates respectively.

Mushrooms have been used as food supplement from time immemorial not only for their flavor , aroma and nutritive values , and but also for their medicinal properties . In the present day world they are known for culinary values due to their high -quality proteins , vitamins , fibers and many medicinal properties . The chemical nature of the bioactive compounds present in the mushroom includes : polysaccharides lipopolysaccharides , proteins , peptides , glycoprotein , nucleoside , lectins ,lipid and their derivatives. Mushrooms are used in folk medicine throughout the world since ancient times as the ultimate health food.(Yashvant *et.al*,2012).

The phytochemical screening of different extracts of Pleurotus florida revealed the presence of different secondary metabolites. Pleurotus florida fruiting bodies were found to contain carbohydrates, alkaloids, glycosides, flavonoids, tannins, saponins and steroids (Nirmala, 2014).

Mushrooms constitude an integral part of the normal human diet and in recent times , the amounts of consumption have been raised greatly , which include variety of species. The oyster mushroom is popularly consumed by all over the world due to their taste , flavor , high nutritional value and medicinal properties . Because of the presence of numerous nutritional composition and various active ingredients in oyster mushroom have been reported to have antidiabetic , antibacterial , antioxidant , eye health and antiviral activities (Krishnamoorthy ,2014).

The use of mushrooms as food is probably as old as civilization and mushrooms currently have greater importance in the diet of mankind. Mushrooms contain reasonable amounts of proteins, carbohydrates, minerals, vitamins and fiber.

MATERIALS AND METHODS

Materials and methods

Collection of substrates (Plate 1)

The different substrates like, paddy straw, banana leaf and sugarcane bagasse were collected from paddy field, banana cultivars and sugarcane juice vendors respectively. The selected substrates were dried under sunlight and stored in bags for further experiments.

Spawn

Spawn of *Pleurotus spp.* prepared on Sorghum grains were collected from (MSM Mushroom Corner, Mushroom cultivation training and seeds sale, Tirunelveli).

Substrate preparation (Plate 1)

The selected substrates (paddy straw, banana leaf and sugarcane bagasse) were grouped into six treatments of 500g each. The treatments include paddy straw (T1), banana leaf (T2), sugarcane bagasse (T3), 1:1 mixture of paddy straw + banana leaf (T4), 1:1 mixture of banana leaf + sugar cane bagasse (T5) and 1:1 mixture of paddy straw + sugarcane bagasse (T6).

Construction of beds and spawning (Vanathi *et al.*, 2016) (Plate 1)

Treatments were soaked individually for 24 hrs and sterilized at 121°C for 20 - 30 minutes. The sterilized substrates were allowed to cool and filled in polypropylene bags with alternative rows of substrate and spawn. Holes were made on the bags using stainless steel needle or knife for aeration. Then the bags were hanged with ropes inside the spawn running room. Inside the room, temperature range of 24°C – 29°C and relative humidity of 60 – 90% was maintained by spraying water on the sides and floor of spawn

Plate 1: Collection of substrates, substrate preparation, construction of beds and spawning of *Pleurotus spp.*

running room 2 – 3 times per day. To avoid errors each treatment has been carried out in three times and the results are presented in mean values.

Harvesting

After the formation of mature fruiting bodies the pileus edges started to fold and the mushrooms were ready for harvesting. Usually it was done in the morning and stored for further use.

Data collection

The mushroom bags were frequently observed from its packing to harvesting to record different parameters such as number of days for starting and completion of spawn running, time required for pinhead initiation, number of primordia, number of effective fruiting bodies, mushroom pileus diameter (cm) and mushroom stipe length (cm), Mushroom yield (g) (Dlamini *et al.*, 2012), biological efficiency (%), production rate (%) and organic mass loss (%) (Carvalho *et al.*, 2012) were calculated by using the following formula:

$$\text{Biological efficiency} = \frac{\text{Fresh weight of mushroom}}{\text{Dry weight of substrate}} \times 100$$

$$\text{Organic mass loss} = \frac{\text{Initial substrate dry mass - residual}}{\text{Initial substrate dry mass}} \times 100$$

Sample preparation

The collected mushrooms from various treatments was cut into slices and shade dried. Then the dried samples were powdered in a mixer grinder and sieved to get uniform particles. The sample powder was stored for further use.

Extract preparation

The powder was steeped in water, methanol and chloroform (5gm/100ml) in a closed flask for twenty hours separately, shaking them frequently during six hours and allowed to stand at room temperature. The clear supernatant of the each extract was decanted and used to determine the phytochemical constituents.

Biochemical analysis of mushroom (Harborne, 1998)

Test for Alkaloids

Mayor's test:

Dissolved filtrate 1 ml treated with Mayor's reagent (Potassium mercuric iodide). Formation of a yellow coloured precipitate indicatedthe presence of alkaloids. (Mercuric chloride + few drops of Iodine solution)

Test for Terpenoids

Crude extract 2 ml was dissolved in 2 ml of chloroform and evaporated to dryness. To this 2 ml of Con H_2SO_4 was added and heated for about 2min. A grayish colour indicated the presence of terpenoids.

Test for Phenol and Tannin

Crude extract was mixed with 2 ml of 2% solution of $FeCl_3$. A blue green (or) black colorization indicated the presence of phenol and tannin.

Test for sugar

The little amount of substance mixed with equal volume of Fehling's A and B solution heated in water bath. Formation of red colour indicated the presence of sugar.

Test for Saponins (Froth test)

To 3 ml of extract were diluted with 2 ml of distilled water and this test tube was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicated the presence of saponin.

Test for Quinines

To the 1% test substance 2% sodium hydroxide was added. Blue green (or) red colour indicated the presence of quinines.

Test for Protein

The 4% extract were treated with few drop of concentrated nitric acid. Formation of yellow colour indicated the presence of protein.

Test for Sterols

The 3 ml of crude extract was mixed with 2 ml of chloroform and con H_2SO_4 was added sidewise. A red colour is produced in the lower chloroform layer indicated the presence of steroids.

Moisture content (Randive, 2012)

Moisture was determined by drying the fresh mushrooms at 80°C to a constant weight in an oven. Moisture content was determined by following formula

$$\textbf{Moisture content} = \frac{\textbf{Initial weight} - \textbf{final weight}}{\textbf{Initial weight of sample}} \times 100$$

Nutrient analysis

Estimation of carbohydrates (Hedge and Hofreiter, 1962)

Principle

Carobohydrate is first hydrolysed into simple sugars using dilute hydrochloric acid. In hot acidic medium glucose is dehydrated to hrdroxymethyl furfural. This compound forms with anthrone a green coloured product with absorption maximum at 630 nm.

Reagents:

A. 2.5 N HCl

B. Anthrone reagent: 0.2% anthrone was dissolved in ice cold concentrated sulphuric acid. Prepared fresh before use.

Procedure

Weighed 100mg of the sample into a boiling tube, hydrolysed by keeping it in a boiling water bath for three hours with 5 ml of 2.5N HCl and cooled to room temperature. Neutralised it with soild sodium carbonate until the effervescence ceas and take 0.2 to 1.0ml for analysis. Then added 4 ml of anthrone reagent, heated for eight minutes in a boiling water bath, colled rapidly and read the green to dark green colour at 630 nm.

Estimation of total soluble protein (Lowry *et al.*, 1951)

Reagents

- 10% trichloroacetic acid (TCA).
- 0.2N sodium hydroxide
- Alkaline copper reagent:

Solution A: 2% sodium carbonate in 0.1N sodium hydroxide

Solution B: 2% 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 – Ciocalteau reagent

  Commercial reagent was prepared freshly in the ratio of 1:1 with distilled water.

Extraction and estimation of protein:

One gram of plant tissue was weighed and ground it with 10 ml of distilled water. The plant extract was filtered through a filter paper. To the filtrate, 10 ml of cold 5% TCA (trichloro acetic acid) was added to precipitate protein. It was centrifuged and the pellet was collected that contains protein. The pellet was dissolved in 10 ml of 0.1N NaOH. To 1 ml of this protein extract, 5 ml of alkaline copper reagent and 0.5 ml of Folin – Ciocalteau reagent were added. The absorbance was measured using red filter (650nm). The absorbance extrapolated in the standard graph and the amount of protein present in 1 gm of plant tissue was calculated.

Estimation of free Amino acids (Jayaraman, 1981)

Principle

The ninhydrin is powerful oxidizing agent. All amino acids in the presence of ninhydrin get decorboxylated and deaminated resulting in ammonia, carbondioxide, the corresponding aldehyde, and a reduced form of ninhydrin. The liberated ammonia then reacts which an additional mole of ninhydrin and the reduced ninhydrin to yield a purple substance, which has absorption maxima at 570nm.

Reagents

A. Standard amino acid solution Dissolved 10 mg of aspartic acid in small volume of (0.1N) HCl and make up with distilled water to the 100 ml mark in the standard flask.

B. Ethanol (80%)

C. Ethanol (50%)

D. Ninhydrin reagent

Prepared by dissolving 2g of ninhydrin in 25 ml of acetone. To this 25 ml of 0.2 M acetone buffer (pH 5.5) was added and stored in a brown bottle.

Procedure

10 ml of samples was homogenized with 80 percent ethanol in a pestle and mortar. The homogenate was centrifuged at 5000 rpm. The clear supernatant was made upto a known volume. From this 1 ml was pipetted out in to a test tube and diluted to 4ml with distilled water. To this, 1 ml of ninhydrin reagent was added and kept in boiling water bath for 15 minutes. The test tubes were then cooled and 1 ml of 50 percent ethanol was added. The purple colour developed was measured in spectronic 20 at 540nm.

Determination of total lipid (Folch *et al.*, 1957)

To 500 mg of dried algal powder taken in a screw capped test tube, 10 ml of 2:1 CHCl3: CH3OH solvent mixture was added. The tube was loosely capped and heated in a water bath at 60°C for 30 min. After cooling the solution, the volume was made up to 10 ml with the solvent mixture. 0.4 ml of the extract was pipetted in a separate test tube, allowed to dry completely and digested with 0.4 ml of cone. H_2SO_4 by boiling in a water bath for 10 min. After cooling the tube, 5 ml of phosphovanillin reagent was added and allowed to stand for 30 min for colour development. The absorbance was then measured

at 520 nm against a reagent blank using UV-Visible spectrophotometer. Cholesterol was used as standard.

RESULT AND DISCUSSION

Result and Discussion

Spawn running, and pin head formation:

The average number of days taken for spawn running was significantly different among different treatment. Time required for completion of spawn running and pin head initiation is presented in **Table 1**. Lowest time required for completion of spawn running was observed in T2, T3 and T6 (12 days) followed by T1, T4, T5 (13 days) respectively. Our results are in accordance with the findings of Tan (1981) who reported that *Pleurotus ostreatus* took 7 to 21 days for complete spawn running. Bhatti *et al.*, 1987 stated that the number of days to complete spawn running on different substrates might be due to variation in C: N ratio. Mondal *et al.*, (2010) also supported the results, who found that the presence of right proportion of alpha-cellulose, hemi-cellulose and lignin is responsible for higher mycelium running rate in banana leaves and rice straw.

Similar to mycelial colonization, pin head formation was significantly different among various treatments. The first pin head was recorded in T1, T2 and T6 (14 days) after spawn inoculation. The maximum duration of pin head appearance was observed in T5, T4 (15 days) and T3 (19). The results were indicated in **Table 1**. According to the report of Fridaus *et al.*, 2015 the number of days for the emergence of pin head and fruiting bodies after spawn running were about 3 – 4 days and 6 – 7 days respectively. Ruhl *et al.*, 2008 also reported 4 days for pin head appearance after spawn running.

Number of pin head, primordia and effective fruiting bodies (Plate 2a and 2b):

Table 2 showed the number of pin head, primordia and fruiting bodies formed during different treatments. The results showed that the highest number of pin head (202), primordia

Table 1: Effect of different treatments on spawn running and pin head formation of *Pleurotus spp.*

S. No.	Treatments	Spawn running (Day)	Pin head formation (Day)
1.	T1	13	14
2.	T2	12	14
3.	T3	12	19
4.	T4	13	15
5.	T5	13	15
6.	T6	12	14

*Mean

T1: Paddy straw
T2: Banana leaf
T3: Sugarcane Bagasse
T4: Paddy straw + Banana leaf
T5: Banana leaf + Sugarcane bagasse
T6: Paddy straw + Sugarcane bagasse

Table 2: Effect of different treatments on number of pin head, primordia and number of effective fruiting bodies of *Pleurotus spp.*

S. No.	Treatments	Number of pinhead	Number of primordia	Number of effective fruiting bodies
1.	T1	132	70	60
2.	T2	155	123	48
3.	T3	202	95	12
4.	T4	172	162	136
5.	T5	125	109	35
6.	T6	114	49	22

*Mean

T1: Paddy straw
T2: Banana leaf
T3: Sugarcane Bagasse
T4: Paddy straw + Banana leaf
T5: Banana leaf + Sugarcane bagasse
T6: Paddy straw + Sugarcane bagasse

Table 3: Comparison of growth performance of *Pleurotus spp.* on different treatments

S. No.	Treatments	Mushroom pileus diameter (cm)	Mushroom stipe length (cm)	Mushroom yield (g)
1.	T1	8.3	2.9	229.3
2.	T2	7.5	2.5	252.67
3.	T3	8.3	3.8	68.3
4.	T4	11	4	462
5.	T5	8	2	199
6.	T6	5.5	2	208

*Mean

T1: Paddy straw
T2: Banana leaf
T3: Sugarcane Bagasse
T4: Paddy straw + Banana leaf
T5: Banana leaf + Sugarcane bagasse
T6: Paddy straw + Sugarcane bagasse

Plate 2a: Different stages of *Pleurotus spp.* grown in various treatments

T1: Paddy straw
T2: Banana leaf
T3: Sugarcane Bagasse
T4: Paddy straw + Banana leaf
T5: Banana leaf + Sugarcane bagasse
T6: Paddy straw + Sugarcane bagasse

Plate 2b: Different stages of *Pleurotus spp.* grown in various treatments

T6

T1: Paddy straw
T2: Banana leaf
T3: Sugarcane Bagasse
T4: Paddy straw + Banana leaf
T5: Banana leaf + Sugarcane bagasse
T6: Paddy straw + Sugarcane bagasse

(162) and fruiting bodies (136) were found in T3 and T4 whereas the lowest number of pin head and primordia were observed in T6 (114 and 149) and fruiting bodies were noted in T3 (12).

Mushroom pileus diameter and stipe length

The stipe length and pileus diameter of the mushroom depend on the environmental conditions (Sánchez, 2004) and the moisture holding capacity (Chukwurah *et al*..2013) of the substrates. However, mushrooms with relatively bigger pileus and wider stipes are the marketable quality. Pileus diameter and stipe length of harvested mushroom among various treatments were measured with ruler and results are indicated in **Table 3**. Mushroom pileus diameter ranged from 11 to 5.5 cm. Similarly, stipe length of mushrooms from different treatments was ranged in 4 to 2 cm. The mushroom harvested from banana leaf combined with paddy straw (T4) showed a significantly increased pileus diameter (11 cm) and stipe length (4 cm).

Mushroom Yield:

Banana leaf with paddy straw treatment had great effect on mushroom yield. The highest biological yield (462g on fresh weight) was found in T4 which was followed by T2 (252.67g), T1 (229.3g), T6 (208g), T5 (199g) and T6 (68.3g) which was indicated in **Table 3.** Similar results were also recorded by Mondal *et al*., 2010. The results suggested that the maximum number and yield of mushroom was recorded on the substrate paddy straw combined with banana leaf. Mijan Hossain, 2017 found that the highest number of fruiting bodies was harvested from paddy straw substrate followed by banana leaf, wheat straw and sugarcane bagasse. Haque (2004) and Al Amin (2004) also supported the same result.

Biological efficiency

Biological efficiency (BE), which represents conversion percentage of the substrate into fungal biomass (mushrooms) and are presented in **figure 1a**. Banana leaf combined with paddy straw (T4) showed highest percentage of biological efficiency (92.4%), whereas other treatments showed relatively least percentage (T2: 50.53%, T1: 45.86, T6: 41.6, T5: 39.8 and T3: 13.66) of biological efficiency. Adenipekum and Omolaso, 2015 stated that the substrate which possesses highest yield shows a maximum percentage of biological efficiency.

Organic mass loss

The organic matter loss (OML) was also evaluated, which represents the decomposition of the substrate by the fungus, determined by the formula. The organic mass loss (OML) represents the decomposition of the substrate by the fungus. The organic mass loss for our study was expressed in **figure 1b**. The result showed that organic mass loss was higher in T4 (38.5) and minimum in T3 (14.6). Zadrazil *et al.*, 1978 reported that OML is due to removal of CO_2 and H_2O from substrates by the fungus. Ruegger *et al.*, 2001 stated that the highest organic mass loss was related to biological efficiency. Our study report also expressed the same that organic mass loss was related with biological efficiency. The treatment (T4) which possesses highest organic mass loss (38.5%) shows a maximum percentage of biological efficiency (92.4%).

Moisture content

Figure 2 illustrated the moisture content of mushroom harvested from different treatments. Fresh mushroom contain about 85 – 95% (Ashok Kumar *et al.*, 2013). Our findings are in accordance with Tulek, 2011. The moisture content of mushroom harvested from various treatments was found about mostly greater than 90%.

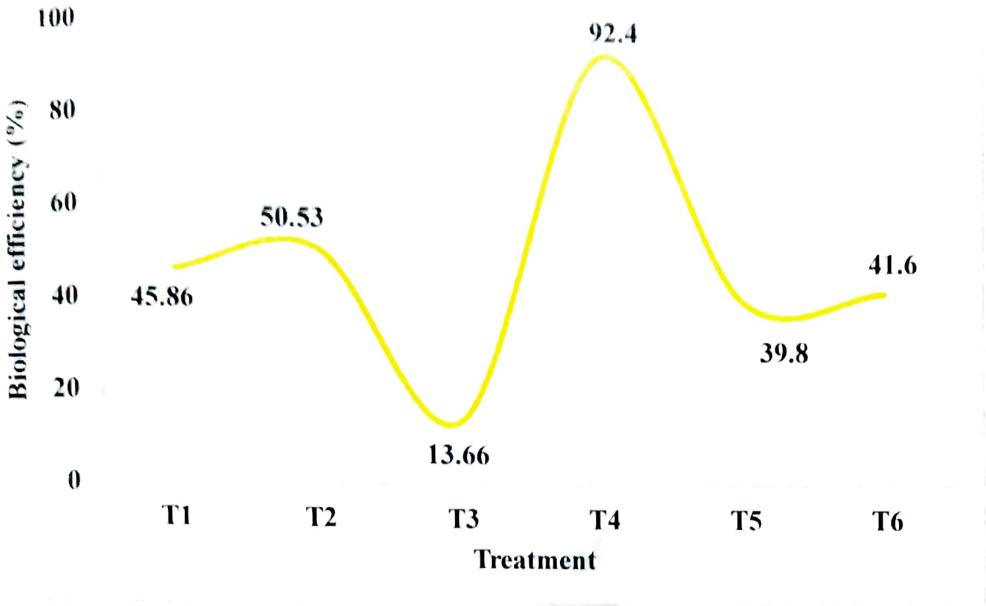


Figure 1a: Effect of different treatments on biological efficiency of *Pleurotus spp.*

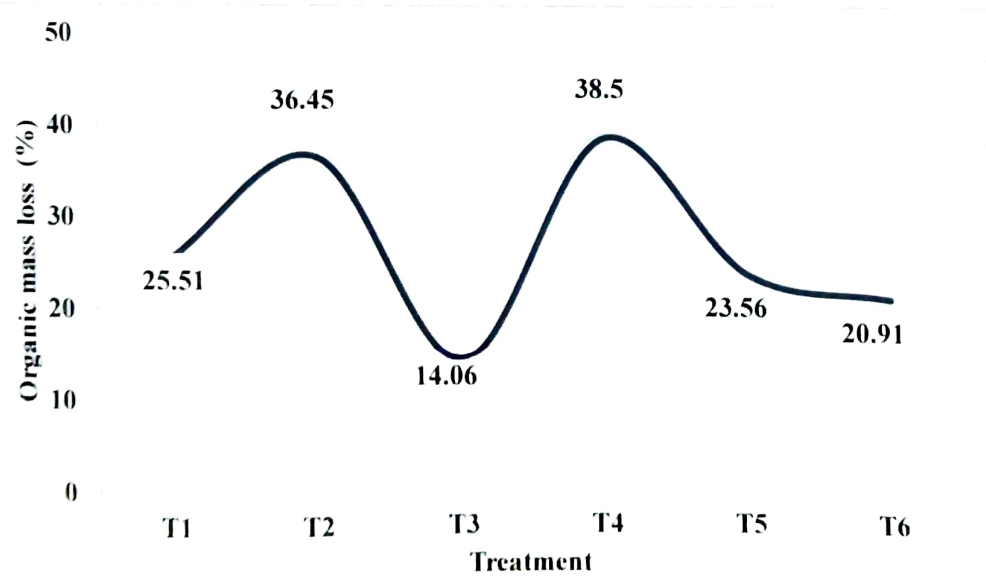


Figure 1b: Effect of different treatments on organic mass loss of *Pleurotus spp.*

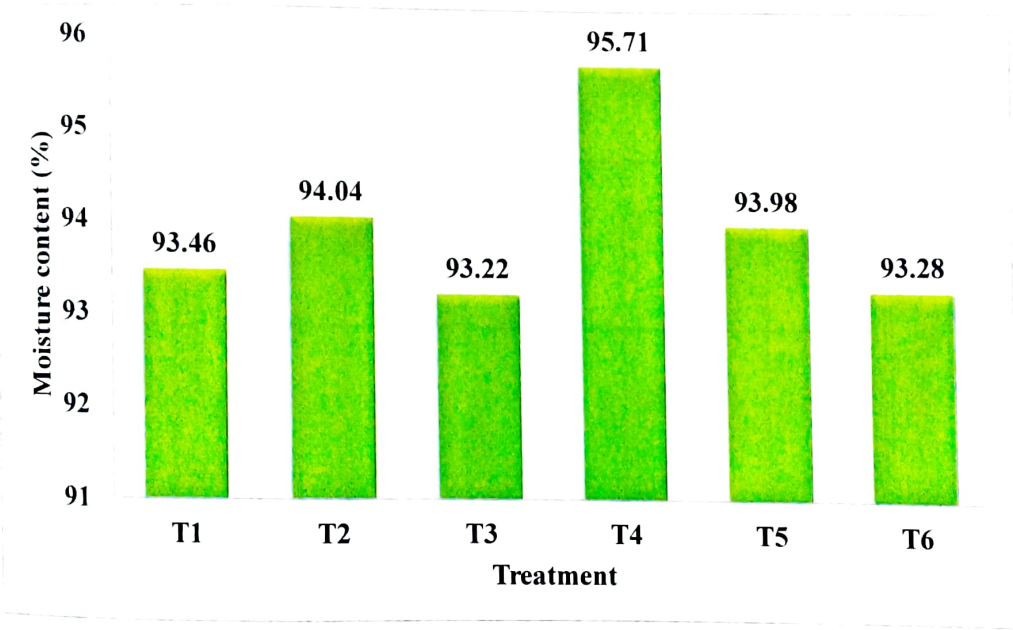


Figure 2: Effect of different treatments on moisture content of *Pleurotus spp.*

Table 4: Biocemical analysis of mushroom harvested from different treatments

S.NO	Treatment	Solvent tested	Alkaloids	Terpenoids	Phenol and tannin	Saponins	Quinines	Protein
1	T1	Water	+	-	+	+	-	+
		Chloroform	-	+	-	+	-	+
		Methanol	+	+	+	+	-	+
2	T2	Water	+	-	-	+	-	+
		Chloroform	-	+	-	+	-	-
		Methanol	+	+	+	-	-	+
3	T3	Water	-	-	-	+	-	+
		Chloroform	-	+	-	-	-	-
		Methanol	+	+	+	-	-	+
4	T4	Water	-	-	-	+	-	+
		Chloroform	-	+	-	-	-	-
		Methanol	+	+	+	+	-	+
5	T5	Water	+	-	-	+	-	+
		Chloroform	-	+	-	+	-	-
		Methanol	+	+	+	+	-	+
6	T6	Water	-	-	+	+	-	+
		Chloroform	-	+	-	+	-	-
		Methanol	-	+	+	+	-	+

T1: Paddy straw
T2: Banana leaf
T3: Sugarcane Bagasse
T4: Paddy straw + Banana leaf
T5: Banana leaf + Sugarcane bagasse
T6: Paddy straw + Sugarcane bagasse

Biochemical analysis

Biochemical analysis of mushroom harvested from various treatments disclosed the presence of alkaloids, terpenoids, phenol, tannin, saponins and protein. Among the different solvent tested methanol extract showed more phytoconstituents than chloroform and water which are presented in **Table 4**. Our results were coinciding with the results of Parihar *et al.*, 2015 revealed that methanolic extract showed the presence of alkaloids, saponins, phenols, glycosides and terpeoids. Joshi *et al.*, 2014 also stated the presence of saponins, tannins, flavonoids, alkaloids, phenols, steroidal glycosides and terpenoids in methanolic and aqueous extract.

Nutritional analysis

Carbohydrates are the important component of dry mushroom and are helpful for proper functioning of digestive tract (Kalac, 2012). Total carbohydrate contents of mushroom were presented in **figure 3**. It is clear that, T2 (12.5 ± 0.04 mg/g DW), T3 (12.6 ± 0.02 mg/g DW) and T5 (12.3 ± 0.03 mg/g DW) possessed maximum amount of carbohydrate among various treatments tested. It was followed by T1 (8.2 ± 0.03 mg/g DW), T4 (8.3 ± 0.04 mg/g DW) and T6 (7.8 ± 0.02 mg/g/ DW).

Total protein content of mushrooms grown on different substrates varied from 10.6 ± 0.1 mg/g DW (T1) for T4 to 7.8 ± 0.05 mg/g DW for T4 which was showed in **figure 4**. Akyuz and Kirbag 2010, reported that mushrooms are rich source of diverse proteins, whereas, the content of protein may vary according to strains, physical and chemical differences of the substrates.

Figure 5 shows the amount of amino acid variation amount variety of treatments. The result reveals that the highest amount was noted in T3 (206 ± 4 mg/g DW) and the least amount was in T6 (144 ± 3 mg/g DW). On the other hand Guo *et al.* (2007), the amino acids in dried *Pleurotus spp.* was 8440 mg and 19,200 mg/100 g dry matter respectively.

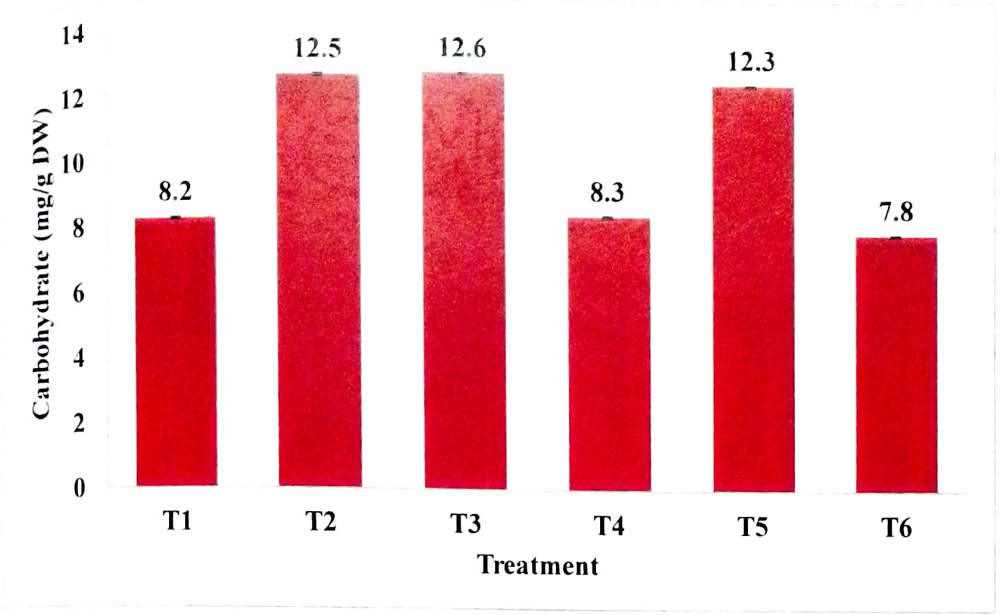


Figure 3: Effect of different treatments on carbohydrate content of *Pleurotus spp.*

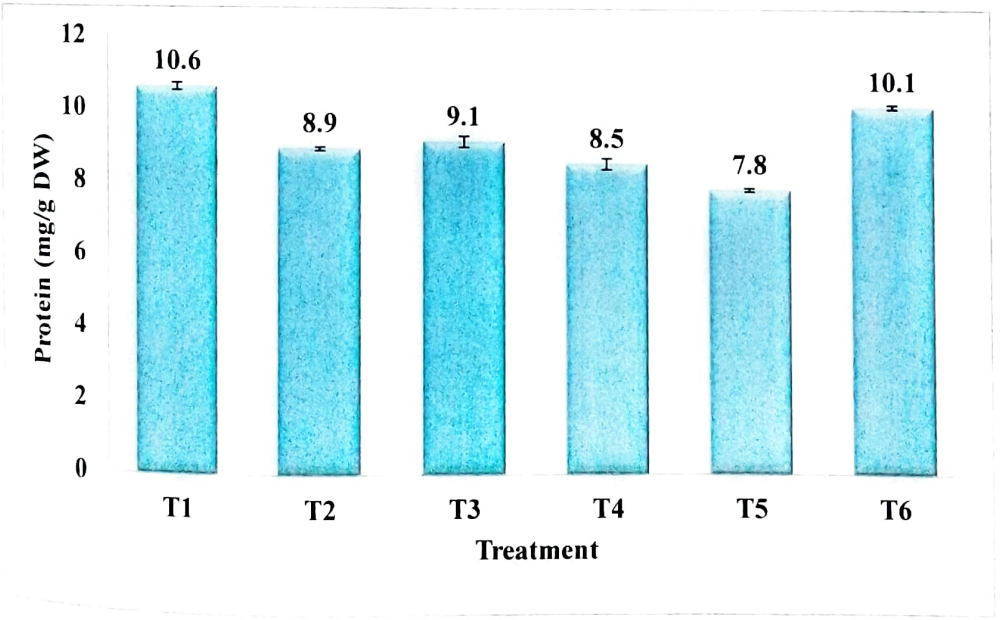


Figure 4: Effect of different treatments on protein content of *Pleurotus spp.*

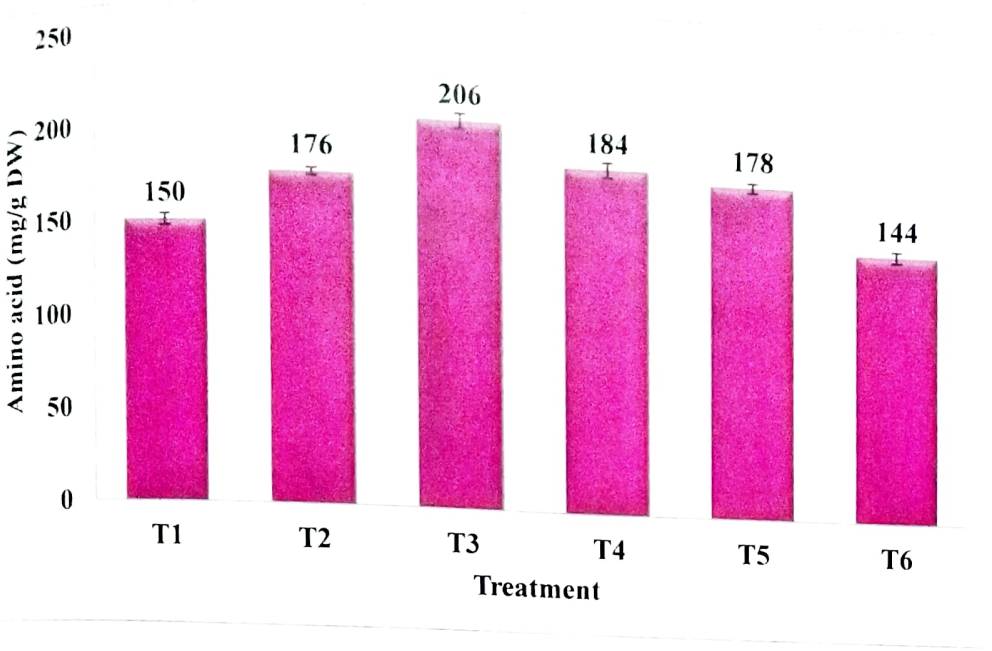


Figure 5: Effect of different treatments on amino acid content of *Pleurotus spp.*

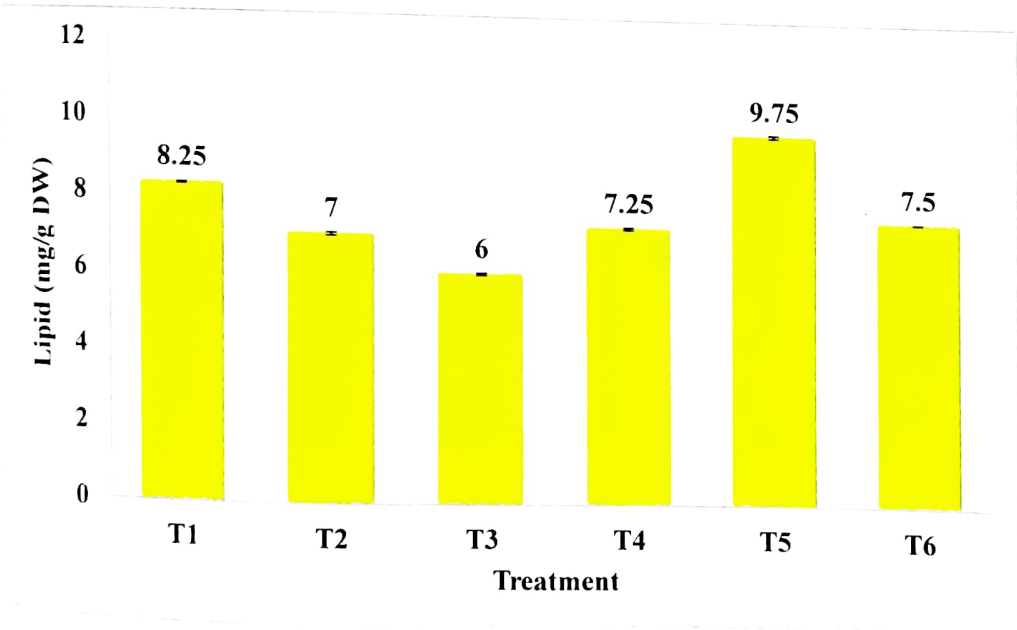


Figure 6: Effect of different treatments on lipid content of *Pleurotus spp.*

Pleurotus mushroom are usually low in fat content. In the present study, *Pleurotus spp.* harvested from T5 was gave highest amount of total lipid (9.75 ± 0.04 mg/g DW) and the lowest amount was noted in T6 (6 ± 0.03 mg/g DW) and are presented in **figure 6.** Our results are related with the lipid content of *Pleurotus* ranged from 0.2 to 8g/100g dried fruit bodies (Hossain *et al.* 2007).

SUMMARY AND CONCLUSION

Summary and Conclusion

Mushroom have been a widely used as food and are considered as an important food time concerning human, health, nutrition and disease prevention. (Chang, 1996). Dietary mushrooms provide a wide variety of medicinal properties such as anticancer, antibiotic, antiviral activities, immunity and blood lipid lowering effects. Mushrooms are rich in protein, minerals and vitamins and they contain an abundance of essential amino acids (Sadler, 2003). Nutritional composition of the substrate depends on the selected strains, the composition of the substrate, the method of cultivation, stage of harvesting etc. (Benjamin, 1995).

In this study, substrates such as paddy straw, banana leaf and sugarcane bagasse were selected. The selected substrates were tested individually and mix equally (1:1) with other substrates to evaluate the growth parameter of the selected mushroom *Pleurotus spp.*

Among the various substrates tested, banana leaf mix with paddy straw (T4) possess a high degree of spawn running (13 days), pin head formation (19 days), number of pin head (72), primordia (162) and fruiting body development (136) when compared to other treatments. The treatment T4 also produce a significant fruiting body with maximum pileus diameter (3 cm) and stipe length (4 cm).

Regarding mushroom yield, T4 treatment produce maximum yield of 462g per 500g of dry weight substrate. Biological efficiency and organic mass loss are more or less related with each other in our study. The treatment (T4) which possesses a high biological efficiency also shows maximum organic mass loss.

Biochemical analysis of the harvested mushroom revealed the presence of alkaloids, terpenoids, phenol, tannin, saponins and protein in methanolic extract when compared to other

solvent. Nutritional analysis of mushroom confirmed the presence of essential nutrients like carbohydrate, protein, amino acid and lipid. The variation in their nutrient content may because of the composition of the substrate, spawn quality, the quantity of spawn used for constructing beds, environmental influences etc.

From the above findings, the lignocellulosic nature of the substrate banana leaf and paddy straw may suitable for the growth of *Pleurotus spp*. Thus in conclusion, the substrate banana leaf combined with paddy straw employed as a vital substrate for mushroom cultivation.

BIBLIOGRAPHY

BIBLIOGRAPHY

Adenipekum, C. O., and Omolaso, P.O., "comparative study on cultivation , yield performanceand proximate composition of Pleurotus pulmonarius Fries (Quelet) on rice straw and banana leaves, *World Journal of Agricultural Science., vol. 11, pp. 151- 158. 2015.*

Ahmed, M., Abdullah, N., Ahmed, K. U., and Bhuyan, M. H. M. B., 2013. Yield and nutritional composition of oystermushroom strains newly introduced in Bangladesh. *Pesquisa Agropecuária Brasileira. 48: 197 – 202.*

Ahmed, S.A., Kadam, J.A., Mane, V.P., Patil, S.S., and Baig, M.M.V., (2009). Biological efficiency and nutritional contents of *Pleurotus florida* (Mont.) Singer cultivated on different agro-wastes. *Nature and science*, 7: 44-48.

Al Amin, M. A., 2004. Studies on mycelium, spawn and production of certain edible mushrooms. *Master of Science Thesis, Department of Biotechnology, Bangladesh Agricultural University, Mymensingh, Bangladesh.*

Anita, T., 2010. Yield Evaluation of Paddy Straw Mushrooms (*Volvariella spp.*) on Various Lignocellulosic Wastes. *International Journal of Applied Agricultural Research* ISSN 0973-2683 Volume 5 Number 3. pp. 317–326.

Ashok Kumar, A., and Gurdeep,S., (2013). Effect of different pretreatments on the quality of mushrooms during solar drying. *Journal of Food Science Technology. 2013.* 50: 165–170.

Beetz, A., and Kustida, M., 2004. Mushroom cultivation and marketing. ATTRA Publication. IP 087.

Bhatti, M.A., Mir, F.A. and Siddiq, M., 1987. Effect of different bedding materials on relative yield of oyster mushroom in the successive flushes. *Pakistan Journal Agricultural Sciences. Res.* 8: 256 - 259.

Carvalho, C. S. M., Aguiar, L. V. B., and Andrade, M. C. N., 2012. Applicability of the use of waste from different banana cultivars for the cultivation of the oyster mushroom. *Brazilion Journal Of Microbiology.* 43: 819 – 826.

Carvalho, M. P., Der Sand, S. T. V., Rosa, E. A. R., Germani, J. C., and Ishikawa, N. K., 2007. Investigation of the antibacterial activity of basidiomycetes. *Biociencias, Porto Alegre*. 15: 173-179.

Chang, S. T., and Quimio, T. H., (Eds.). (1982). Tropical mushrooms: biological nature and cultivation methods. Biological nature and cultivation methods. *Chinese University Press.*

Chukwurah, N.F., Eze, S.C., Chiejina, N.V., Onyeonagu, C.C., Okezie, C.E.A., Ugwuoke, K.I., Ugwu, F.S.O., Aruah, C.B., Akobueze, E.U., and Nkwonta, C.G., Correlation of stipe length, pileus width and stipe girth of oyster mushroom (*Pleurotus ostreatus*) grown in different farm substrates. *Journal of Agricultural and Biotechnology Sustainable Development. 2013;5(3):54–60.*

Dahmardeh, M., Hossienabadi, R., and Safarpoor, H., 2010. *Journal of Food Agricultural Environment,* 8, 996.

Dlamini, B. E., Earnshaw, D. M. and Masarirambi, M. T., 2012. Growth and Yield Response of Oyster Mushroom (*Pleurotus ostreatus*) Grown on Different Locally Available Substrates 1 *Current Research Journal of Biological Sciences.* 4: 623-629.

Dundar, A., Acay, H., and Yildiz, A., 2009 Effect of using different lignocellulosic wastes for cultivation of *Pleurotus ostreatus* (Jacq.) *P. Kumm.* On mushroomyield, chemical composition and nutritional value. *African Journal of Biotechnology*. 18:662.

Edet, U. O., **Ebana, R. U. B., Etok, C. A. and Udoidiong, V. O., 2016.** Nutrient Profile and Phytochemical Analysis of Commercially Cultivated Oyster Mushroom in Calabar, South-South NigeriaAdvances in Research.

Firdaus, **S.M., ShamsulBahri, A.R., Rahijan, M., AbdWahab Rahman, A., Rohana, M., and Ibrahim, R.,** *Advances in Life Science and Technology. 28 (2015)*

Folch, **J., Lees, M., and Sloane-Stanley, G., (1957).** A simple method for the isolation and purification oftotallipids from animal tissue. *Journal of biologicalcChemistry*. 226: 497- 509.

Fukushima **M. 2000.** LDL receptor mRNA in ratsis increased by dietary mushroom. (*Agaricusbisporus*) fibre and sugar beef fibre. *J.Nutr*., 130:2151-2156

Haque, **A. B. M. A., 2004**. Use of different substrates and methods on the mycelial growth, spawn production and yield of three mushroom species. *Master of Science Thesis, Department of Biotechnology, Bangladesh Agricultural University, Mymensingh, Bangladesh.*

Harborne, **J.B. 1998**. Phytochemical methods: A guide to modern technique of plant analysis', Champman and Hall, London.

Hedge, **J.E., and Hofreiter, B.T., (1962)**. In: Methods in carbohydrate chemistry. Vol 17. (Eds.) **Whistler, R.L., and BeMiller,** *Journal of nutrient academic press*, New York, 420.

Hossain, M. D. M., 2017. Effect of Different Substrates on Growth and Yield of Oyster Mushroom (Pleurotus sajorcaju). *International Journal of Current Microbiology and Applied Sciences.* 6: 760-764.

Imtiaj, **A., and Rahman, S.A., 2008.** Economic viability of mushroom cultivation to poverty reduction in Bangladidesh. *Tropical and Subtropical Agroecosystems.* 8: 93 - 99.

Jayaraman, J., 1981. Laboratory manual in biochemistry, Wiley-Eastern company limited, Madras. 1- 65.

Kang, S.W. 2004. "What is oyster mushroom," in Mushroom Growers Handbook. 48–51, Mushroom World. 1st edition. 48–51.

Kim, B. S. 2004. Mushrooms Worldwide. Part II. Oyster mushrooms. Mushroom Storage and Processing. In: Mushroom Growers' Handbook 1, Oyster Mushroom Cultivation. *MushWorld*. 192-196.

Krishnamoorthy, Deepalakshmi ,Sankaran, Mirunalini. 2014 .A study on the oyster mushroom with nutritional and medicinal properties.718-726.

Lourdes, A.T., Arvin, S.M.A., and Jerremy, B.R. 2008. Agronomic Responses of Oyster Mushroom (*Pleurotus ostreatus*.) on Different Agricultural Wastes as Substrates.

Lowry, O.H., Rosenbrough, N.J., Farr, A.L. and Randall, R.J., 1951. Protein measurement with the Folin-phenol reagent. *Journal Of Biological Chemistry*. 193: 265 – 275.

Mago, P., Gunwal, I., Singh, L., and Awasthi, D., 2014. Commercial production of Oyster mushroom. Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT) e-ISSN: 2319-2402,p- ISSN: 2319-2399.Volume 8, Issue 6 Ver. III.

Manson, J.C., Sims, P.G.G., and Broad, P., (1989): Biological routes to improve digestibility of animal feeds In: Biotechnology in livestock in developing countries. Edited by Hunter, A. G. Proceeding of an International Conference on the Application of Biotechnology to livestock in developing countries. *University of Edinburgh. 4-8 Sept. 1989* organized by the staff of the Center for Tropical Veterinary Medicine.

Mathews, A.V., Mathi, G., and Suharban, M., 1996. Performance evaluation of five species of *Pleurotus* (Oyster mushroom) in Kerala. Mushroom Result. 5 9

Mijan Hossain, M.D., Effect of Different Substrates on Growth and Yield of Oyster Mushroom (Pleurotus sajorcaju. *International Journal of Current Microbiology and Applied Sciences* ISSN: 2319-7706 Volume 6 Number 12 (2017) pp. 760-764.

Mondal, S.R., **Rehana, M.J., Noman, M.S., and Adhikary, S.K. 2010.** Comparative study on growth and yield performance of oyster mushroom (*Pleurotus florida*) on different substrates. *Journal of the Bangladesh Agricultural University.* 8: 213 –220.

Mondal, S.R., **M.J. Rehana, M.S. Noman, S.K. Adhikary,** Comparative study on growth and yield performance of oyster mushroom (Pleurotusflorida) on different substrates *(Agrotechnology Discipline, Khulna University, Bangladesh 2010).*

Nirmala **Gupta. 2014.**Preliminary phytochemical screening of different extracts of *Pleurotus florida. Columbia Journal of Pharmaceutical Sciences.* 1: 23-26.

Obodai, **M., Cleland-Okine, J., and Vowotor, K. A. 2003.** Comparative study on the growth and yield of Pleurotusostreatus mushroom on different lignocellulosic by-products. *Journal of Industrial Microbiology and Biotechnology.* 30:146 –149.

Owaid, **M.N., Al-Saeedi, S.S.S., and Al-Assaffii, I.A. 2015a**. Antimicrobial activity of mycelia of oyster mushroom species (*Pleurotus spp.*) and their liquid filtrates (in vitro). *Journal of Medical and Bioengineering*, Anbar, 4: 376-380.

Owaid, **M.N., Raman, J. Lakshmanan, H., Al-Saeedi, S.S.S., Sabaratnam, V., Al-Assaffii, I.A.A., 2015b.** Mycosynthesis of silver nanoparticles from Pleurotuscornucopiae var. citrinopileatus and its inhibitory effects against *Candida sp.* Materials Letters. *Anbar,* 153: 186 – 190.

Patil, S.S., **2012**. Cultivation of mushroom (*pleurotus sajor-caju*) on soybeans straw, paddy straw, wheat straw, groundnut straw, sunflower stalk & pigeon pea stalk. *Science research reporter*. 225-228.

Ramanathan, G., Vinodhkumar,T., Abinaya pallavi,T., and J.Immanuel suresh,J., 2013. Evaluation Of Effect Of Different Substrates On Mushroom Production And Their Bioactive Potential *International Research Journal of Pharmaceutical* and Applied Sciences. 3:10 - 15.

Randive , S.D., Cultivation and study of growth of oyster mushroom on different agricultural waste substrate and its nutrient. *Advances in Applied Science Research*, 2012, 3 (4):1938-1949.

Ritika K. and Ishita tiwari. 2017. Cultivation of mushroom in agricultural waste. *European journal of experimental biology*.

Ruegger, M.J.S., Tornisielo, S.M.T., Bononi, V.L.R., Capelari, M. (2001). Cultivation of the edible mushroom Oudemansiella canarii (Jungh.) höhn. in lignocellulosic substrates. *Brazillian Journal of Microbiology. 32 (3), 211-214.*

Ruhl, M., Fischer, C., and Kues, U., (2008). Ligninolytic enzyme activities alternate with mushroom production during industrial cultivation of on wheat straw-based substrate. *Pleurotus ostreatusCurrent Trends in Biotechnology and Pharmacy, 2(4), 478-492.*

Sanchez, C., Modern aspects of mushroom culture technology. *Applied Microbiology and Biotechnology. 64 (6) (2004), pp. 756-762.*

Sangwan, M.S., and Saini, L.C., (1995). Cultivation of *Pleurotus sajorcaju* (Fr.) Singer on agro-industrial wastes. Mush Res, 4 33.

Soniya. S., Ram Kailash, P. Y., Chandra P. Pokhrel.2013. Growth and Yield of Oyster mushroom (Pleurotus ostreatus) on different substrates. *Journal on New Biological Reports* 2(1): 03-08 ISSN 2319 – 1104

Stanley,R.P., 2011. Enumerative combinatorics vol 49. *Cambridge university press.*

Tan, K. K., 1981 cotton waste is a fungus (Pleurotus) good substrate for cultivation of (*Pleurotus ostreatus*) the oyster mushroom. *Mushroom Science*, 11: 705-710.

Tulek, Y.,(2011). Drying Kinetics of Oyster Mushroom (Pleurotus ostreatus) in a Convective Hot Air Dryer *Journal of Agricultural Sciences of Technology*. 13: 655-664.

Vanathi, P., Paneerselvam, A. and Senthil Kumar, R., 2016. Studies on Cultivation and Biochemical Characterization of *Pleurotus florida*. *International Journal of Current Microbiology and Applied Sciences*. 5: 342-347.

Yashvant Patel ,RamNaraion,V K Singh ,2012.A study on the medicinal properties ofoyster mushroom. *World Journal of Fungi and Plant biology*, 1-5.

Zadrazil, F., Ghang S.T., and Hayes, W.A .,(Eds.), (1978). Cultivation of Pleurotus .*The Biology and Cultivation of Edible Mushrooms. Academic Press. Nova Iorque, pp. 521-557.*

Zhang, Y.H., 2008. Reviving the carbohydrate economy via multiproduct lignocellulose bioefineries. *Journal Industrial Microbiol Biotechnology*. 35: 367-75.

A STUDY ON THE PRELIMINARY PHYTOCHEMICAL SCREENING AND NUTRITIVE ANALYSIS OF SELECTED MUSHROOM SPECIES

A Short Term Project Work Submitted to St. Mary's college (Autonomous) affiliated to Manonmaniam Sundaranar University in Partial Fulfillment for the Degree of

BACHELOR OF SCIENCE IN BOTANY

By

J. Mounisha	**16AUBO32**
Y. Nanthini	**16AUBO33**
J. Narkis Fathima	**16AUBO34**
A. Nirmala	**16AUBO35**
G. Poornisha	**16AUBO38**

DEPARTMENT OF BOTANY

ST.MARY'S COLLEGE (AUTONOMOUS)

THOOTHUKUDI-628001

2018-2019

CERTIFICATE

It is certified that this short term project work entitled **"A STUDY ON THE PRELIMINARY PHYTOCHEMICAL SCREENING AND NUTRITIVE ANALYSIS OF SELECTED MUSHROOM SPECIES"** submitted to **St. Mary's College (Autonomous)** affiliated to **Manonmaniam Sundaranar University** in partial fulfillment of the requirements for the degree of **Bachelor 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 2018-2019 by the following students.

J. Mounisha	**16AUBO32**
Y. Nanthini	**16AUBO33**
J. Narkis Fathima	**16AUBO34**
A. Nirmala	**16AUBO35**
G. Poornisha	**16AUBO38**

29/3/2019

GUIDE

(S. PAULINE JENIFER)

29/3/19

HEAD OF THE DEPARTMENT

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

EXAMINER

PRINCIPAL

St. Mary's College (Autonomous)
Thoothukudi - 62

ACKNOWLEDGMENT

First of all,we thank Lord Almighty for giving us the strength to complete our project successfully.

We express our cordial thanks and deep sense of gratitude to our guide Ms. **S. Pauline Jenifer** , M.Sc., B.Ed., SET, Assistant Professor of Botany, St. Mary's College (Autonomous), Thoothukudi for her inspiring guidance, infinitive help, valuable ideas, critical comments, fruitful discussions and genuine friendliness which led us to the successful completion of our project.

We are greatly indebted to **Dr. Sr. A. S. J. Lucia Rose** M.Sc., PGDCA, M.Phill., Ph.D., Principal and the management of St. Mary's College for allowing us to do the course in St. Mary's College (Autonomous), Thoothukudi.

We are immensely grateful to **Dr. M. Glory** M.Sc., M.Phil., Ph.D., Head of the Department of Botany for providing us the laboratory facilities throughout our project. Thanks are also extended to all the staff members and the laboratory assistants of the Department of Botany and to our friends for their generous help.

Last but not least, we thank our parents for their lovable care, encouragement and constant support during the course of study.

CONTENT

Introduction

Mushroom has fascinated the mankind since ages with their colourful fleshy fruiting bodies. Theophrastus 300 BC was probably the first to mention about mushroom. Mushroom is edible as well as non-edible (poisonous). These have been included in the diet by Greeks and Romans since ancient time. Romans regarded the mushroom as a food of God while Chinese termed them as elixir of life.

Mushrooms are the edible fungi prized for their delicacy and distinctive flavour. Because of their unique nutritional status they are known as "The ultimate health food". The Greeks and Romans described mushrooms as "Gift from God" and were served only on festive occasions. Reference to mushrooms is found in Vedas (Adhikari, 2010). The prominence of fungi can now be seen increasingly evidenced by their use as a major source of pharmaceuticals and medicinal foods (Law, 2001).

More than 140,000 species of mushrooms exists in nature, but less than 25 species are widely accepted as food and only a few have attained the level of an item of commerce (Lindequist *et al*., 2002). Edible mushrooms have long been considered to have medicinal value and devoid of undesirable effects. Due to their high content of protein, vitamins and minerals mushrooms are considered as "poor man's protein" (Pandey, 2004). According to Fukushima, (2000) mushrooms generally possess most of the attributes of nutritious food as they contain many essential nutrients in good quality. Mushrooms can not only convert lignocellulosic waste materials into human food, but also can produce notable nutriceutical products, which have many health benefits. They provide people with an additional vegetable of high quality, and enrich the diet with high quality proteins, minerals and vitamins which can be of direct benefit to the human health and fitness. Edible mushrooms are highly nutritious and

can be compared with eggs, milk and meat (Oei 2003). The spent substrate left after harvesting the mushrooms, which is entangled with innumerable mushroom threads (collectively referred to as mycelia), can also be used as animal feed (more palatable), bio-fertilizer for soil fertility enrichment and biogas (Alice and Kustudia 2004).

The global food and nutritional security of growing population is a great challenge, which looks for new crop as a source of food and nutrition. In this context, mushroom cultivation helps to address the issue of nutritional security and also provides solution for proper recycling of agro-wastes. In addition to good quantity protein, no cholesterol, high fibre, low sodium, good quantity of vitamins and minerals, protein polysaccharide complexes that impart unique medicinal values like anti-cancer and anti-viral properties. Globally, China is the leading producer of mushrooms with more than 70% of the total global production which is attributed to community based farming as well as diversification of mushrooms. In India, owing to varied agro-climate and abundance of farm waste, different types of temperate, tropical and sub-tropical mushrooms are cultivated throughout the country. With ever increasing demand for quality food, mushroom cultivation is now emerging as an important activity in different parts of our country (Ambili and Nithya, 2014).

The mushroom cultivation has grown up in almost all the parts of the world and during last decades, the world mushroom production achieved the growth rate of about 10%. In India, owing to varied agro-climate and abundance of farm waste, different types of temperate, tropical and sub-tropical mushrooms are cultivated throughout the country (Shah *et al*., 2004).

Mushrooms are prized for their exclusive flavour and deliciousness, they are rich in proteins, contain less fat, less carbohydrates and salt and rich in fibres and

have high vitamin B12 and folic acid which are uncommon in vegetables. High availability of lysine and tryptophan and other amino acids usually absent in cereals make them ideal for food for patients suffering from hypertension, diabetes and obesity (Park *et al*., 2014).

Mycelia growth was very sparse on wheat straw and apple pomace was more dense but grew slowly. Dense, fast growing mycelia were produced on the mixture of substrates (Josephine, 2014).

Mushroom cultivation can be a labour-intensive agro-industrial activity, thus can help generate income and employment, particularly for women and youth in developing countries. Mushrooms are relatively fast growing organisms, thus, mushroom cultivation as a short return agricultural business can be of immediate benefit to the community. While land availability is usually a limiting factor in most types of primary production, mushroom cultivation requires relatively little space; they can be stacked using shelf-like culture systems. It is, therefore, hoped that the avocation of mushroom farming will become a very important cottage industry in integrated rural development programs. This will lead to the economic betterment of not only small-holder farmers but also of landless labourers and other weak sections of communities (Alam and Raza 2001)

Edible mushrooms are recommended by the FAO as food, contributing to the protein nutrition of developing countries dependent largely on cereals. The most important edible mushroom is the *Pleurotus* species (Oyster Mushroom) (Gyorfi and Hajdu., 2007) Mushroom cultivation is a profitable agribusiness and Oyster mushroom (*Pleurotus ostreatus*) is an edible mushroom having an excellent taste and flavour. It belongs to the class Basidiomycetes, subclass

Hollobasidiomycetidae, order Agricales. It grows wild in the forest and is cultivated in the temperate and sub tropical regions of the world (Shah *et al*., 2004).

Pleurotus ostreatus is the second most cultivated edible mushroom worldwide after *Agaricus bisporus*. It has economic and ecological values and medicinal properties. Mushroom culture has moved toward diversification with the production of other mushrooms. Edible mushrooms are able to colonize and degrade a large variety oflignocellulosic substrates and other wastes which are produced primarily through the activities of the agricultural, forest, and food-processing industries. Particularly, *P. ostreatus* requires a shorter growth time in comparison to other edible mushrooms. The substrate used for their cultivation does not require sterilization, only pasteurization, which is less expensive. Growing oyster mushrooms convert a high percentage of the substrate to fruiting bodies, increasing profitability. *P. o s t r e a t u s* demands few environmental controls, and their fruiting bodies are not often attacked by diseases and pests, and they can be cultivated in a simple and cheap way. All this makes *P. ostreatus* cultivation an excellent alternative for production of mushrooms when compared to other mushroom (Carmen, 2010).

Calocybe indica, popularly known as Milky mushroom or summer mushroom, is a relatively new introduction from India to the world of mushroom growers. *Calocybe indica* is one of the promising mushrooms cultivated in summer introduced by Purkayastha *et al*., in 1974. The name is derived from the ancient Greek terms kalos “pretty” and cubos “head”. In Orissa it is known as dudha chhatu and in some places they are called kuduk. Around nine species of *Calocybe* are found in neotropical regions (Nilson *et al*., 1997)

Krishnamoorthy (1997), identified a potential strain of *Calocybe indica* occurring in a sugarcane field near Coimbatore, later it was released as a new variety called APK2 from Tamil Nadu Agricultural University. Natural occurrence of this mushroom *Calocybe* in the plains of Tamil Nadu and Rajasthan has also been reported. Geetha (2011), reported a high yielding strain of *Calocybe gambosa* from western ghat region of Kerala.

Among eighty edible mushrooms are considered for commercial exploitation, milky mushroom has become the focal point of exploitation in India as it grows in hot humid climate and suitable for cultivation almost throughout the year. The milky mushroom is considered as a better proxy for oyster mushroom notably in tropical regions with longer shelf life of 3-4 days and offers wide export potential. Cultivation of milky mushroom has become popular in Tamil Nadu, Kerala, Karnataka and Andra Pradesh.

Recently *Calocybe indica* have become an attractive functional food mainly because of their chemical composition. Chang *et al.* 2004 reported that milky mushroom known for its delicacy, flavour, and aroma. Nutritionally it is considered as a valuable vegetable, consisting of protein (10-40 per cent) carbohydrate (13-70 per cent) fat less than (1-8 per cent) minerals and significant amount of essential amino acids. Rahul *et al.* (2010) reported that *Calocybe indica* is rich in protein, carbohydrates, and vitamins and contain abundant amount of essential amino acids and fibre.

Since milky mushroom gained spectacular growth in the commercial front, its potential to boost health is to be explored. In this context the present study is taken up to investigate the nutritional and medicinal value of milky mushroom, so that the

commercial cultivation of milky mushroom could be promoted and popularized further.

Scope and Objectives

Mushroom can play an important role contributing to the livelihoods of rural and peri-urban dwellers, through food security and income generation. Mushrooms can make a valuable dietary addition through protein and various micronutrients and, coupled with their medicinal properties, mushroom cultivation can represent a valuable small-scale enterprise option. There has been 1200 species of fungi that considered to mushrooms, with at least 200 species showing various degree of edibility (Chang, 1999).

Based on the availabily and delicacy the two mushroom varieties, Oyster and Milky mushroom were selected for the present study. The main objectives were,

1. To grow the varieties of this two different species in the same environment.
2. To analyze the preliminary phytochemical screening of the mushroom species.
3. To quantitatively analyze the nutrient content of each mushroom species.

Mushrooms have been consumed by humans as nutritious and delicious food since ancient times. The Greeks, the Romans and the Chinese regarded them as valuable healthy food. Recently there is an increased demand for edible mushrooms globally as they are low in calories, carbohydrates, fat and sodium and rich in proteins, minerals and vitamins and are free from cholesterol. Mushrooms are reported to be useful in preventing and treating Parkinson's disease, Alzheimer's disease, diabetes, hypertension and high risk of stroke (Shialaja and Radhika, 2018)

Mushroom cultivation is considered as an alternative source of uplift the living standards of poor farmers and also to add high quality protein in their daily diets to eradicate malnutrition problems . Mushroom cultivation includes cultivation techniques , spawn preparation , substrate preparation , marketing of fresh product , preservation etc (Biswas, 2014).

The cultivation of edible fungi is a controlled bioconservation of agro industrial lingo-cellulosic waste and residues . The oyster mushroom containing more nutrient . Mushroom cultivation fits in very well with sustainable forming and has several advandages . The mushroom are good each crop . The development of oyster mushroom production methologies an agricultural waste like paddy straw and wheat straw gives very high yield as well the nutrients contain like carbohydrates , protein , ash , aminoacids , crude fiber , magnesium , lipid were checked (Sonali ,2012).

Numerous species of wild growing mushrooms are widely consumed as a delicacy in central and eastern Europe. Credible evaluation of their nutritional

value has so far been limited due to fragmentary knowledge of their composition and mainly due to the very limited information on the availability of their constituents. Dry matter content is usually about 100 g kg^{-1}. Structural polysaccharides and proteins comprise the main components of dry matter, while the lipid content is low (Pavel, 2009)

Edible mushrooms and wild mushrooms were cultivated using paddy straw and wheat straw. The effect of mushroom size , spawn inoculation level and type of substrate on mushroom yield and biological efficiency. Comparing to wheat straw, rice straw yield about 10% more mushrooms (Abulude *and,* 2014)

Mushrooms have been used as food supplement from time immemorial not only for their flavor , aroma and nutritive values , and but also for their medicinal properties . In the present day world they are known for culinary values due to their high -quality proteins , vitamins , fibers and many medicinal properties . The chemical nature of the bioactive compounds present in the mushroom includes : polysaccharides lipopolysaccharides , proteins , peptides , glycoprotein , nucleoside , lectins ,lipid and their derivatives. Mushrooms are used in folk medicine throughout the world since ancient times as the ultimate health food. (Yashvant *et.al*,2012)

Mushrooms constitude an integral part of the normal human diet and in recent times , the amounts of consumption have been raised greatly , which include variety of species. The oyster mushroom is popularly consumed by all over the world due to their taste , flavor , high nutritional

value and medicinal properties . Because of the presence of numerous nutritional composition and various active ingredients in oyster mushroom have been reported to have antidiabetic , antibacterial , antioxidant , eye health and antiviral activities (Krishnamoorthy,2014).

Mushrooms had long been used for medicinal and food purposes since decades . Modern pharmacological research confirms large parts of traditional knowledge regarding the medicinal effects of mushrooms due to their antifungal , antibacterial , antioxidant and antiviral properties , besides being used as functional foods . This papersumsap diverse beneficial health effects of mushrooms to human , in the form of proteins , carbohydrates , fats , vitamins , minerals , food and drugs and medicine (Ahmad *et al,* 2010)

Oyster mushroom is consumed all over the world due to its taste,flavour,high nutritional Value and some medicinal properteies. Many species of this genus are rich in proteins with essential amino acids, polysaccharides, essential amino acetic acid, dietary fibers, important minerals and some vitamins.Because of these nutritional composition and presence of bioactive molecules oyster mushroom have been reported to have anticancer, antihypertensive, anti diabetic and antioxidant.The high nutritional value and potent medicinal uses suggests that *pleurotus* mushrooms are important functional foods or nutraceuticals (Savita and Anjana,2017)

Three distinct oyster mushroom strains including *Pleurotus florida* (PF), *Pleurotus eous (PE) and Pleurotus sajor-caju* (PS) were successfully cultivated on cattail weed substrate. A comparative analysis of different parameters viz., biological efficiency (BE) and protein, carbohydrate, crude fibre and fat content

in fruitbodies were evaluated. According to biological efficiencies obtained PF (90%) was superior strain, while order can be represented as 90%> 89%> 82% respectively in PF>PS>PE (Ram and Bharti, 2017)

The phytochemical screening of different extracts of *Pleurotus florida* revealed the presence of different secondary metabolites. *Pleurotus florida* fruiting bodies were found to contain carbohydrates, alkaloids, glycosides, flavonoids, tannins, saponins and steroids (Nirmala, 2014)

The greatest difficulty in feeding man is to supply a sufficient quantity of the body building material protein. The other three nutritional categories are: the source of energy food carbohydrates and fats, accessory food factors vitamins and inorganic compounds which are indispensable to good health (Shimizu and Anzai 2001). Furthermore, mushroom protein contains all the nine essential amino acids required by man. In 14 addition to their good proteins, mushrooms are a relatively good source of the following individual nutrients: fat, phosphorus, iron, and vitamins including thiamine, riboflavin, ascorbic acid, ergosterine and niacin. They are low in calories, carbohydrates and calcium. Mushrooms also contain a high proportion of unsaturated fat

The use of mushrooms as food is probably as old as civilization and mushrooms currently have greater importance in the diet of mankind. Mushrooms contain reasonable amounts of proteins, carbohydrates, minerals, vitamins and fiber. Mushrooms generally possess most of the attributes of nutritious food as they contain many essential nutrients in good quantity (Fukushima, 2000).

Nutritional value of mushrooms lies between that of meat and vegetables. The rich source of proteins, vitamins and minerals and low in fat content (2-8%) unique chemicals constitution of mushrooms makes them low calorie food 8 and choice diet for those suffering from hypertension, arthrosclerosis, diabetes, obesity etc. ((Fukushima, 2000)

The carbohydrate content of mushrooms represents the bulk of fruiting bodies accounting for 50 to 65 per cent on dry weight basis. Free sugars amounts to about 11 per cent (Florezak et al., 2004). The calorific value of mushrooms is quite low compared to other foods. When compared to oyster mushroom milky mushroom had more carbohydrate protein, and fat. (Krishnamoorthy, 1997). Milky mushroom consists of 13-70 per cent of carbohydrate. There for milky mushroom can be recommended for "slimming diet"

Mushrooms are excellent source of high quality proteins as compared to most of the vegetables; they are in easily digestible form. Quality of protein is comparable with meat, egg and milk. (Aletor, 1995). The protein value of mushrooms is twice as that of asparagus and potatoes four times as that of tomatoes and carrots, six times as that of oranges. *Calocybe indica* consists of about 15- 40 per cent protein (Tapasya and Rashmi, 2011).

Mushrooms normally contain 19- 35% protein. Mushroom proteins contain all the essential amino acids and are especially rich in lysine and leucine, which are lacking in most staple cereal food. The low total fat content, and high proportion of polyunsaturated fatty acids (72-85%) relative to total fatty acids, is considered a significant contribution to the health value to

mushrooms. Fresh mushrooms contain relatively large amount of carbohydrate and further range from 51-88% and 4- 20% mushroom appear to be a good source of vitamin including thiamine, riboflavin, niacin, biotin and ascorbic acid

Verma et al. (1987) reported that mushrooms are very useful for vegetarian because they contain some essential amino acids which are found in animal proteins.

Pushpa and Purushothama (2010), reported that *C.indica* is rich in protein and fiber with low fat content, which make the mushroom as a low energy, healthy food stuff. They were also of the opinion that milky mushroom can also be used as protein supplementing diet. Digestibility of milky mushroom protein is 72-83 per cent.

Mushroom is a low fat food containing 1.1 to 8.3 per cent fat on dry weight basis, however it contain all the classes of lipids including free fatty acids, glycerides, sterols and phospholipids. The fat content of *Calocybe indica* is 1 to 8 per cent. When compared to oyster mushroom, milky mushroom contain more protein, carbohydrate and fat (Krishnamoorthy *et al*., 1997).

Jacob (2010), reported that one type of fibre found in mushrooms called beta- glucan is similar to that of main oat products, which is beneficial for sugar and blood cholesterol management. According to Kalac and Svoboda (2009), cooked mushrooms contain more fibre because they are more concentrated. Mushrooms contains about 4-9 per cent and 22-30 per cent soluble and insoluble fibre respectively. An average serving of mushrooms (100g) guarantees 940 per cent of the daily recommendations of dietary fiber (Manzi, 2001). According to Doshi *et al.* (1988) due to its alkaline ash and high fiber

content, milky mushroom is highly suitable for people with hyperacidity and constipation.

Dietary mushrooms are considered as valuable health foods since they are known for proteinacious food, consisting of about 75 per cent proteins and are low in calories and fat. (Murugkar and Subbalakshmi, 2005). Mushrooms have been valued throughout the world both as food and medicine for thousands of years. They are effective functional foods with wide spectrum of pharmacological potentials. They are good source of high quality protein comprising all the amino acids (Tapasya and Rashmi 2011).

Edible mushrooms contain interesting functional components, particularly beta glucans, homo and hetero glucans with glucosidic linkages that are responsible for some health properties of mushrooms (Pamela, 2010).

Usha (2007), reported that Calocybe indica on different substrates shows the presence of altogether eighteen fatty acids especially eicosapentaenoic acid and docasohexaenoic acid. These two omega3 PUFAs known to decrease the incidence of coronary heart diseases, stroke and rheumatoid arthritis. The bioactive substances with immunomodulating effects of mushrooms include polysaccharides, glycoprotein, terpenoids, and fungal immunomodulatory proteins. The biologically active polysaccharides have antitumor and immunostimulating properties (Chang, 2004).

Materials and Methods

Materials:

The different mushroom spawns were collected from the certified mushroom cultivator,Mr. Abdul Rahim, MSM Mushroom corner,Mushroom cultivation training and seeds sale, Rediyarpatti, Tirunelveli for the present study. The spawns were of 3 different species of *Pleurotusspp.* (Oyster mushroom) and two species of*Calocybe spp.*(milky mushroom). The five different spawns were noted as *Pleurotusspp.* Var. 1, *Pleurotusspp.* Var. 2, *Pleurotusspp.* Var. 3, *Calocybe spp.* Var. 1 and *Calocybe spp.* Var. 2 respectively.

Methods:

Cultivation of mushroom:

The standard procedure for the cultivation of oyster and milky mushroom instructed by Tamil Nadu Agricultural University was followed to grow the mushroom specimens. They were shown in **Plates 1 - 3**

Substrate preparation:

Oyster mushroom was grown on the basic substrate paddy straw. Since paddy straw is easily available and cheap, it is widely used. Paddy straw used was fresh and well dried.

Soaking:

The paddy straw was chopped into 3-5 cm pieces and soaked in fresh water for 8-16 hours. Excess water from straw was drained off by spreading it on filter paper.

Heat Treatment:

Heat treatment of substrate results in minimizing contamination problem and gives higher and almost constant yields. It can be done by pasteurization.

Pasteurization:

The soaked paddy straws were removed and filled in a pressure cooker to heat them at high temperature. The cooker lid was closed and allowed to cook the straw till two whistles. Now the paddy straws become saturated and were free of contamination.

Spawning:

When the pasteurized substrate had cooled down to room temperature, it was ready for filling and spawning. At this stage, substrate moisture content was about 70%. Polypropylene bags (35 x 50 cm) were used for its cultivation. Spawning can be done using layer spawning method.

In this method, substrate was filled in bag, pressed to a depth of 8-10 cm and broadcasted with a handful of spawn above it. Similarly, 2nd and 3rd layers of substrate were put and simultaneously after spawning the bags were closed. After that it was gently pressed, and the bags were sealed for spawn running (development).Spawned bags were hanged inside the mushroom cultivation unit and the temperature and humidity were maintained by pouring water on the sack walls four to five times in a day.It took 8-10 days when bags were fully covered with white mycelium.

Cropping and harvest:

After 14-18days, when bags were with fully grown mushroom, they were hand plucked and were stored in perforated polythene bags to maintain their freshness. It loses freshness after about 6 hours, which can be enhanced by keeping them in refrigerator.

Data collection

The mushroom bags were frequently observed from its packing to harvesting to record different parameters such as number of days for starting and completion of spawn running, time required for pinhead initiation, number of primordia, number of fruiting bodies. Mushroom yield (%) (Dlamini*et al*., 2012) ,biological efficiency (%) and production rate (Carvalho*et al*., 2012) were calculated by using the following formula:

$$\text{Mushroom yield} = \frac{\text{Fresh weight of mushroom}}{\text{Fresh weight of substrate}} \times 100$$

$$\text{Biological efficiency} = \frac{\text{Fresh weight of mushroom}}{\text{Dry weight of substrate}} \times 100$$

$$\text{Production rate} = \frac{\text{Biological efficiency}}{\text{Total days of production}} \times 100$$

Sample preparation

The collected mushrooms from various treatments was cut into slices and shade dried. Then the dried samples were powdered in a mixer grinder and sieved to get uniform particles. The sample powder was stored for further use.

Extract preparation of the mushroom samples

For the present study, the cold extract was prepared using the dried mushroom samples in various solvents like Methanol, Acetone and water. I gmof the dried samples was mixed with 20 ml of solvents and kept in at shaking at regular intervals for 24 hours. After that the content was filtered through filter paper and the extract was used for the phytochemical analysis.

Preliminary phytochemical screening of mushroom samples (Harborne, 1998)

Test for Alkaloids

Mayor's test:

Dissolved filtrate 1 ml treated with Mayor's reagent (Potassium mercuric iodide). Formation of a yellow coloured precipitate indicatedthe presence of alkaloids. (Mercuric chloride + few drops of Iodine solution)

Test for Terpenoids

Crude extract 2 ml was dissolved in 2 ml of chloroform and evaporated to dryness. To this 2 ml of Con H_2SO_4 was added and heated for about 2min. A grayish colour indicated the presence of terpenoids.

Test for Phenol and Tannin

Crude extract was mixed with 2 ml of 2% solution of $FeCl_3$. A blue green (or) black colorization indicated the presence of phenol and tannin.

Test for sugar

The little amount of substance mixed with equal volume of Fehling's A and B solution heated in water bath. Formation of red colour indicated the presence of sugar.

Test for Saponins (Froth test)

To 3 ml of extract were diluted with 2 ml of distilled water and this test tube was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicated the presence of saponin.

Test for Quinines

To the 1% test substance 2% sodium hydroxide was added. Blue green (or) red colour indicated the presence of quinines.

Test for Protein

The 4% extract were treated with few drop of concentrated nitric acid. Formation of yellow colour indicated the presence of protein.

Test for Sterols

The 3 ml of crude extract was mixed with 2 ml of chloroform and con H_2SO_4 was added sidewise. A red colour is produced in the lower chloroform layer indicated the presence of steroids.

Quantitative analysis of mushroom samples

Moisture content (Randive, 2012)

Moisture was determined by drying the fresh mushrooms at 80˚C to a constant weight in an oven. Moisture content was determined by following formula

$$\text{Moisture content} = \frac{\text{Initial weight} - \text{final weight}}{\text{Initial weight of sample}} \times 100$$

Estimation of total soluble protein (Lowry *et al.*, 1951)

Reagents

- 10% trichloroacetic acid (TCA).

- 0.2N sodium hydroxide
- Alkaline copper reagent:

  Solution A: 2% sodium carbonate in 0.1N sodium hydroxide

  Solution B: 2% 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 – Ciocalteau reagent

  Commercial reagent was prepared freshly in the ratio of 1:1 with distilled water.

Extraction and estimation of protein:

One gram of plant tissue was weighed and ground it with 10 ml of distilled water. The plant extract was filtered through a filter paper. To the filtrate, 10 ml of cold 5% TCA (trichloro acetic acid) was added to precipitate protein. It was centrifuged and the pellet was collected that contains protein. The pellet was dissolved in 10 ml of 0.1N NaOH. To 1 ml of this protein extract, 5 ml of alkaline copper reagent and 0.5 ml of Folin – Ciocalteau reagent were added. The absorbance was measured using red filter (650nm). The absorbance extrapolated in the standard graph and the amount of protein present in 1 gm of plant tissue was calculated.

Estimation of carbohydrates (Hedge and Hofreiter, 1962)

Principle

Carobohydrate is first hydrolysed into simple sugars using dilute hydrochloric acid. In hot acidic medium glucose is dehydrated to hrdroxymethyl

furfural. This compound forms with anthrone a green coloured product with absorption maximum at 630 nm.

Reagents:

A. 2.5 N Hcl

B. Anthrone reagent: 0.2% anthrone was dissolved in ice cold concentrated sulphuric acid. Prepared fresh before use.

Procedure

Weighed 100mg of the sample into a boiling tube, hydrolysed by keeping it in a boiling water bath for three hours with 5 ml of 2.5N Hcl and cooled to room temperature. Neutralised it with soild sodium carbonate until the effervescence ceas and take 0.2 to 1.0ml for analysis. Then added 4 ml of anthronereagent, heated for eight minutes in a boiling water bath, colled rapidly and read the green to dark green colour at 630 nm.

Estimation of free Amino acids(Jayaraman, 1981)

Principle

The ninhydrin is powerful oxidizing agent. All amino acids in the presence of ninhydrin get decorboxylated and deaminated resulting in ammonia, carbondioxide, the corresponding aldehyde, and a reduced form of ninhydrin. The liberated ammonia then reacts which an additional mole of ninhydrin and the reduced ninhydrin to yield a purple substance, which has absorption maxima at 570nm.

Reagents

A. Standard amino acid solution Dissolved 10 mg of aspartic acid in small volume of (0.1N) HCl and make up with distilled water to the 100 ml mark in the standard flask.

B. Ethanol (80%)

C. Ethanol (50%)

D. Ninhydrin reagent

Prepared by dissolving 2g of ninhydrin in 25 ml of acetone. To this 25 ml of 0.2 M acetone buffer (pH 5.5) was added and stored in a brown bottle.

Procedure

10 ml of samples was homogenized with 80 percent ethanol in a pestle and mortar. The homogenate was centrifuged at 5000 rpm. The clear supernatant was made upto a known volume. From this 1 ml was pipetted out in to a test tube and diluted to 4ml with distilled water. To this, 1 ml of ninhydrin reagent was added and kept in boiling water bath for 15 minutes. The test tubes 38 were then cooled and 1 ml of 50 percent ethanol was added. The purple colour developed was measured in spectronic 20 at 540nm.

Determination of total lipid (Folch*et al*., 1957)

To 500 mg of dried algal powder taken in a screw capped test tube, 10 ml of 2:1 CHCl3: CH3OH solvent mixture was added. The tube was loosely capped and heated in a water bath at 60°C for 30 min. After cooling the solution, the volume was made up to 10 ml with the solvent mixture. 0.4 ml of the extract was pipetted in a separate test tube, allowed to dry completely and digested with 0.4 ml of cone. H_2SO_4 by boiling in a water bath for 10 min. After cooling the tube, 5 ml of phosphovanillin reagent was added and allowed to stand for 30 min for colour development. The absorbance was then measured at 520 nm against a reagent blank using UV-Visible spectrophotometer. Cholesterol was used as standard.

Results and Discussion

The different samples were grown in the mushroom unit and are carefully analyzed for various parameters. The cumulative result of the all the studies undertaken in the present study is clearly given and discussed in this part.

The spawn running and pinhead formation are the most important phases in the cultivation of mushroom. The days taken for this process are given in Table 1. It is evident that in *Pleurotus* varieties it took one week for the spawn running process. Similar results were observed by Tan (1981) and Shah *et al*. (2004). In the same way in *Calocybe* varieties, it had taken 2-3 weeks for the spawn to run. Onuha (2007) also observed the same type of spawn running period in the *Calocybe spp.*

The time taken for the pin head formation was shown in Table 1. It took about 6-7 days in both the mushroom varieties to produce the pin head. Vetayasuporn (2007) reported the same type of result when he worked with different mushroom species including oyster and milk mushroom.

The number of pin head, primordia and fruiting body formation in mushroom samples were shown in Table 2 and Plate 1-3. The number of pin head formed was more in *Calocybe spp*Var. 1 and least number is present in *Pleurotus spp* Var. 2. The number of primordia formed was lesser than the pin head formation. The number of primordia was more in *Calocybe spp*Var. 1 and lesser in *Calocybe spp* Var. 2. The fruiting body produced were even more lesser than the pin head produced. In this also *Calocybe spp*Var. 1 produced more number of fruiting body. These similar results were observed with the

works done by Chandra 2016 while working with the oyster mushroom and Celik and Perkker(2003) in milky mushroom.

Mushroom yield percentage of different mushroom samples was given in Figure 1. Higher amount of mushroom yield was noted in *Pleurotusspp* Var. 2 followed by *Calocybespp*Var. 1. The least yield was observed in *Pleurotusspp* Var. 1. Bolton and Blair (1982) and (Fasidi, 1996), reported that rice husk is good for the production of V. esculenta because of its richness in oils and vitamins which are good stimulants for high mushroom yield. Substrate structure is an important factor for the growth of the mycelium as it should be suitable for penetration of the mycelium.

Biological efficiency and rate of production of mushroom samples were shown in Figure 2,3. The biological efficiency and rate of production of the samples were based on their mushroom yield percentage. In this also, *Pleurotusspp* Var. 2 followed by *Calocybe spp*Var. 1 showed high biological efficiency and rate of production. The least biological efficiency and rate of production were observed in *Pleurotusspp* Var. 1. A higher Biological efficiency was observed in supplemented with paddy straw substrate support the work of (Fasidi, and Kadiri, 1993 and Royse, 1996) on Pleurotustuberregium and Lentinussubnudu, respectively.

The preliminary phytochemical screening of mushroom samples with different extract was shown in Table 3. Alkaloid was present only in the aqueous samples of *Pleurotus spp* Var. 2, *Pleurotus spp* Var. 3, *Calocybe spp*Var. 1, *Calocybe spp* Var. 2, whereas alkaloid was absent in other extracts of mushroom samples. The medicinal value of mushroom were attributed to the

presence of alkaloids. Saponin and terpenoids were present in all the mushroom samples of the present study but were not observed in all the extracts. Tannins were reported in the aqueous extract of *Pleurotus spp* Var. 2 and *Calocybe spp*Var. 1 and were absent in all the other extracts of mushroom samples. Quinone is present only in the aqueous sample of *Pleurotus spp* Var. 1 where as it was not present in any other extracts of mushroom samples.Protein was present in all the samples of the mushroom samples. This work was supported by Patil *et al*., 2008

The moisture content of the mushroom samples was shown in Figure 4. The moisture content was high in *Pleurotus spp* Var. 3 followed by *Calocybespp* Var. 2, *Pleurotus spp* Var. 2, *Pleurotus spp* Var. 1 and *Calocybespp*Var. 1 in the order. This work was supported by (Alam, 2004)Alam N, Khan A, Hossain MS, Amin SMR, Khan LA. Nutritional analysis of dietary Mushroom- Pleurotusflorida Eger and Pleurotussajor-caju (Fr.) Singer. Bangladesh Journal of Mushroom.2007; 1:1-7.

The total amount of protein in the mushroom samples was shown in Table 4 and Figure 5. High amount of protein was observed in *Pleurotusspp* Var. 1 followed by *Calocybespp*Var. 1, *Pleurotusspp* Var. 2, *Calocybespp* Var. 2 and *Pleurotusspp* Var. 3 in the decreasing order. This shows that the protein content of edible mushrooms, ingeneral,is about twicethatofasparagusandcabbageand4timesand12times respectively (Chang, 1980).

The total amount of amino acids in the mushroom samples was shown in Table 5 and Figure 6. The total amount of protein in the mushroom samples was

shown in Table 4 and Figure 5. High amount of protein was observed in *Pleurotusspp* Var. 1 followed by *Calocybespp*Var. 1, *Pleurotusspp* Var. 2, *Calocybespp* Var. 2 and *Pleurotusspp* Var. 3 in the decreasing order. Essential amino acids (lysine, methionine, tryptophan, threonine, valine, leucine, isoleucine, histidine, and phenylalanine) were present in protein . The proteins of commonly cultivated mushrooms contain all nine amino acids essential for man (Ouzouni et al., 2009).

The total amount of carbohydrates in the mushroom samples was shown in Table 6 and Figure 7. High amount of carbohydrates were observed in *Pleurotusspp* Var. 1 followed by *Pleurotusspp* Var. 3, *Pleurotusspp* Var. 2, *Calocybespp*Var. 1 and *Calocybespp* Var. 2 in the decreasing order. This result was supported by Barros*et al.*, 2007.

The total amount of lipids in the mushroom samples was shown in Table 7 and Figure 8.*Pleurotusspp* Var. 3 showed high amount of lipid content which was followed by *Calocybespp* Var. 2, *Calocybespp*Var. 1, *Pleurotusspp* Var. 2 and *Pleurotusspp* Var. 1 respectively. In general, the crude fat of mushrooms represents all classes of lipid compounds including fatty acids ,triglycerides, sterols, sterolesters, and phospholipids. At least, 72% of the total fatty acids were found to be unsaturated in each one of these mushrooms, thus making mushrooms a health food as they are essential in our diet, whereas saturated fatty acids may be harmful to our health (Barros *et al.*, 2007).

Table 1: Days taken to produce spawn run and pin head formation in the mushroom samples

Sample	Days taken to produce	
	Spawn run	Pin head
*Pleurotus spp.*Var. 1	7	13
Pleurotus spp Var. 2	5	11
Pleurotus spp Var. 3	8	14
Calocybe spp Var. 1	8	16
Calocybe spp Var. 2	10	18

Table 2: Number of pin head, primordia and fruiting body in the mushroom samples

Sample	No. of Pin head	No. of Primordia	No. of fruiting body
Pleurotus spp Var. 1	34	24	20
Pleurotus spp Var. 2	30	23	18
Pleurotus spp Var. 3	32	23	17
Calocybe spp Var. 1	45	30	23
Calocybe spp Var. 2	35	20	18

Figure 1 Yield percentage of mushroom samples

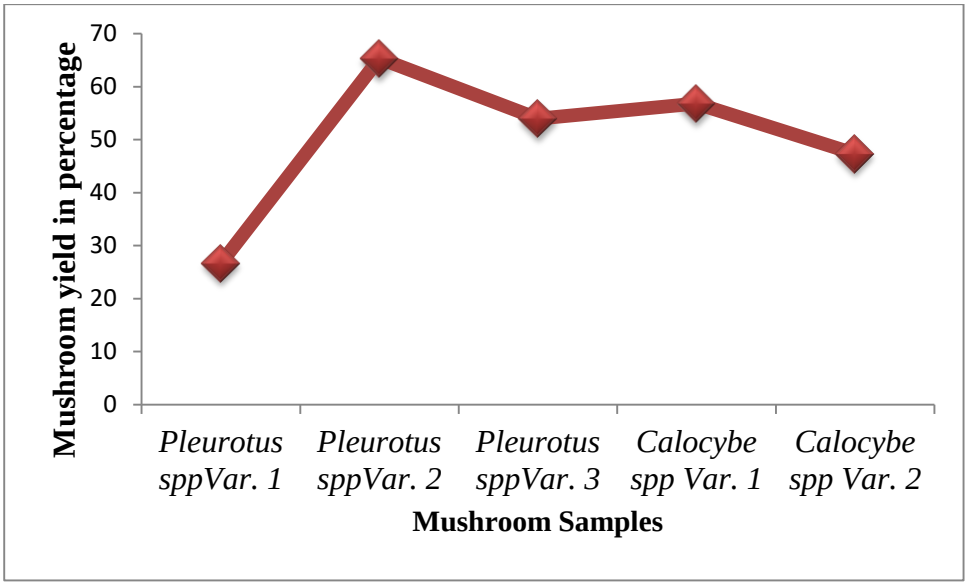


Figure 2 Biological efficiency of mushroom samples

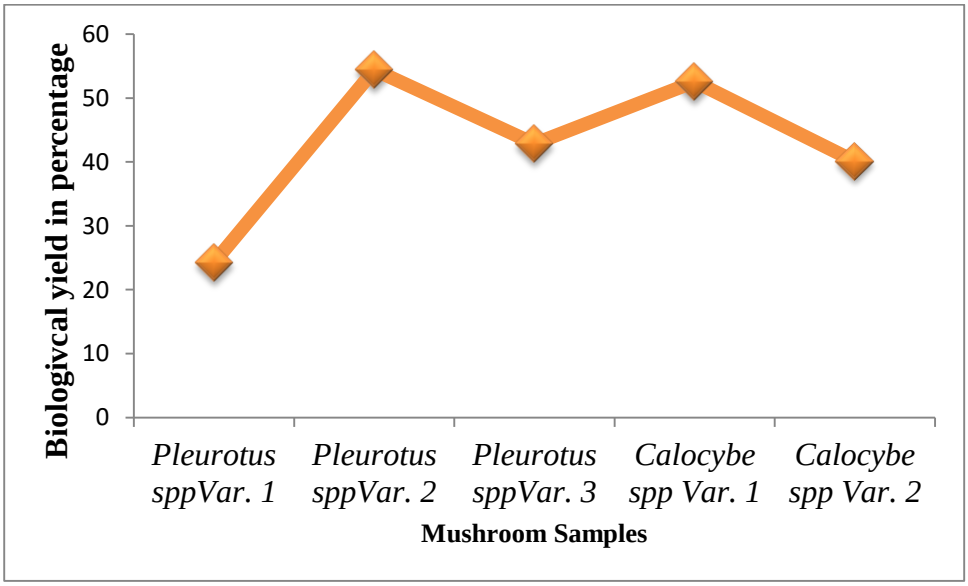


Figure 3 Production rate of mushroom samples

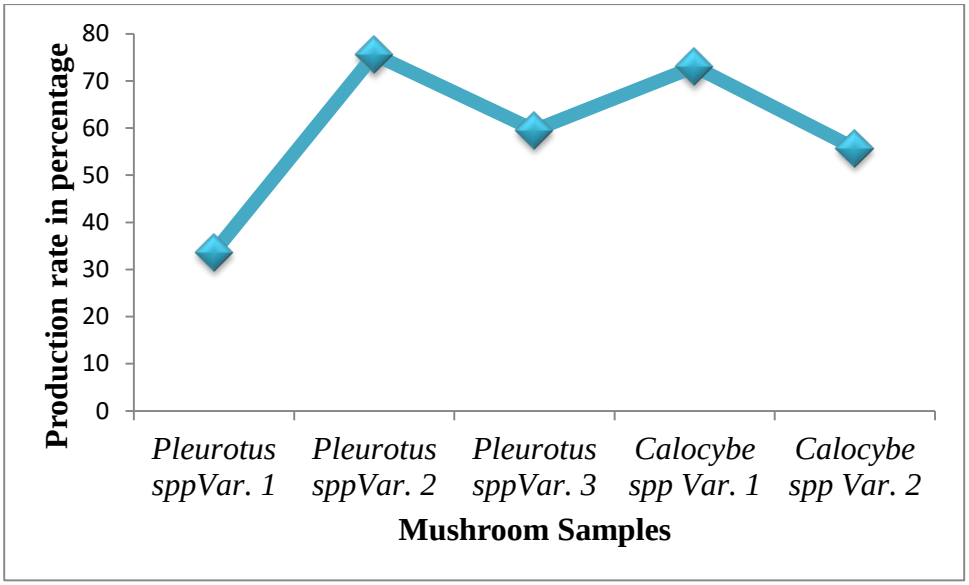


Table 3 Preliminary Phytochemical screening of the mushroom samples

Plant Samples	Extract	Alkaloid	Saponin	Terpenoid	Tannin	Quinone	Protein
Pleurotus spp Var. 1	Acetone	-	-	+	-	-	+
	Methanol	-	+	+	-	-	+
	Aqueous	-	+	-	-	+	+
Pleurotus spp Var. 2	Acetone	-	-	+	-	-	+
	Methanol	-	+	+	-	-	+
	Aqueous	+	+	-	+	-	+
Pleurotus spp Var. 3	Acetone	-	-	+	-	-	+
	Methanol	-	+	+	-	-	+
	Aqueous	+	+	-	-	-	+
Calocybe spp Var. 1	Acetone	-	-	+	-	-	+
	Methanol	-	+	+	-	-	+
	Aqueous	+	-	-	+	-	+
Calocybe spp Var. 2	Acetone	-	-	+	-	-	+
	Methanol	-	+	+	-	-	+
	Aqueous	+	+	-	+	-	+

+ indicates Presence

- indicates Absence

Table 4: Total amount of protein in the mushroom samples

S.No	Sample	Total amount of protein mg/g DW
1.	*Pleurotus spp* Var. 1	2.35 ±0.049943
2.	*Pleurotus spp* Var. 2	1.39 ±0.0141745
3.	*Pleurotus spp* Var. 3	1.1 ±0.041892
4.	*Calocybe spp* Var. 1	1.38 ±0.0127279
5.	*Calocybe spp* Var. 2	1.2 ±0.0540185

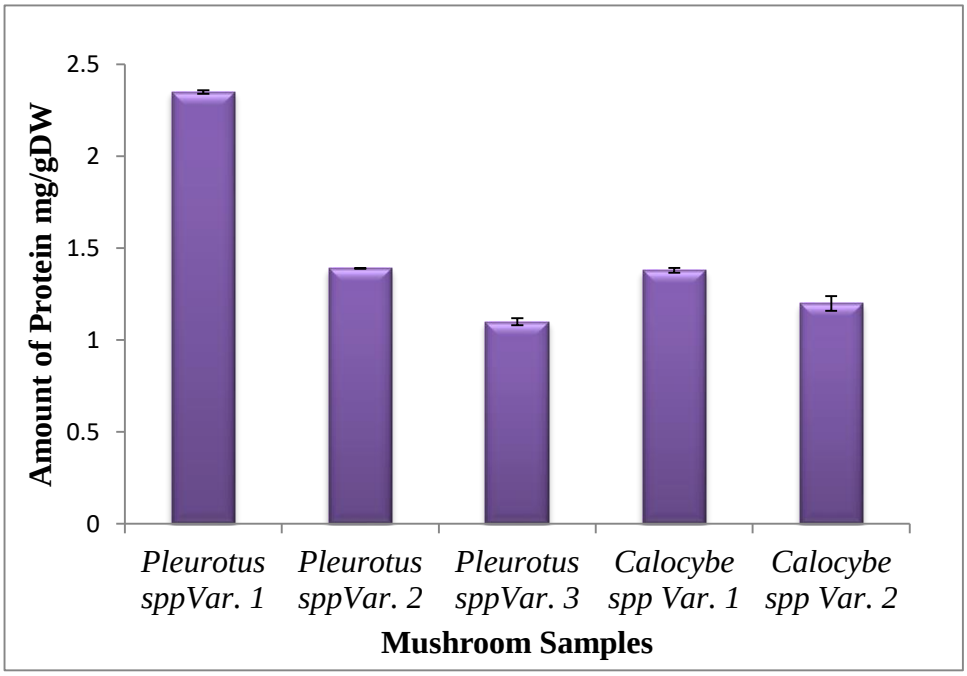


Figure 5 **Total amount of protein in the different mushroom samples**

Table 5: Total amount of amino acid in the mushroom samples

S.No	Sample	Total amount of amino acid mg/g DW
1.	*Pleurotus spp* Var. 1	1.8 ±0.0244948
2.	*Pleurotus spp* Var. 2	1.11 ±0.012333
3.	*Pleurotus spp* Var. 3	0.52 ±0.011435
4.	*Calocybe spp* Var. 1	1.2 ±0.049888
5.	*Calocybe spp* Var. 2	0.65 ±0.013144

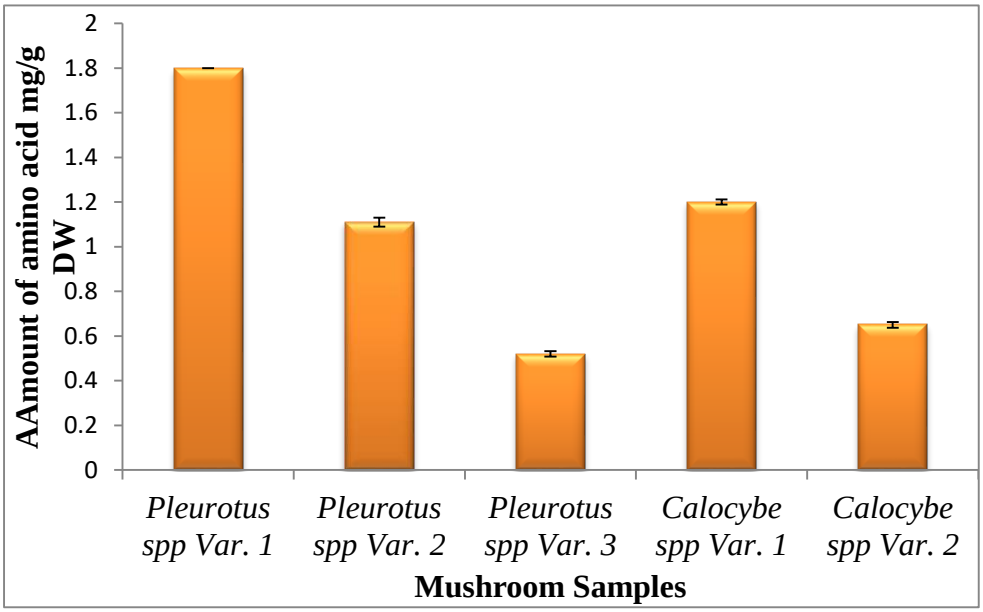


Figure 6 Total amount of amino acid in the mushroom samples

Table 6: Total amount of Carbohydrates in the mushroom samples

S.No	Sample	Total amount of carbohydrates mg/g DW
1.	*Pleurotus spp* Var. 1	9.84 ±0.16490896
2.	*Pleurotus spp* Var. 2	5.89 ±0.21460304
3.	*Pleurotus spp* Var. 3	7.29 ±0.2399005
4.	*Calocybe spp* Var. 1	5.78 ±0.3723088
5.	*Calocybe spp* Var. 2	2.59 ±0.133735

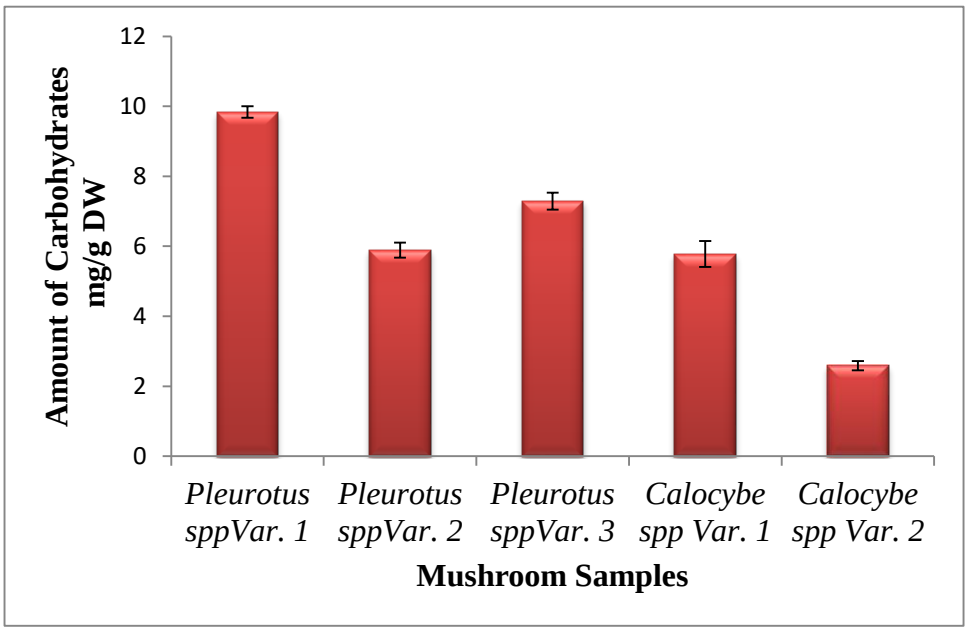


Figure 7 Total amount of carbohydrates in the mushroom samples

Table 7: Total amount of lipids in the mushroom samples

S.No	Sample	Total amount of lipids mg/g DW
1.	*Pleurotus spp* Var. 1	3.07 ±0.02766608
2.	*Pleurotus spp* Var. 2	3.50 ±0.0245678
3.	*Pleurotus spp* Var. 3	6.51 ±0.0134768
4.	*Calocybe spp* Var. 1	4.06 ±0.0217865
5.	*Calocybe spp* Var. 2	4.49 ±0.0244446

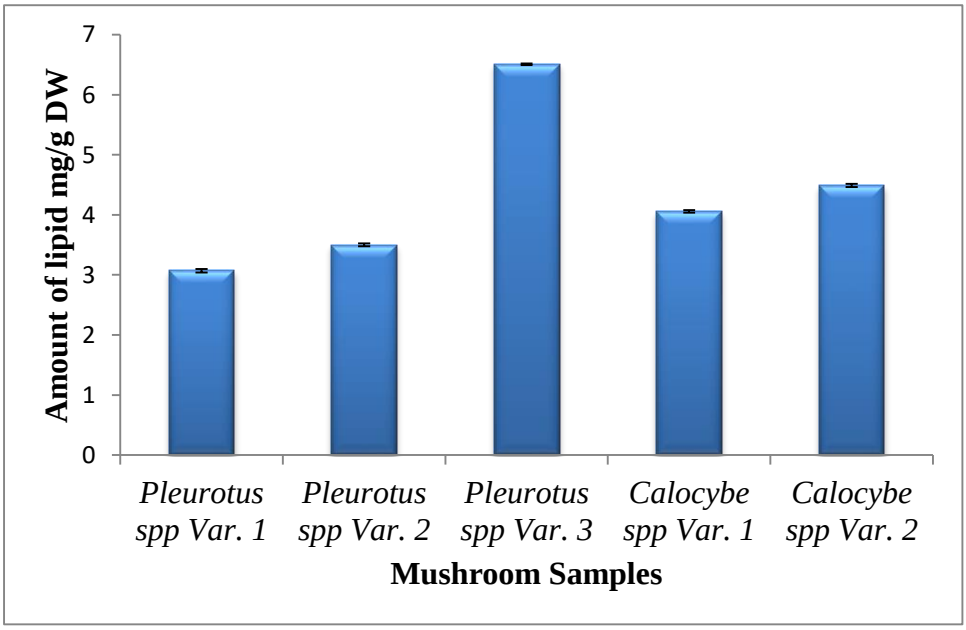


Figure 8 Total amount of lipids in the mushroom samples

Figure 4: Moisture content of the mushroom samples

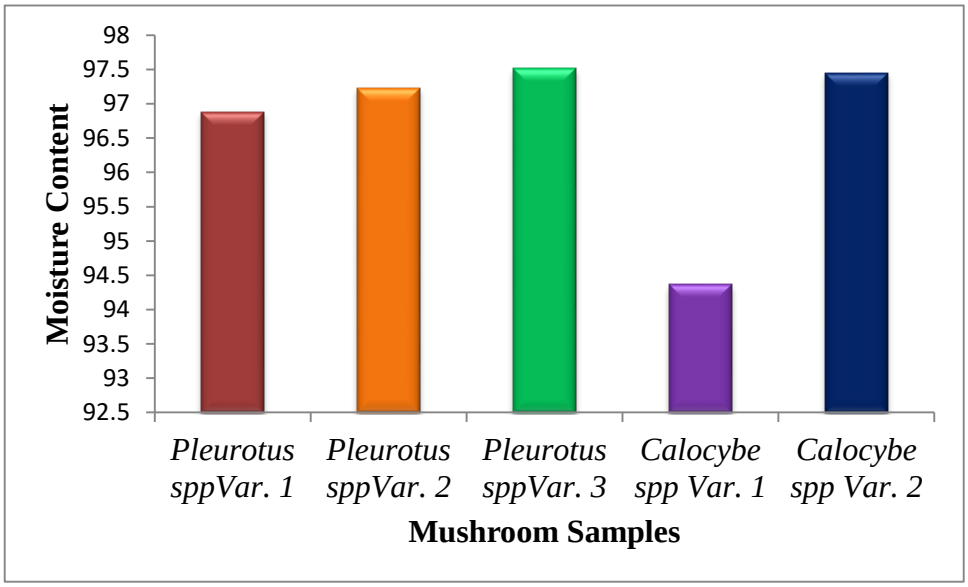


Plate 1 Methods of mushroom cultivation

Spawn

Substrate preparation

Soaking of substrate

Pasteurization

Plate 2 Packing of mushroom bags

Pasteurized substrate

Usage of polypropelene bag

Layering of substrate

Spawning

Plate 3 Packing of mushroom bags

Packed mushroom bag

Mushroom unit

Plate 3 Spawn run, pinhead formation and fruiting body development of mushroom samples

***Pleurotus spp.* Var. 1**

***Pleurotus spp.* Var. 2**

Plate 4 Spawn run, pinhead formation and fruiting body development of mushroom samples

***Pleurotus spp.* Var. 3**

***Calocype spp*. Var. 1**

Plate 5 Spawn run, pinhead formation and fruiting body development of mushroom samples

***Calocype spp*. Var. 2**

Summary and Conclusion

Mushrooms are the richest source of protein in vegetarian diet. They are largely cultivated though out the urban areas. They became the greatest source of income as well for the women at home.

For the present study, the different varieties of *Pleurotus spp.* (Oyster mushroom) and *Calocybe spp.* (milky mushroom) were taken and were analyzed under various parameters.

The number of pin head, primordia and fruiting body development were analyzed and observed for the freshness of mushroom. Once the fruiting body turns brown, they were plucked and analyzed biochemically.

The preliminary phytochemical screening of the samples revealed the presence of alkaloid, terpenoids, tannins and proteins in the samples. The qualitative presence of proteins led way to quantitatively measure them.

The moisture content analysis exhibited the amount of fibre content in the samples. Out of oyster mushroom varieties *Pleurotus spp* Var. 3 showed high moisture content and out of milky mushrooms *Calocybe spp* Var. 2 had high moisture content.

The quantitative measurement of protein, amino acids, carbohydrates and lipids were clearly depicted their nutrient contents. The different mushroom samples differed in their nutrient content as well. In all that *Pleurotus spp* Var. 1 showed maximum nutrient content where as *Pleurotus spp* Var. 3 showed least amount of nutrients.

When compared to the milky mushroom varieties, the oyster mushroom varieties showed good results in their nutrient quality. In Oyster mushrooms, the *Pleurotus spp* Var. 1 stood out in all the nutrient efficiency.

Though in the present study, the spawns were purchased commercially, future scope of the study includes preparation of parental spawn from the existing mushrooms also to learn their anti microbial activity.

Bibliography

Abulude Olawale and Yusuf Abdularasheed. 2014. Attitudes of Nigerian towards mushroom. *Continental J. Microbiology*. 8 (1) 20-25

Adhikari, M K. 2010. Mushrooms of Nepal edited by G. Durrieu, P.V Printers, Battishputali, Kathmandu, Nepal. 236.

Ahmad Bilal, Wani, R H Bodha, A B Hamid Wani. 2010. A study on the nutritional and medicinal importance of mushroom .*Journal of medicinal plant research* ,2598-2604.

Alam S M, Raza M S. 2001. Importance of mushroom. *Industry and Economy, NIA, Tandojam,* Pakistan.

Aletor V A. 1995. Compositional studies on edible tropical species of mushrooms. *Food Chem*., 54:265– 268.

Alice B and Kustudia M. 2004. Mushroom cultivation and marketing. NCAT ATTRA, Publication No. IP087.

Ambili S and T P Nitiya. 2014. Oyster mushroom cultivation-A study in Palakkad district, Kerela *International Journal of Management and Social Science Research Review* 104-105

Barros L, P Baptista, D M Correia, S Casal, B Oliveira, and I C F R Ferreira. 2007. Fatty acid and sugar compositions ,and nutritional value of five wild edible mushrooms from Northeast Portugal. *FoodChemistry*,vol.105,no.1,pp.140–145,2007

Biswas M K 2014.A study on the oyster mushroom cultivation ..*International Journal of Management and Social Science Research Review 104-105.*

Bolton W and R Blair. 1982. Poultry Nutrition (Ministry of Agriculture, fisheries and food reference book 174 pp 4th ed. London: Her Majesty's Stationery office pp 115,118 ,121

Carmen Sanchez, 2010. Cultivation of *Pleurotus ostreatus* and other edible mushrooms. *Appl. Microbiol. Biotechnol*. 85:1321–133

Carvalho C S M, Aguiar L V B, and Andrade M C N. 2012. Applicability of the use of waste from different banana cultivars for the cultivation of the oyster mushroom. *Bio-resource*,432-435.

Celik Y and Pekker K. 2009. Benefit/Cost analysis of mushroom production for diversification of income in developing countries. *Bulgarian Journal of Agricultural Science*15(3): 228-237.

Chandra P Pukerel, Cultivation of oyster mushroom: a sustainable approach of rural development in Nepal JIST 2016, 21(1) : 56-60

Chang S T. 1980. Mushrooms as human food. *BioScience*, Vol. 30, no.6,pp.399–401.

C Chang, S.T. (1999). *Global impact of edible and medic*inal mushrooms on human welfare in the 21st Century: non-green revolution. *Brazilion Journal Of Microbiology.* 43: 819 – 826.

Chang S.T, and Miles P G. 2004. Mushrooms cultivation, nutritional value, medicinal effects and edible mushrooms. *Curr. Scie*.550.

Dlamini, B E, D M Earnshaw, and M T Masarirambi. 2012. Growth and Yield Response of Oyster Mushroom (*Pleurotus ostreatus*) Grown on Different

Locally Available Substrates.*Current Research Journal of Biological Sciences* 4(5): 623-629.

Doshi A, J F Munot and B P Chakravarthy.1988. Nutritional status of an edible *Calocybe indica* P&C. *Int. J. Mycol. Pl Pathol*. 18:301-302

Fasidi, I. O. 1996, Studies on *Volvariella esculenta* (mass) singer: Cultivation on Agricultural wastes and Proximate Composition of Stored Mushrooms. *Food Chemistry*, 55(2) ,161 – 163.

Fasidi I O and Kadiri M. 1993, Effect of sporophore maturity on chemical composition of *Volvariella esculenta*. *Die Nahrung* 37, 269-273.

Florezak J, Karmnska A, Wedzisz A. 2004. Comparison of the chemical contents of the selected wild growing mushrooms. *Bromatol.Chem.Toksykol.*37:365-371.

Folch J, Lees M, and Sloane-Stanley G. 1957. A simple method for the isolation and purification of totallipids from animal tissue. *Journal Of Biological Chemistry*. 226: 497- 509.

Fukushima M. 2000. LDL receptor mRNA in ratsis increased by dietary mushroom (*Agaricusbisporus*) fibre and sugar beef fibre. *J.Nutr*., 130:2151-2156..

Geetha D. 2011. Final report of WGDP Collection, identification, cultivation and popularization of edible/medicinal mushrooms of Western Ghats of Kerala.p8

Gyorfi J and C S Hajdu. 2007. Casing-material experiments with *P. eryngii*. *International J.Horticultural Sci*., 13(2): 33-36

.**Harborne J B. 1998**. Phytochemical methods: A guide to modern technique of plant analysis', *Champman and Hall, London.*

Hedge JE and Hofreiter B T. 1962. In: Methods in carbohydrate chemistry. Vol 17, (Eds.) **Whistler R L, and BeMiller,** *Journal Of Nutrient Academic Press*, New York, P. 420.

Jacob R A. 2010. Passive smoking induces oxidant effect of antioxidant vitamins. *Intr. J.Nutr*., 126.

Jayaraman J. 1981. Laboratory manual in biochemistry, Wiley-Eastern company limited, Madras. 1-65

Josephine M. 2014. Solid waste management through mushroom cultivation – an ecofriendly approach. *Int. J. Biol. Biomol. Agric. Food Biotechnol.Eng*, 8(2): 115- 117.

Kalac P and Svoboda L. 2009. A review of trace element concentrations in edible mushrooms. *Food Chem* 69:273–281.

Karim M Rezaul, Islam M Farhadu.2010. A study on the Phytochemical and Cytotoxic Investigation of Oyster mushroom.

Krishnamoorthy A.S. 1995. PhD Thesis, Taminnadu Agriculture University, Coimbatore, pp.222

Krishnamoorthy, Deepalakshmi ,Sankaran, Mirunalini. 2014 .A study on the oyster mushroom with nutritional and medicinal properties.718-726.

Law S K and T B N G. 2001. Antifungal santiprdiferatine activities from fruiting bodies of edible mushrooms. *Biochem.Biophys.Res.Commu.*285: 1071-1075.

Lindequist U, T H Niedermeyer and W D Julich. 2002. The pharmacological potential of mushrooms. *eCAM*, 2:285-299.

Lowry O.H, N J Rosenbrough, A L Farr and R J Randall. 1951.Protein measurement with the Folin-phenol reagent .*Journal Of Biological Chemistry*. 193: 265 – 275.

Manzi, P. 2001. Nutritional value of mushrooms widely consumed in Italy. *Food Chemistry* 73(3):321325

Mary Josephine R and B Sahana,**2014.** .A study on the cultivation of milky mushroom using paddy straw waste. *International Journal of currentMicrobiology*, 404-408.

Murugar A D and G Subbulakshmi. 2005. Nutritional value of edible wild mushrooms collected from the Khasi hills of Meghalaya, *Food Chem*; 89:599-603.

Nilson S and O Person .1997. Fungi of Northern Europe: Gill-fungi. Penguin, 24

Nirmala Gupta. 2014. Preliminary phytochemical screening of different extracts of *Pleurotus florida*. *Columbia Journal of Pharmaceutical Sciences* Vol 1 23-26.

Oei P. Mushroom cultivation, appropriate technology for mushroom growers. *Leiden : Backhugs Publishers;* 2003.

Pamela Manzi, 2010. Nutritional value of mushrooms widely consumed in Italy. *Food Chemistry*. Vol73(3). Pp.321-325.

Pandey N. 2004. Chemical analysis of mushroom of Kathmandu valley. Submitted to University Grant Commision

Park C H, Lim J, Lee Y, Lee B, Kim S, Lee J. and Kim, S. 2007. Optimization and morphology for decolorization of reactive black 5 by Funaliatrogii. *Enzy. Microb. Technol.* 40: 1758- 1764.

Pavel Kalac 2009. Chemical composition and nutritional value of European species of wild A review. *Food Chemistry* Volume 113, Issue 1, 9-16

Ouzouni P K, D. Petridis, W.-D. Koller, and K. A. Riganakos, "Nutritional value and metal content of wild edible mushrooms collected from West Macedonia and Epirus, Greece, *Food Chemistry*,vol.115,no.4,pp.1575–1580,2009.

Patil SS, Kadam RM, Shinde SL, Deshmukh SA. Effect of different substrate on productivity and proximate composition of P fl orida.Int J Plant Sci. 2008; 3: 151-153

Growing mushrooms: A review. *Food Chemistry*Volume 113, Issue 1, 9-16

Pushpa, H and Purushothama 2010. Nutritional analysis of wild and cultivated edible medicinal mushrooms. *World J. of Diary & Food Scie*.5(2):140-144.

Purkayastha and Chandra, A 1976. Studies on some edible mushroom mycelia as dietary components for laboratory animals. *Indian J. Exp.Biol*.14 pp.63-64

Rahul Amin, **Abul Khair, Nuhu Alam and Tae Soo Lee. 2010**. Effect of different substrates and casing materials on the growth and yield of *Calocybe indica*. *Mycobiology* 38(2): 97-101

Ram Naraian and Bharti Dixit. 2017. Nutritional Value of Three Different Oyster Mushrooms Grown on Cattail Weed Substrate. *Archives of Biotechnology and Biomedicine* Vol 5 61-66

Randive S D. Cultivation and study of growth of oyster mushroom on different agricultural waste substrate and its nutrient. *Advances in Applied Science Research*, 2012, 3 (4):1938-1949.

Boda R H,A H Wani, M A Zargar, B A Ganie, B A Wani, and S A Ganie, "Nutritional values and antioxidant potential of some edible mushrooms of Kashmir valley, *Pakistan Journal of PharmaceuticalSciences*,vol.25,no.3,623–627,2012

Royse D.J. 1996, Yield stimulation of shiitake by millet supplementation of wood chip substrate, *Mushroom Biol. Mushroom Prod*., 2, 277-283.

Savita and Anjana. 2017. Mushroom uses waste substrate and its nutrient. PharmaceuticalSciences,vol.25,no.3,.623–627,

Shailaja B and B Radhika (2018).A review on production of edible mushrooms and their applications. *International Journal of Pharmacy and Biological Sciences* Volume 8 | Issue 2265-283

Shah, Z.A., M. Ashraf and M. Ishtiaq, 2004. Comparative study on cultivation and yield performance of oyster mushroom (*Pleurotusostreatus*) on different substrates (wheat straw, leaves, saw dust). Pak. J. Nutr., 3: 158-160eaves, saw dust). Pak. J. Nutri., 3 (3): 158-160.

Shimizu and Anzai. (2001). Concentration of 137 Cs in dried Mushroom. *Journal of oral science***.** 43 (2): 145 - 149

Sonali D Randive , 2012. A study on the cultivation and study of oyster mushroom on different agricultural waste substrate and its nutrient analysis. *Advances in Applied Science Research.* 1938 - 1949.

Tan, K.K., 1981. Cotton waste is a fungus (*Pleurotus*) good susbtrate for cultivation of *Pleurotus ostreatus* the oyster mushroom. *Mush. Sci*., 11: 705-710.

Tapasya Anand and Rashmi Kapoor. **2011**. Antioxidant profile of selected herbs in comparison to mushroom. *J. Dairying, Food & H.S* 30(2):122-125

Usha, S 2007. Fatty acid analysis of *Calocybe indica* cultivated in various bio wastes. Mushroom researches 16(1):27-29

Varinea Singh,Gurpaul Singh Dhingra, A study of theTaxanomy,Physicochemical features and constituents of a few edible mushroom.

VashishtaB R, A study of the Botany part II fungi .1-3

Verma,.R.N, Singh G.B and Bilgrami K.S 1987. Fleshy fungal flora of N. E. H. India- I. Manipur and Meghalaya India mush .sci,2;414-421.

Yashvant Patel and Ram Naraion,V K Singh ,2012.A study on the medicinal properties of oyster mushroom. *World Journal of Fungi and Plant biology,*1-5.

.

PHYTOCHEMICAL SCREENING AND ANTIBACTERIAL ACTIVITY OF *CLEOME VISCOSA* LINN. AND *CRATAEVA RELIGIOSA* HOOK & FROST

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

Bachelor of Science in Botany

By

R. RAMYA	-	**16AUBO39**
A.REBIBHA	-	**16AUBO40**
M.SANTHIYA RAICHEL	-	**16AUBO41**
P.SARANYA	-	**16AUBO42**
V.SHEEBA	-	**16AUBO43**

DEPARTMENT OF BOTANY

ST.MARY'S COLLEGE (Autonomous)

THOOTHUKUDI-628001

APRIL, 2018 – 2019

CERTIFICATE

It is certified that this short term project work entitled **"PHYTOCHEMICAL SCREENING AND ANTIBACTERIAL ACTIVITY OF *CLEOME VISCOSA* LINN. AND *CRATAEVA RELIGIOSA* HOOK AND FROST"** submitted to St. Mary's college (Autonomous) affiliated to **MANONMANIAM SUNDARANAR UNIVERSITY** in partial fulfilment of the requirements for the degree of Bachelor 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 2018 – 2019 by the following students.

By

R. RAMYA	-	**16AUBO39**
A.REBIBHA	-	**16AUBO40**
M.SANTHIYA RAICHEL	-	**16AUBO41**
P.SARANYA	-	**16AUBO42**
V.SHEEBA	-	**16AUBO43**

GUIDE

29/3/19

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

EXAMINER

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

ACKNOWLEDGEMENT

It is our humble attempt to present this project **"PHYTOCHEMICAL SCREENING AND ANTIBACTERIAL ACTIVITY OF *CLEOME VISCOSA* LINN. AND *CRATAEVA RELIGIOSA* HOOK & FROST"** First and foremost our sincere gratitude belongs to **Dr. Sr. A.S.J. LUCIA ROSE., M.Phil., PGDCA., Ph.D.,** Principal, St. Mary's College (Autonomous) for providing us an opportunity to do this project.

With deep sense of thanks to **Dr. M. GLORY M.Sc., M.Phil., Ph.D.** Head of the Department of Botany, St. Mary's College, Thoothukudi for her encouragement and support.

We take great pleasure in expressing our heartfelt thanks to **Dr. F. Dayana Lobo** Assistant professor of Botany, St. Mary's College, Thoothukudi for suggesting this topic, for providing necessary information, timely suggestions, guidance and sustained interest throughout the period of investigation and for the perusal of this report.

Thanks are also due to the guiding hands of all the staff members and the laboratory assistants of Botany, and also my friends for their encouragements.

We would like to thank and extend our heartfelt thanks to **Robert Xerox** for the execution of the work.

Above all we humbly bow in gratitude to the **GOD LORD** for showering abundant graces on us and for helping us to yield fruitful results

CONTENTS

CHAPTER - I

INTRODUCTION

Since ancient times, plants have been source of crude material for medicines. A rich heritage of knowledge of medicinal importance of plants is available in India amongst the common people. Generally, it is estimated that more than 3,500 plant species in India are useful as a source of crude drug. Near about 2,500 plants are ethno medicinally important. There are too many plant species which are considered as a weed, but they are also medicinally important. The products obtained from plants are used in various traditional and modern methods of therapy. The trend of using natural products as a medicine is ever increasing. The invention of modern biotechnological and bioinformatics techniques have helped new drug discoveries. Medicinal plants are the basis of modern pharmaceuticals used today for various ailments, as it has a good source of natural antioxidants for medicinal use and related to radical mechanism (Peter *et al.*, 2012).

Medicinal plants are expensive gift from human to nature. The approval of traditional medicine as an alternative form of health care and the improvement of microbial resistance to the existing antibiotics has lead researchers to scrutinize the antimicrobial compounds. Herbal medicines are safer than synthetic medicines because the phytochemicals in the plant extract target the biochemical pathway. Medicinal plants have been used all over the world for the treatment and prevention of various ailments, particularly in developing countries where infectious disease is endemic and modern health facilities and services are inadequate. The tribal communities of many countries are still using medicinal plants to cure sickness (Balaji *et al.*, 2009)

Phytochemicals are compounds that are produced by plants "phyto" means "plant". They are found in fruits, vegetables, grains, beans and other parts. Some of these phytochemicals are believed to protect cells from damage that could lead to cancer. Some scientists think that you could reduce cancer risk by as much as 40% by eating more vegetables, fruits and other plant foods that have certain phytochemicals in them. Plants, the most wonderful gift from nature have been used as an origin of drugs. Various types of drugs are obtained from them. These types of plants are known as medicinal plants. We use one or more of its organ for therapeutic purpose as a precursor of synthesizing of many useful drugs. According to some generous estimates, almost 80% of the present day medicines are directly or indirectly obtained from plants (Vidhya and Umavandhana, 2016)

Capparidaceae family comprises various important medicinal properties distributed in tropical and subtropical India, whose medicinal usage has been reported in the traditional systems of medicine such as Ayurvedha, Siddha and Unani. The Capparidaceae family contains so many active constituents such as alcohol, alkaloids, amino acids, amyrin, anthocyanins, betulin, carbohydrates, flavonoids, glycosides, saponins, steroids, sterol and terpenes were reported in various researches.

Cleome viscosa Linn. (wild or dog mustard), family (Capparaceae) is a widely distributed sticky herb with yellow flowers and long slender pods containing seeds, which similar those of mustard (Hindi), Hurhuria (Bengali), Nayikkadugu (Tamil) in South Asian folk medicine, found throughout the larger part of Indian Subcontinent, often in waste places. The plant finds its use in the traditional system of local medicine as a laxative and diuretic. It is reported to be useful in the treatment of malarial fevers, fever due to indigestion, skin diseases, leprosy, blood diseases, and uterine complaints. In the Unani system of medicine, the seeds of the plant are

documented as anthelmintic and detergent, and are given to treat fever and diarrhoea. The seeds are used for anthelmintic while the leaves are useful for healing wounds. In Ayurveda system of medicine, in the plant is used in fever, inflammation, liver diseases and diarrhoea. The rural people use the fresh juice of the crushed seed for infantile convulsions and mental disorder. The juice of the plant diluted with water is given internally in small quantities in fever and the seeds and leaves are useful in wound healing. The pungent seeds and seed pods are used as a mustard substitute in curries.(Lakshmi and Bindhu, 2011)

Crataeva religiosa Hook& Frost belonging to family capparidaceae (capparaceae) is a tree usually found in the vicinity of temples of central and eastern India. It is known as pasugandha in Sanskrit, three legs in capper in English, Varunain hindi. *Crataeva religiosa* is globally distributed in India, Myanmar, Sri Lanka, Malaysia, Indonesia and China. In India, it is found in Penisular India, Western India, Gangetic plains and Eastern India, upto Tripura and Manipur. The plant part used for the medicinal purpose includes leaves, stem bark and root bark. Plant is used ethnopharmacologically as diuretic, laxative, lithonotriptic, antirehumatic, antiperiodic, bitter tonic, rubifacient and counterirritant. The counterirritant bark is used in the urinary disorders including kidney and bladder stones, antiemetic and calculous affections and as an antidote in snakebite (Nandakami, 1997). Scanty literature is available on bactericidal properties of this *Cleome viscosa* and *Crataeva religiosa* and hence both plants are screened for the bactericidal potential.

CHAPTER - II

SCOPE AND OBJECTIVES

From old age, plants are used as crude material for drugs. In India, rich knowledge of medicinal importance of plants is available to the common people. About 3,500 plant species are useful as a source of crude drug in India. Medicinally important plants are about 2,500 in number. Some plant species are considered as a weed, but they are also medicinally important too. In various traditional and modern methods of therapy, plants products are used. In current era, the trend of using herbal medicine is in scope. New drugs are discovered by the invention of modern biotechnological and bioinformatics techniques. In current era, herbal medicines are used more often as human believe in natural therapies is increasing day by day. Natural products play a dominant role in the development of novel drug leads for the treatment and prevention of diseases. 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 phytochemicals present in the stem and leaf of *Cleome viscosa* and *Crataeva religiosa*
- Elucidating the effectiveness of medicinal plants (*Cleome viscosa* and *Crataeva religiosa*) in controlling human pathogenic bacteria such as *Escherichia coli*, *Bacillus subtilis*, *Salmonella typhi*, *Serratia marcescens* and *Klebsiella pneumoniae*.

The results of the study also underline the cost effective, bio friendly resources which could be tapped for development of effective drugs in future.

CHAPTER-III

LITERATURE REVIEW

Plants are used medicinally in different countries and are a source of many potent and powerful drugs (Srivastava *et al*., 2008). A number of herbs with significant antimicrobial activity have been reported in different traditional literatures (Sathish *et al*., 1999). Many medicinal plants are used daily in Ayurvedic practices. In India more than 7000 medicinal plants are known. According to a report of World Health Organisation, more than 80% of World's population depends on traditional medicine for the primary healthcare needs (Umamaheswari *et al*., 2008). The less availability and high cost of new generation antibiotics necessitates looking for the substances from alternative medicines with claimed antimicrobial activity (Poovendran *et al*., 2011).

DISTRIBUTION AND MEDICINAL VALUES OF PLANTS

Crataeva religiosa commonly known as Mavilankai in tamil is a common medicinal plant distributed throughout India and is known to have versatile medicinal values. The plant parts used for the medicinal purposes include leaves, stem bark and root bark. The plant is used ethnopharmacologically as a diuretic, laxative, lithonotriptic, antirheumatic, antiperiodic, bitter tonic, rubifacient and counter irritant (Nandakami, 1997). The fruit juice, leaves and bark are used to cure snakebite, infected wounds and cuts. It increases the appetite and controls other skin diseases (Sapkota, 2003)

Cleome viscosa L., the most commonly occurring species of *Cleome* is an annual herb, seen along the roadsides, wastelands and forest undergrowth commonly known as 'wild mustard', it is referred to as 'Araiveli', 'Vela', 'Naivela' in

Malayalam. The plant is reported to possess rube facient, vesicant, expectorant, astringent, antispasmodic, contact insecticidal, repellent, antifeedant, nematicidal and anthelmintic properties (Fletchers, 1999; Lakshmi and Bindu, 2011). The leaves and seeds are used to treat viral infections, fever, rheumatism and headache. The roots are a remedy for scurvy. The seeds have a pleasant flavour and in India they are used as a condiment substitute for mustard seed and cumin in the preparation of pickling spices, sausages, vegetables, curries and pulses.

Cleome burmanni W. and A., is an erect herb commonly known as 'Kattukadku' in Malayalum. The plant possesses anthelmintic properties (Lakshmi and Bindu, 2011). Apart from this, no other reports exist for the medicinal value of the plant.

Cleome gynandra is used as a medicinal plant and can be found in all over world. It grows as a weed in paddy fields and also in roadsides and in open grass lands. In India it is never cultivated but grows spontaneously everywhere. Different species of *Cleome* can be found in all states of India. This article briefly reviews the botany, pharmacology, biochemistry, folkloric, traditional medical applications of the plant and also different possible medical and therapeutic applications established through various laboratory researches and papers. The medicinal application of this plant is also described in Ayurvedic pharmacopoeia of India and also in other ancient medical texts. In Ayurvedic medicine it is a chief constituent in Narayana Churna. In Ayurveda it is used as an Anthelmentic, in ear disease, pruritis and several other diseases like gastro intestinal disorders and gastrointestinal infections etc. This is an attempt to compile and document information on different aspects of *Cleome gynandra* and highlight the need for research and development.(Mishra, 2011)

Cleome rutidosperm possesses diuretic, laxative, anthelmintic, anti-microbial, analgesic, anti-inflammatory, antipyretic, antioxidant, anti-arthritic and antiplasmodial activities (Bose *et al.*, 2010; Chakraborty and Roy, 2010). Abundance in nature and their proven medicinal worth are two factors that make it likely that these plants will be used in Ayurvedic drug preparation in due course though presently they are consumed merely as cooked vegetables or as decoctions. Therefore a proper evaluation of the plant powder is warranted.

Crataeva religiosa is found in the forest galleries of African Sudanese area, in India and Burma (Adjanohoun *et al.*, 1989). It is found in many parts of Africa where it has different uses. In Benin, the plant has different vernacular names: goriguiberou (Bariba, northern region), wonton-zonzwen (Fon and Goun, southern region), tanilabia and tcharouwenwe (Yoruba, middle and southern region). The leaves were used in combination with other plants by traditional healers and local populations as analgesic, antispasmodic, antimalarial and antidiarrheic. The decoction of fresh leaves and branches is taken orally to treat hypertension (Adjanohoun *et al.*, 1989). In rural areas in India, it is reported that *C. religiosa* is currently used for the treatment of different diseases (Khan *et al.*, 2003). In Benin, the decoction of this species was used by traditional breeders to treat the digestive disorders of the bred animals such as ruminants and *Thryonomys swinderianus*. This study looks into the investigation of the *in vitro* antimicrobial activity of extracts and fractions obtained from *C. religiosa* against five pathogenic micro-organisms that cause the most common causes of digestive infectious diseases in *T. swinderianus* Temminck breeding in the republic of Benin.

Antimicrobial and phytochemical activity of *Cleome viscosa*

Chandrasekaran Swaminathan, 2017 studied the phytochemical analysis, antibacterial activity and antioxidant property of *Cleome viscosa* using ethanol, acetone and methanol extracts of leaves. Phytochemical analysis revealed the presence of carbohydrates, alkaloids, phytosterols, fixed oils, saponins, phenolic compounds and flavonoids. Though all the extracts exhibited antibacterial activity, methanol extracts of leaves registered highest antibacterial activity against *Staphylococcus aureus*. The strong free radical scavenging effect was observed with ethanol extract at 100 µg/ml which was comparable to that of standard ascorbic acid. This study confirmed the broad spectrum antimicrobial activity and free scavenging activities of *Cleome viscosa* L. and could be used as a potential alternative for treatment of various ailments.

Dhanalakshmi *et al.*, 2011 investigated the antimicrobial activity of methanolic extract of *Cleome viscosa* against pathogenic bacteria and fungi responsible for common infections. The present investigation may be concluded that the plant *C. viscosa* is endowed with significant antimicrobial due to the presence active constituents, there by justifying its use in the indigenous system of medicine.

Suttijit Sriwatcharakul, 2016 analysed the antibacterial activity of *Cleome viscosa* Linn. using 6 bacterial strains such as *Salmonella typhimurium, Pseudomonas aeruginosa, Staphylococcus aureus, Streptococcus epidermis, Escherichia coli* and *Bacillus subtilis*. The minimum inhibition concentration with gram +ve strains revealed that leaf crude extract give the best result of the lowest concentration compared with other plant parts to inhibit the growth *of S. aureus, S. epidermis* and *B. subtilis*. According to the bioactivities results the leaf crude extract of *Cleome*

viscosa Linn. is the most interesting plant part for further work to search the beneficial of this weed.

Cleome viscosa L. is well known for its medicinal properties. Lactam nonanoic acid [2-amino-9-(4-oxoazetidin-2-yl)-nonanoicacid; $C_{12}H_{22}N_2O_3$, mol. Wt. 242] has been isolated from the roots exudates of *Cleome viscosa*. The aqueous solution of this pure compound has been tested on bacteria (*Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*) and fungi (*Aspergillus fumigates*, *A. niger* and *A. tamarii*). At a dosage of 500 ppm and above, *P. aeruginosa* and *S. aureus* were totally inhibited while *E. coli* remained unaffected. On the other hand, growth of *A. niger* and *A. tamarii* was stimulated while there was no effect on *A. fumigatus*. This pure compound showed concentration-dependent inhibitory activity on rice, gram and mustard seeds (Anirban Jana and Suprana Mandal Biswas, 2011).

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.

Lakshmi sreekumar pillai and Bindu Rajeshwary nair, 2012 studied the pharmacognostical and phytochemical characterization of two common road side

weeds, *Cleome viscosa* L. and *C. burmanni* W. & A. The study includes the macroscopical, microscopical, histochemical, fluorescence, physico-chemical and phytochemical analysis .Both the species could be distinguished clearly on the basis of all the pharmacognostic parameters studied.

Bose *et al*., 2007 studied the antimicrobial activity of the ethanol extract and its various fractions of *Cleome rutidosperma* DC. The study revealed the antimicrobial activity of the ethanol extract and its different fractions against the tested strains of micro organisms. The activities were found to be potentiated by fractionation of mother extract (ethanol extract) with highest activity for diethyl ether fraction. The present study indicates the potential usefulness of *C. rutidosperma* in the treatment of various pathogenic diseases.p

Sudhakar *et al*., 2005 studied the ethanolic extracts of the leaves and flowers of *Cleome viscosa* and roots of *Gmelina asiatica* were tested for antimicrobial activity. The two plants exhibited a broad spectrum of antimicrobial activity, particularly significative against *Escherichia coli, Proteus vulgaris* and *Pseudomonas aeruginosa*. The leaf extract of *C. viscosa* showed moderate activity against pathogenic fungi.

Asis Bala *et al*., 2010 evaluated the anticancer activity of methanol extract of *Cleome gynandra* on Ehrlich Ascites Carcinoma treated mice.

Antibacterial and phytochemical activity of *Crataeva religiosa*

Gowsalya and Saravanababu, 2013 studied the Phytochemical and antibacterial activity of chloroform, ethanol and hexane extract of *Crataeva religiosa* bark samples with respect to three pathogenic bacteria species *Enterococcus faecalis*,

E. coli and *Staphylococcus*. The ethanolic extract of bark was effective than chloroform and hexane extract. The bacterium *Enterococcus faecalis* has more inhibition than two other bacterial species studied. Preliminary phytochemical analysis revealed the presence of alkaloids, glycosides, flavanoids, tannins, saponins, steroids and anthroquinone glycosides. Among the phytochemicals, saponins, glycosides and anthroquinone glycosides was shown best in all extracts.

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.

Latifou Lagnika *et al.*, 2011 screened the antibacterial activity of leaves of *Crataeva religiosa* used in Benin traditional veterinary medicine against bacterial infection of *Thryonmys swinderians*. Seven extracts from *C. religiosa* were screened for the bacterial activity was evaluated micro test method and bioautography method. The minimum inhibitory concentration and the total were determined. The screening experiment revealed that ethyl acetate extract was more potent than other extracts. The results provide an evidence for the traditional use of *C. religiosa* for the treatment of infective diseases of *T. swinderianus*.

Udaysing Hari Patil and Dattatraya Gaikwad, 2011 reported the antibacterial activity of methanolic extract of *Crataeva religiosa* bark samples with *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoneae*, *Salmonella typhi*, *Proteus mirabilis* and *Micrococcus sp*. The methanolic extract of apical bark was effective than the middle bark and mature bark in inhibiting the growth of all bacteria. The bacterium *Staphylococcus aureus* was most effective among all the bacterial species studied. Preliminary phytochemical analysis revealed the presence of alkaloids, glycosides, flavanoids, phenols, saponins and terpenoids. The concentrations secondary metabolites was found higher in the apical stem bark than the middle and mature stem bark. The percent extract yield was maximum in apical stem bark.

Antibacterial and phytochemical activity of *Gynandropsis gynandra* and *capparis sp*

Rajaselvam and Basil Rose, 2016 investigated the antimicrobial and phytochemical analysis of different extracts by agar well diffusion method. Five bacterial pathogen such as Gram positive – *Bacillus subtilis, staphylococcus aureus* and Gram negative – *Escherichia coli, Pseudomonas aeruginosa* and *Proteus mirabilis* were used as test organisms. Among the extract prepared in four solvents ethanolic extracts were found to possess highest antimicrobial activity against *E. coli, Proteus mirabilis* and *Pseudomonas aeruginosa.* Acetone and chloroform extracts showed moderate inhibitory potency and no inhibitory activity was observed when tested in the aqueous extract. The phytochemical analysis revealed the presence of tannis, terpenoids, saponin and protein.

Ogunmefun *et al.*, 2013 reported the phytochemical and antifungal studies of two members of the cappardidaceae family which are *Gynandropsis gynandra* and *Buchholzia coriacea*. These two plants were screened for the presence of their active constituents. The antifungal activities of the leaves and stem were tested using some fungus. The activity of the extract at 200 mg/100ml of methanol was compared with methanol as the control and Tioconazole as reference standard. The result of the antifungal assay of the plant extracts justifies their use in traditional medicine.

Ajaiboeya, 2000 examined the leaves and stems of *Gynandropsis gynandra L.* (Briq.) and *Buchholzia coriaceae* Engl. (A. Chev.). Two plants were screened phytochemically for the presence of secondary metabolites and *in vitro* antibacterial and antifungal properties respectively. The main secondary metabolites indicated in both plants were alkaloids, cyanogenetic glycosides and steroidal nuclues. Anthraquinones were slightly indicated. Hexane and methanolic extracts of the plant materials of the two plants were screened for antimicrobial properties using eleven human pathogenic microorganisms. At a concentration of 200 mg/ml, the extracts displayed in both bioassays. Of the eight extracts investigated, *B. coriaceae* stem hexane extract displayed the highest activity. Ampicillin and tioconazole were used as standard reference drugs while methanol was included as a solubilising agent as well as a negative control in the study. Diameters of zones of inhibition were in the range of 10-24 mm for the extracts and drugs.

Kalpana *et al.*, 2015 tested the ethanolic extracts of *Capparis sepiaria* L. leaves and fruits for antibacterial activity against six species of bacteria, using disc diffusion technique. Ethanolic fruit extracts showed higher activity than ethanolic leaf extracts of *Capparis sepiaria*. The results showed that in 1000 ppm leaf extract, a maximum of 2.1 cm ZI was observed against *Bacillus subtilis* followed by 2.0 against

Enterococcus faecalis. The maximum zone was recorded in ethanolic fruit extract against *Pseudomonas aeruginosa*.

Carla Boga *et al*., 2010 studied the antibacterial activity of *Capparis spinosa* L. roots, which shows an interesting bacteriostatic activity on the growth of *Deinococcus radiophilus*. Heterocyclic compounds were also recovered from the chloroformic extract of the roots.

Deepa priya *et al.*, 2012 evaluated the antimicrobial activity of hexane, ethyl acetate, methanol and aqueous extracts of the leaves of *Capparis zeylanica*. Based on the results obtained from the zone of inhibition, antifungal activity of crude extracts was performed using broth dilution method. Maximum inhibition was recorded in ethyl acetate extract. In case if antifungal activity of the aqueous extract has high inhibited high growth. In preliminary phytochemical screening of aqueous extract have more positive results when compared to other extracts. The results of this study present the evidence that *Capparis zeylanica* given either systematically or topically has antimicrobial properties.

Jigna Parekh *et al*., 2007 studied the aqueous and methanol extracts of 12 plants each belonging to different families were evaluated for antibacterial activity against medically important bacteria. The *in vitro* antibacterial activity was performed by agar disc diffusion and agar well diffusion method. The aqueous extracts were inactive but methanol extracts showed some degree of antibacterial activity against the tested bacterial strains. Amongst the plant species screened, methanol extract of *Bauhinia variegata* bark showed best antibacterial activity.

CHAPTER-IV

MATERIALS AND METHODS

Collection and processing of the plant materials

Crataeva religiosa and *Cleome viscosa* were collected from the St. Mary's College (Autonomous) Thoothukudi. The Leaf and stem of *Crataeva religiosa* and *Cleome viscosa* 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 250 ml of different solvents like petroleum ether, benzene, methanol, acetone, ethanol and 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 phytochemical analysis and antibacterial activity.

***a) Cleome viscosa.* Linn**

Common Name	:	Pili, Talavani
Class	:	Dicotyledons
Subclass	:	Polypetalae
Series	:	Thalamiflorae
Order	:	Parietales
Family	:	Capparidaceae

Plate 1 : *Cleome viscosa*.Linn

Plate 2 : *Crataeva religiosa* Hook & Frost

Description

Cleome viscosa Linn. is an annual, erect, 30-90 cm high plant. Stem of plant is grooved, densely clothed with glandular and simple hairs. Leaves of the plant are 3-5 foliolate. Lower leaves petioles are 2.5-5 cm long gradually becoming shorter upwards. The bracts are subsessile. Leaflets are elliptical–oblong or obovate, acute or obtuse. Petioles are short and hairy. Flowers are yellow in colour, axillary, growing out into a lax raceme. Pedicels are slender, terete and hairy. Sepals are 4.5 cm long oblong–lanceolate, glandular–pubescent outside. Petals are oblong–obovate, about 12 mm long, veined. Stamens are more than 20 in number. Capsules 5-6.3 by 0.4 cm, erect, hairy, obliquely striate, compressed, tapering towards both ends, terminated by a style 3 mm. long. Seeds are brown–black in colour when ripe, finely transversely striate, subglobose

***b) Crataeva religiosa* G. Forst**

Common Name	:	Maralingam
Class	:	Dicotyledons
Subclass	:	Polypetalae
Series	:	Thalamiflorae
Order	:	Parietales
Family	:	Capparidaceae

Description

A small or medium sized tree with broad terminal corymbs of white flowers, which turn yellow soon after opening; filaments purple. Berry 1-2 in thick globose or

ovate. Found here and these occasionally in almost all districts, often planted, frequent along river banks. Wood yellowish white, even grained but not double. vern. Tam. marvillinga; Tel. Uskia man, Voolemara; kan Nirvala; mal. Nir mathalam.

Preliminary phytochemical analysis:

The qualitative phytochemical test for steroids, quinones, terpenoids, saponins, alkaloids, flavonoids, tannins, phenol and coumarin were carried out on the concentrated extracts using the standard procedures to identify the constituents in leaf and stem of *Cloeme viscosa* and *Crataeva religiosa*. The chemical test for various phytoconstituents in the extracts were carried as described below.

Alkaloids (Mayers test)

To 1 ml of leaf extract, 6 drops of Mayer's reagent was added. The formation of yellowish creamish precipate indicated the presence of alkaloids (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)

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)

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).

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)

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)

Phenols

To 1 ml of the leaf extract was mixed with 3% ferric chloride solution was added. Appearance of deep blue colour indicated the presence of phenol (Kokate, 2000)

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)

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)

Antibacterial activity – Disc diffusion Assay (Bauer *et al.*, 1966).

Antibacterial activity of each plant extract was analysed using human pathogens., Gram positive bacteria, *Bacillus subtilis* and Gram negative bacteria *Escherichia coli*, *Salmonella typhi*, *Pseudomonas aeruginosa* and *Klebsiella pneumonia* and *Serratia marcescens* obtained from the Department of Botany: St. Mary's College (Autonomous), Thoothukudi. Each bacterial pathogen was subcultured in agar medium and maintained. What man No.l sterile filter paper discs (6mm) were impregnated with different plant extract concentrations0.5 mg/ml, 1 mg/ml, 1.5 mg/ml, 2 mg/ml, 2.5 mg/ml and 3 mg/ml 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 hours of ±37°C incubation, the diameter of the inhibition zone was measured. For positive control, streptomycin disc (100 µg/ml) was used, whereas for negative control, respective solvents were loaded on sterile discs. All the assays were carried out in triplicates. The leaf and stem extract concentration which has effected minimum inhibition (MIC = 2.5 mg/ml) was used for further studies.

CHAPTER-V

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 result of the phytochemical screening of the leaves and stem of *Cleome viscosa* and *Crataeva religiosa* and the yield of each extract are presented in Table (1 to 4). Methanol, ethanol, benzene, acetone and petroleum ether of *Cleome viscosa* and *Crataeva religiosa* were tested with various reagents to detect the presence of phytochemicals. Majority of the compounds were present in the ethanolic and methanolic extracts of *Cleome viscosa* and *Crataeva religiosa*. Saponins, flavonoids, phenol, terpenoids, steroids, coumarins and tannins were detected in both *Cleome viscosa* and *Crataeva religiosa*.

Saponins have great pharmaceutical importance as it has antioxidant, anticancer, anti-inflammatory and antifungal properties and is used to treat hyper-chlosterolaemia and hyper-glycemia (De Lucca *et al.*, 2005). Flavonoids have been reported to possess many useful properties, including anti-inflammatory, oestrogenic,

Table 1 : Prelimilary phoytochemical analysis of *Cleome viscosa* leaf extracted with different solvent

Phytochemical	Methanol	Ethanol	Benzene	Acetone	Petroleumether
Phenol	–	–	+	+	+
Tannin	–	+	–	+	–
Coumerins	–	+	–	+	–
Saponins	+	–	–	–	+
Quinons	+	–	–	+	–
Steroied	+	+		+	–
Flavonoied	+	–	+	+	+
Alkaloied	+	+	+	+	+
Terpenoied	+	+	+	+	+

+ = indicates presence of phytochemicals.

− = indicates absence of phytochemicals

Table 2 : Preliminary phytochemical analysis of *Cleome viscosa* of stem extracted with different solvent

Phytochemical	Methanol	Ethanol	Benzene	Acetone	Petroleumether
Phenol	–	–	–	–	–
Tannin	–	+	–	+	–
Coumerins	–	+	–	+	–
Saponins	+	–	–	–	+
Quinons	+	–	–	+	–
Steroied	–	+	+	–	+
Flavonoied	+	+	+	+	+
Alkaloied	+	+	+	+	+
Terpenoied	+	+	+	+	+

+ = indicates presence of phytochemicals.

– = indicates absence of phytochemicals

Table 3 : Preliminary phytochemical analysis of *Crataeva religiosa* of leaf extracted with different solvent

Phytochemical	Methanol	Ethanol	Benzene	Acetone	Petroleumether
Phenol	+	+	+	+	+
Tannin	–	+	+	–	+
Coumerins	+	–	+	–	–
Saponins	–	+	+	+	–
Quinons	–	+	+	+	+
Steroied	+	+	–	+	+
Flavonoied	+	+	–	+	+
Alkaloied	+	+	+	+	+
Terpenoied	+	+	+	+	+

+ = indicates presence of phytochemicals.

– = indicates absence of phytochemicals

Table 4 : Preliminary phytochemical analysis of *Crataeva religiosa* of stem extracted with different solvent

Phytochemical	Methanol	Ethanol	Benzene	Acetone	Petroleumether
Phenol	+	+	+	+	+
Tannin	+	+	+	–	+
Coumerins	+	–	–	–	+
Saponins	–	+	+	–	+
Quinons	+	+	+	–	–
Steroied	+	+	–	+	+
Flavonoied	+	–	–	+	+
Alkaloied	+	+	+	+	+
Terpenoied	+	+	+	+	+

+ = indicates presence of phytochemicals.

– = indicates absence of phytochemicals

enzyme inhibition, antimicrobial, anti-allergic, antioxidant, vascular and cytotoxic antitumour activities (Haysteen, 1990). Phenols are of great importance as they protect the human body from the oxidative stress, which cause many disease, including cancer, cardiovascular problems and ageing (Robards *et al.*, 1990). Steroids are also of interest in pharmacy dye to their relationship with sex hormones. Terpenoids are attributed for analgesic and anti-inflammatory activities (Santhi *et al.*, 2011). Tannins have received considerable attention in the field of nutrition, health and medicine due to their antioxidant, antimicrobial, and anti-inflammatory properties (Santos-Buelga and Scalbert, 2000). Coumarins are potential antioxidants (Tseng, 1999). Emodins have many biological effects such as anti-cancer, anti-microbial and anti- inflammatory effects (Wang *et al., 2007).*

Flavonoids, terpenoids and alkaloids were found in all five extracts of *Cleome viscosa*. Terpenoids and phenols were found in all five extracts of *Crateava religiosa*. Rests of the phytochemicals was moderate / trace amounts. Compared to *C. viscosa*, *C. religiosa* showed the presence of more phytochemicals. Hence this plant should be evaluated further to assess its phytotherapeutic properties.

The presence of antibacterial substance in the higher plants is well established (Srinivasan, 2001). Plants have provided a source of inspiration for novel drug compounds as plants derived medicines have made significant contribution towards human health. Phytomedicine can be used for the treatment of diseases as is done in case of Unani and Ayuredic system of medicines or it can be the base for the development of a medicine, a natural blueprint for the development of a drug (Didry *et al.*, 1998). Successive isolation of botanical compounds from plant material is largely dependent on the type of solvent used in the extraction procedure. The traditional healers use primarily water as the solvent but we found in this study the

Table 5 : Antibacterial activity of *Cleome viscosa* leaf extracted with different solvents against human pathogens

S. No.	Solvent	Inhibition Zone in mm				
		E. coli (2.5mg/ml)	*B. subtilis* (2.5mg/ml)	*K. Pneumonia* (2.5mg/ml)	*S. typhi* (2.5mg/ml)	*S. Marcesens* (2.5mg/ml)
1	Ethanol	10	8	11	9	NS
2	Benzene	4	8	10	9	8
3	Acetone	10	10	7	6	9
4	Methanol	9	8	4	9	7
5	Petroleum ether	6	9	6	NS	10
6	Streptomycin	11	15	10	10	11

Control = Streptomycin (100 µg/ml)

Plant extract = 2.5 mg/ml (effective concentration)

NS = No sensitivity

Table 6 : Antibacterial activity of *Cleome viscosa* stem extracted with different solvents against human pathogens

S. No.	Solvent	Inhibition Zone in mm				
		E. coli (2.5mg/ml)	*B. subtilis* (2.5mg/ml)	*K. Pneumonia* (2.5mg/ml)	*S. typhi* (2.5mg/ml)	*S. Marcescence* (2.5mg/ml)
1	Ethanol	16	NS	15	6	11
2	Benzene	NS	7	8	9	9
3	Acetone	10	12	10	6	7
4	Methanol	NS	5	NS	10	10
5	Petroleum ether	NS	11	5	5	12
6	Streptomycin	10	13	11	15	10

Control = Streptomycin (100 µg/ml)

Plant extract = 2.5 mg/ml (effective concentration)

NS = NO Sensitivity

Table 7 : Antibacterial activity of *Crataeva religiosa* leaf extracted with different solvents against human pathogens

S. No.	Solvent	Inhibition Zone in mm				
		E. coli (2.5mg/ml)	*B. subtilis* (2.5mg/ml)	*K. pneumoniam* (2.5mg/ml)	*S. typhi* (2.5mg/ml)	*S. Marcescens* (2.5mg/ml)
1	Ethanol	11	14	14	5	6
2	Benzene	11	7	12	9	9
3	Acetone	7	NS	11	11	4
4	Methanol	14	11	9	9	11
5	Petroleum ether	10	9	7	NS	9
6	Streptomycin	11	15	10	11	10

Control = Streptomycin (100 µg/ml)

Plant extract = 2.5 mg/ml (effective concentration)

NS = NO Sensitivity

Table 8 : Antibacterial activity of *Crataeva religiosa* stem extracted with different solvents against human pathogens

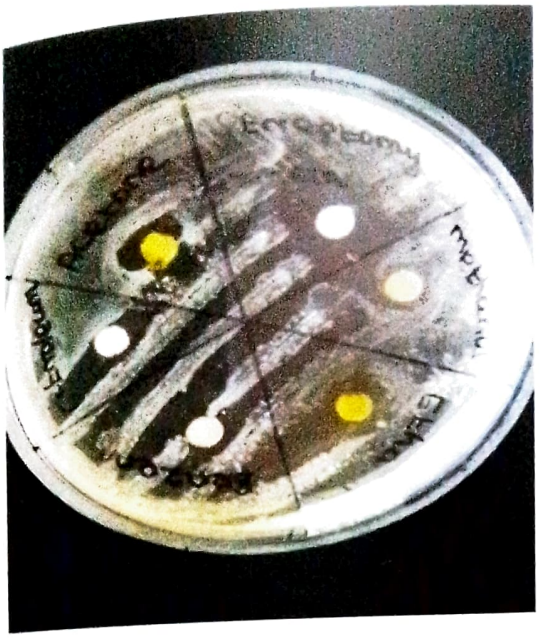
S. No.	Solvent	Inhibition Zone in mm				
		E. coli (2.5mg/ml)	*B. subtilis* (2.5mg/ml)	*K. Pneumonia* (2.5mg/ml)	*S. typhi* (2.5mg/ml)	*S. Marcescens* (2.5mg/ml)
1	Ethanol	10	15	15	NS	7
2	Benzene	10	15	10	10	8
3	Acetone	10	8	13	6	5
4	Methanol	10	13	0	NS	9
5	Petroleum ether	8	10	5	10	10
6	Streptomycin	11	10	15	16	10

Control = Streptomycin (100 µg/ml)

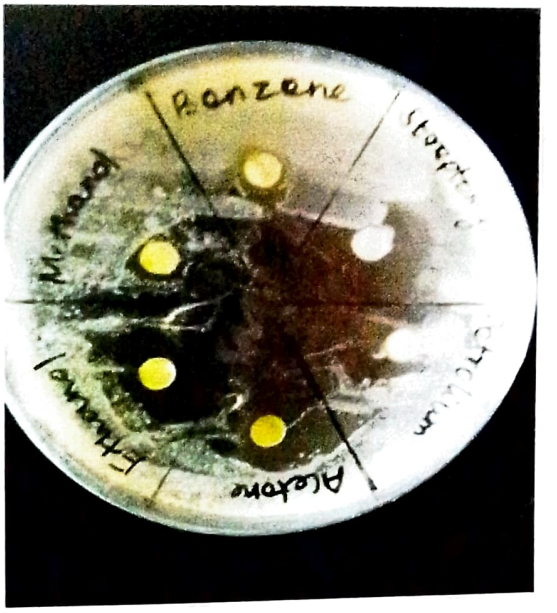
Plant extract = 2.5 mg/ml (effective concentration)

NS = NO Sensitivity

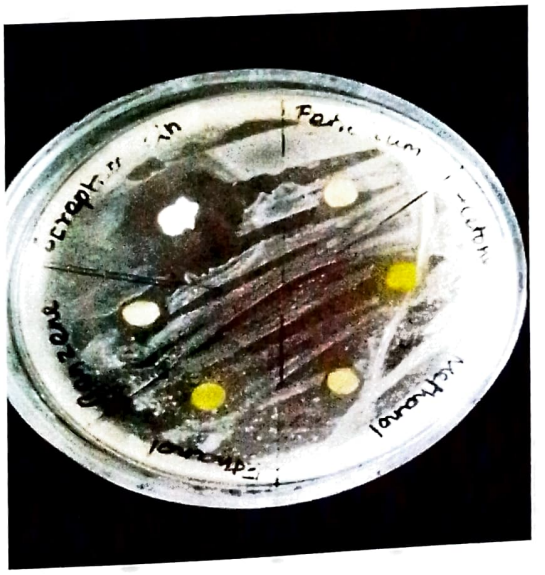
Plate 3 : In *vitro* antibacterial activity of *Cleome viscosa* leaf and stem extract against human pathogens



Cleome viscosa* stem extract against *E.coli



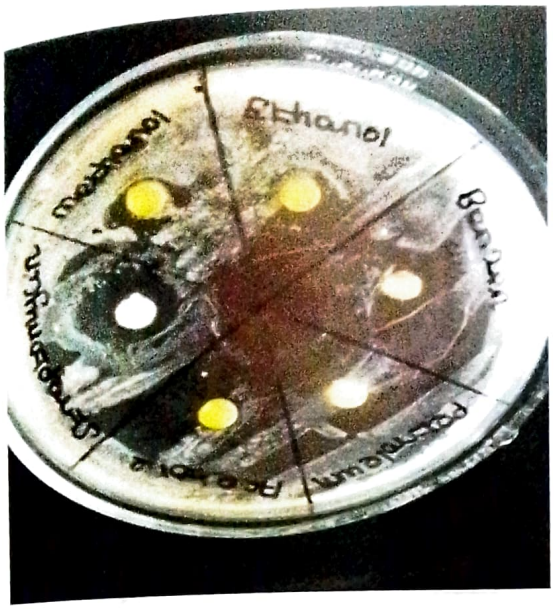
Cleome viscosa* leaf extract against *K. pneumonia



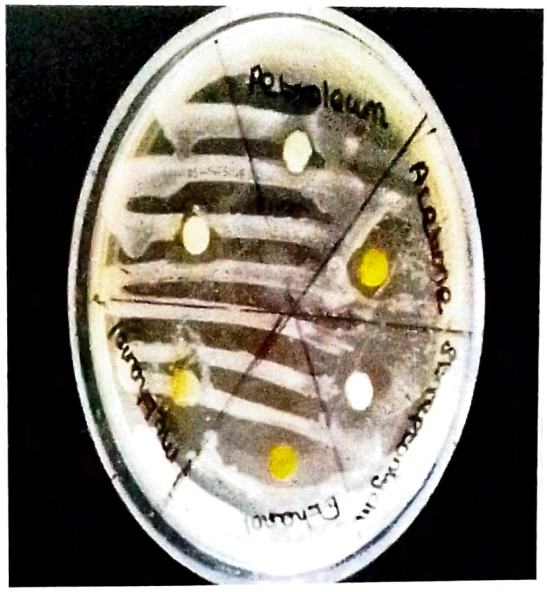
Cleome viscosa* stem extract against *S. marcescens

Antibacterial activity is revealed as clear zone around the disc and is represented as zone of inhibition. E – Ethonal, B – Benzene, A – Acetone, M – Methonal, P – Petroleum ether, extract of plants (2.5mg/ml). S – Streptomycin (100 µg/ml) - positive control.

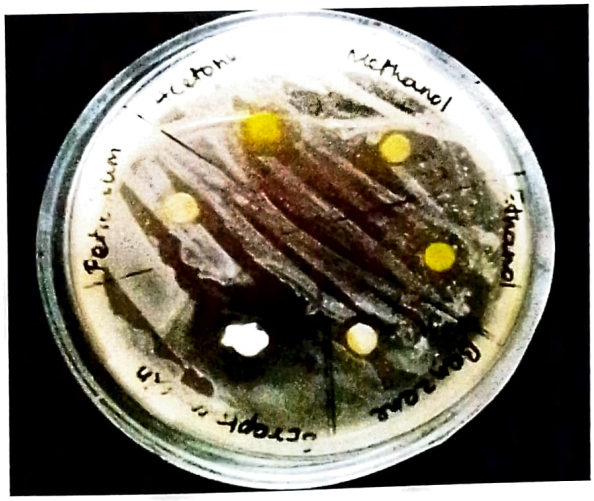
Plate 4 : In *Vitro* antibacterial activity of *Crataeva religiosa* leaf and stem extract against human pathogens.



Crataeva religiosa* leaf extract against *B. Subtilis



Crataeva religiosa* leaf extract against *E.coli



Crataeva religiosa* stem extract against *B.Subtilis

Antibacterial activity is revealed as clear zone around the disc and is represented as zone of inhibition. E – Ethonal, B – Benzene, A – Acetone, M – Methonal, P – Petroleum ether, extract of plants (2.5mg/ml). S – Streptomycin (100 µg/ml) - positive control.

plant extracts by ethanol provided more consistent antimicrobial activity compared to those extracted by water.

In the present study, antibacterial activity of two plant extracts (*Cleome viscosa* and *Crataeva religiosa*) of five different solvents were tested against five human pathogenic bacteria viz., *Escherichia coli*, *Bacillus subtilis*, *Klebsiella pneumonia*, *Salmonella typhi* and *Serratia marcescens* were presented in table (5 to 8). The diameter of the inhibition zones against these species ranged from 4 to 16 mm. As shown in Table 6, ethanolic extract of *Cleome viscosa* stem exhibited maximum activity against *E. coli* (16 mm). Similarly petroleum ether and acetone extract of *Cleome viscoa* stem inhibited the growth of *S. marcescens* and *B. subtilis* by showing 12 mm of inhibition zone. *K. pneumonia* was seemed to be more sensitive to ethanolic extract of *Cleome viscosa* stem in comparison with other pathogens. In case of leaf extract of *Cleome viscosa*, the highest activity (zone of inhibition of 11 mm) was demonstrated against *K. pneumonia* while the lowest activity (inhibition zone of 4 mm) was demonstrated against *E. coli*. The results obtained from the study concurred with earlier reports (Saradha and Subba Rao, 2010).

The methanolic and ethanolic extracts of *Crateva religiosa* leaf shown the most effective and highest activity against *B. Subtilis*, *E. coli*, and *K. pneumonia* (14 mm) followed by benzene extract with highest activity against *K. pneumonia* (12 mm) *E. coli* (11 mm), *S. typhi* (9 mm) and *B. Subtilis* (7 mm). The acetone extract showed slight activity against test bacterial pathongens with the zone size ranging from 4 mm to 11 mm. Ethanol and benzene extract of *Crateva religiosa stem it showed* an inhibition zone of 15 mm diameter against *B. subtilis* (Table 8). In case of stem extract of *Crateva religiosa*, the highest activity (inhibition zone of 15 mm).

The acetone extract of *Crateva religiosa* stem showed lowest activity against *S. Marcescens* (5 mm).

Various workers have already shown that Gram positive bacteria are more susceptible towards plants extracts as compared to Gram negative bacteria (Lin *et al.*, 1999; Parekh and Chanda, 2006). These differences may be attributed to fact that the cell wall in Gram positive bacteria is of a single layer, whereas the Gram negative cell wall is multilayered structure (Yao *et al.*, 1995). Alternatively, the passage of the active compound through the Gram negative cell wall may be inhibited. It is though that observed differences may result from the does used in this study. In addition, microorganisms show variable sensitivity to chemical substances related to different resistance levels between strains (Cetin and Gurler, 1989).

Variation in antibacterial activity may be due to the method of extraction, solvent and season at which samples were collected. Therefore it is concluded that *Cleome viscosa* and *Crataeva religiosa* plants used in the present study could be effectively processed to be utilized as a source for antibacterial therapeutic drug preparations.

CHAPTER-VI

SUMMARY AND CONCLUSION

India is richly endowed with a wide variety of plants having medicinal value. These plants are widely used by all sections of the society either directly as folk remedies or indirectly as pharmaceutical preparation of modern medicine. Phytochemicals with biological activity have a great utility as pharmaceuticals and pharmacological actions. The Preliminary phytochemical studies revealed the presence of phytoconstituents such as terpenoids, flavonoids, alkaloids, coumarins, phenols, steroids, quinones, saponins and phenols. In this study the leaf and stem of *Cleome viscosa* and *Crateava religiosa* have a various chemical groups in their chemical composition. It revealed some differences in the constituent of the two parts of the plant tested. Flavonoids, terpenoids and alkaloids were found in all five extracts of *Cleome viscosa*. Terpenoids and phenols were found in all five extracts of *Crateava religiosa*. Rests of the phytochemicals was moderate / trace amounts. Compared to *C. viscosa*, *C. religiosa* showed the presence of more phytochemicals. Hence this plant should be evaluated further to assess its phytotherapeutic properties.

Medicinal plants have antimicrobial properties. The extracts of *Cleome viscosa* and *Crateava religiosa* have the antibacterial activity. As shown in Table 6, ethanolic extract of *Cleome viscosa* stem exhibited maximum activity against *E. coli* (16 mm). In case of stem extract of *Crateva religiosa*, the highest activity (inhibition zone of 15 mm). The acetone extract of *Crateva religiosa* stem showed lowest activity against *S. Marcescens* (5 mm). In case of leaf extract of *Cleome viscosa*, the highest

activity (zone of inhibition of 11 mm) was demonstrated against *K. pneumonia* while the lowest activity (inhibition zone of 4 mm) was demonstrated against *E. coli*. The methanolic and ethanolic extracts of *Crateva religiosa* leaf shown the most effective and highest activity against *B. Subtilis*, *E. coli*, and *K. pneumonia* (14 mm). It is concluded that, in the present study, both the plants contain potential antibacterial components that may be useful for evolution of pharmaceutical for the therapy of ailments. Although the extract active plant principles such as flavonoids, alkaloids, phenol and tannins were observed in these extracts. The study has therefore justified the use of the plant is ethanomedicine. Further studies are needed with this plant to identify the unknown functional groups, isolate, characterize and elucidate the structure of the bioactive compounds which are responsible for the antimicrobial activity and other medicinal values.

REFERENCE

Ajaiboeya, E.O. 2000. Original article Phytochemical and antimicrobial studies of*Gynanandropsis gynandra* and *Buchholizia coriaceae* extracts. *Afr. J. Biomed.* Res., 3:161 - 165.

Anirban Jana and Suparna Mandal Biswas, 2011. Lactam nonanic acid, a new substance from *Cleome viscosa* with allelopathic and antimicrobial properties. *Journal of Bioscience.* 36:27-35.

Asis Bala, Biswakanth Kar, Pallab, K., Haldar, Upal, K., Mazumder and Samit Bera, 2010. Evaluation of anticancer activity of *Cleome gynandra* on Ehrlich's Ascites Carcinoma treated mice. *J. Ethnapharm.* 129:131-134

Balaji. R, V. Suba, N. Rekha and M. Deecaramam, 2009. Hepatoprotective activity methanolic fraction of *Jatropha curcas* on aflatoxin b_1 induced hepatic Carcinoma, International journal of physical sciences vol.2:287-296

Bauer, A.W., W.M.Kirpy, J.C.Sherris and M.Turcck. 1996. Antibiotic susceptibility testing of a standardized single disk method. Am.J.Clin. Pathol., 45(4):493-496

Bose, A., Mondal, S., Gupta, J.K., Ghoshi, T., Si, S. and Debbhuti., D. 2007. A study on antimicrobial activity of *Cleome rutidosperma DC*, *Journal of natural remedies*. 7(1): 13

Bose, A., Smith, P.J., Lategan, L.A., Gupta, J.K. and Sudam, SI. 2010. Studies on anti-plasmodial activity of *Cleome rutidosperma*. *Acta poloniae Pharmaceutica-Drug Research*., 67(3): 315-318.

Carla Boga, Luciano Forlani, Rocco Calienni, Teresa Hindley, Alejandro Hochkoeppler, Silvia Tozzi and Nicola Zanna. 2011. On the antibacterial activity of roots of *Capparis spinosa L. Nat. Prod. Res.* 25(4):417-421.

Chandrasekaran Swaminathan, 2017. Evaluation of Antibacterial and Antioxidant properties of *Cleome viscosa L. Indo Amer. J. Pharmaceut. Res.*. ISSN NO:2231-6876:8473-8478

De lucca, A., Cleveland, T., Rajasekara, K., Bouc, S. and Brown, R. 2005. Fungal properties of CAY-1, a plant of saponin, for emerging fungal pathogens. 45^{th} *interscience conference in antimicrobial agents and chemotherapy abstract.* F-490, 180.

Deepa priya, R., Jacintha Jasmine, D. and Agastian, P. 2012. *In Vitro* Antibacterial and Antifungal Activity of *Capparis zeylancia Linn.* Leaf Extracts. *J. Pharm. Res.*, 5(8):4449-4453. ISSN: 0974-6943.

Dhanalakshmi, D., Sathis Kumar, M., Sravan Prasad, Venkateshwarlu Koli, B., Pawan Kumar and Harani., A. 2011. Antimicrobial activity evaluation of *Cleome vicosa* Linn. *Pelagia research library Eurobean journal of experimental biology.* 1 (1):103-110.

Fletchers, R. 1999. *Cleome gynandra.* The Australian new crops News letter, 11.

Gowsalya, P. and Saravanababu, 2013. Phytochemical and antimicrobial activity of selected microorganism of bark extract of the plant *Crataeva religiosa*

International journal of pharmaceutical & biological archives 1(6):179-18. ISSN 0976-3333

Harbone, J.B. 1999. Phytochemical Method. Chapman and Hall. London. 60-66.

Havsteen, B. 1990. Flavonoids a class of natural products for antimicrobial agents. *Eur. J. Clin. Microbial. Infect. Dis.*. 9. 455-61.

Jegede, I.A., Ibrahim, J.A. and Kunle, O.F. 2011. Phytochemical and pharmacognostic studies of the leaf and stem bark of Anthocleista vogelii. *JMPR. Academic Journals*. 5(26):6136-6139.

Jigna parekh and Sumitra Chanda, 2007. Antibacterial and Phytochemical studies on twelve species of Indian medicinal plants. *Afr. J. biomedi. Res.* 10:175-181. ISSN 1119-5096

Kalpana, B. and Prakash, M. 2015. Original Research Article Antibacterial Activity of *Capparis sepiaria L.* (Capparidaceae) Leaves and Fruits *Int. J. Curr. Microbiol. App. Sci.*, 4(1): 1007-1012.

Kokate, C.K. 2000. Practical Pharmacognosy, Vallabh Prakashan. Delhi. 107-111.

Lakshmi Sree Kumar Pillai and Bindu Rajeswary Nair, 2012. Pharmacognostical standardization and phytochemical studies in *Cleome viscosa L.* and *Cleome burmanni* W.and A. (Cleomaceae). *J. Pharm. Res.*, 5(2):1231-1235.

Latifou Lagnika, Eugenie Anago, Menove Atindehou, Brice Adjahoutonon, Karim Dramane and Ambaliou Sanni, 2011. Antimicrobial activity of *Crateava religiosa* Forst against bacteria isolated from *Thryonomys swinderianus* Temminck. *Afr. J. Biotech.*, 10(49), pp. 10034-10039. ISSN 1684-5315.

Lutterodt, G.D., Ismail, A., Basheer, R.H. and Baharudin, H.M. 1999. Antimicrobial effects of guajava extract as one mechanism of its antidiaharrhoel action. *Malay. J. Med. Sci.*, 6:17-20.

Nandakani, A.K. 1997. Indian material medica. *popular publication Pvt. Ltd.* Mumbai. 387.

Ogunmefun, O.T. and Ajaiyeoba, E.O. Phytochemical analysis and Antifungal activities of *Gynandropsis gynandra* (spider flower)and *Buchholzia coriacea* (musk tree) (Family:Capparidaceae) on some common fungal isolates *Journal of biological sciences and bioconservation*. 5(1) : 75-85. ISSN: 2277-0143

Perumal Samy R, Ignacimutu S, Patric Raja D, 1999. Preliminary screening of ethnomedicinal plants from India. *J. Ethnopharm.*, 16:235-240.

Poovendran, P, vidhya, N. and Murugan. 2011 Antimicrobial activity of Mirabilis Jalapa and Dichrotachys cinera against biofilm and extended spectrum of beta lactamase(ESPL) producing uropathogenic *Escherichia coli*. 5(22) : 3620-3623

Rajaselvam, J. and Basil Rose, M.R. 2016. *In vitro* antibacterial activity and phytochemical analysis of *Cleome gynandra* Linn leaf extracts against human pathogens. *International journal for research in applied science & engineering technology (IJRASET)* , Volume 4 issue VIII ISSN:2321-9653 IC Value :13.98. : 793-796

Rajesh, C., Patil, Swati, D., Wavhal, Sunil, S., Yadav and Vaibhav, Deshpande, D. 2012. Antibacterial and bioenhancing Activity of Ethyl Acetate Extract of *Cleome rutidosperma* Leaves. *J. Pharm. Res.*, 5(1):557-559; ISSN:0974-6943

Robards, K., Prernzler, P.D., Tucker, G., Swatsitang, P. and Glover, W. 1990. Phenolic compounds and their role in oxidative processes in fruits. *Food Chem.*, 66:401-36.

Santhi, R., Lakshmi, G., Priyadharshini, A.M. and Anandaraj, L. 2011. Phytochemical screening of *Nerium oleander* leaves and *Momordica charantia* leaves. *Inter. Res. J.Pharm.* 2:131-135.

Santos-Buelga, C. and Scalbert, A. 2000. Proanthocyanidins and tannin like compounds-nature, occurrence, dietary intake and effects on nutrition and health. *J. Sci. Food Agri.*, 80:1094-1117.

Sapkota J. 2003. Biologically active pentacyclic triterpenes lupeol and their current medicine significations. *Journal of Applied Biomedical.*31:7-12.

Saradha, J.K. and Subba Rao, S. 2010. In vitro antibacterial activity of *Cleome viscosa* Linn. Pharm Sci Mon.n, 71-78.

Sathish, S, Raveesha, K.A, janardhana, G.R. 1999. Antibacterial activity of plant extracts of phytopathogenic Xanthomonas Campestris pathovars. Lett.App.Microbial., 28 : 145-147.

Sethupandian Geetha, Kokkaiah Irulandi, Sinthia Ganesan and Palanichamy, 2016 Preliminary phytochemical screening of different solvent extracts of leaves and stem of *Crataeva religiosa* hook &frost. *Inter. J. Bot. Stud.* X. Impact factor :RJIF5.12 1(4): 24-26. ISSN:2455-541

Sudhkar, M, Rao, ch.V., Rao, D.B. 2005. Evaluation of antimicrobial activity of *Cleome viscosa* and *Gmelina asiatica* 006. 77(1):47-49.

Suttijit Sriwatcharakul, 2016. Antimicrobial, Antioxidant and cytotoxic activities of *Cleome viscosa* Linn. Crude extracts. *World academy of science, Engineering and technology Inter. J. Bioengineer. Life Sci.*, 10(7):435-438.

Tseng, A. 1999. Chemoprevention of tumors in MTV-H ras transgenic mice with coumarins. *Proc. Am. Assoc. Cancer Res.*, 32:2257.

Udaysing Hari Patil and Dattatrya K. Gaikwad. 2011. Different bactericidal potential and phytochemical evaluation of *Crataeva religiosa* stem bark International journal of Pharma. *Research & development – online (IJPRD), Platform for pharmaceutical researches and developments* ISSN:0974-9446, Publication ref no.: IJPRD/PUB/ARTI/VOV-2/ISSUE-11/012:82-88

Vidhya, R. and Umavandhana, R. 2016. Phytochemical screening and GC-MS analysis of methanolic leaf extract of *Cleome viscosa L. Indo- Asi. J. Multidiscip. Res. (IAJMR)* 2(6):863-868. ISSN-2454-1370.

Umamaheswari, A. Shreevidya, R. and Aparna Nuni. 2008.*In vitro* Antibacterial Activity of Bougainvillea spectabilis leaves Extracts.Advances in biological Research. 2(1-2) :01-05.

Wang, C.H., Gao, Z.Q., Ye, B., Cai, J.T., Xie, C.G., Qian, K.D. and Du, Q. 2007. Effects of Emodin on pancreatic fibrosis in rats. *World J. Gastroenterol.*, 13:378-382.

Yadav, M., Chatterji, S., Gupta, S.K. and Watal, G. 2014. Preliminary phytochemical screening of six medicinal plants used in traditional medicine. *Int. J Pharm Pharm Sci.* 6(5):539-542.

PHYTODIVERSITY SURVEY OF ROADSIDE WEEDS OF THOOTHUKUDI

A Short – term projectsubmitted to

ST.MARY'S COLLEGE (AUTONOMOUS)

Affiliated to

MANONMANIAM SUNDARANAR UNIVERSITY

in partial fulfilment of the requirements for the degree of

BACHELOR OF SCIENCE IN BOTANY

By

S. SNOWBA SERAPHINE	**:16AUBO44**
M. SUBA	**:16AUBO47**
D. SULAMITHIYAL	**:16AUBO48**
M.VALARMATHI	**:16AUBO49**
M. VINCY AGNES	**:16AUBO50**

DEPARTMENT OF BOTANY

ST.MARY'S COLLEGE (AUTONOMOUS)

THOOTHUKUDI -628 001.

APRIL 2018-19

CERTIFICATE

It is certified that this short term project work entitled "**Phytodiversity survey of roadside weeds of Thoothukudi"** submitted to **St. Mary's College (Autonomous)** affiliated to **Manonmaniam Sundaranar SUniversity** in partial fulfilment of the requirements for the degree of Bachelor 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 2018-2019 by the following students.

S. SNOWBA SERAPHINE	**:16AUBO44**
M. SUBA	**:16AUBO47**
D. SULAMITHIYAL	**:16AUBO48**
M.VALARMATHI	**:16AUBO49**
M. VINCY AGNES	**:16AUBO50**

GUIDE

29/3/19

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.

ACKNOWLEDEGEMENT

We offer ourpraise and sincere thanks to the **Almighty God**, for his avalanche of graces and bountiest blessings, enabling us to complete this project.

We wish to express my deep sense of gratitude to **Dr. E. Daffodil D Almeida M.Sc., SET, Ph.D.**, Assistant Professor of Botany for suggesting this research problem, her constant encouragement and inspiring guidance throughout this project work.

We consider it a privilege to express our gratitude to **Dr. Rev. 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 thank all the staff members, the laboratory assistants of Botany Department and also our families and friends for their ready and generous help.

CONTENTS

INTRODUCTION

INTRODUCTION

Roads are the chief way of communication among the different cities for trade and economy, in order to compete with the International positions (Harper, 2001). Hence, the construction and maintenance of roads are considered to be necessary in the context of the economical, political and social development of the country (Dierkes & Geiger, 1999). Roads may act as habitats, as linear corridors or as barriers to the dispersal of animal and plant species (Angold, 1997). Road verges comprise the diverse ecological and environmental conditions. Road verges can support habitats and species of nature conservation value. Some areas are specially designated for their biodiversity. The advantage of road sides for study of vegetation performance is now widely acknowledged (Wilson *et al*., 1992). Some parts of the world particularly, Europe, New Zealand, and North America designated the roadsides as excellent habitats for the examination of vegetation. Small marginal habitats in the rural landscape may play an important role for plant species richness as refugia (Sara, 2006). They provide important corridors for the movement of species, and sometimes support plant including medicinal plants and animal communities which are important in their own right (Jesse *et al*., 2008).

The effect of a road on the environment is complex and includes disturbances during construction, alteration of normal hydrological flows (Forman and Alexander, 1998), the introduction of chemicals, including salts (Spellerberg, 2002) and heavy metals (Storch et al., 2003), and fragmentation of natural habitats (Heilman et al., 2002). Roads are very important to local and national economic development. However, they can also be major causes of biodiversity loss (Trombulak and Frissell, 2000) because the access provided by them to

previously isolated areas increases rates of deforestation (Fearnside *et al.*, 2009). Several researches have shown that solid particle emissions, oxides of carbon, sulphur, nitrogen, and gases such as ozone and ethylene through vehicular emissions (Ball, *et al.*, 1998), may affect plant photosynthesis, composition, competition and growth (Angold, 1997, Larson, 2003). These authors, also reported that anthropogenic disturbances such as trampling, crushing and heavy metal emissions are widely recognized as primary influences on the soil structure and plant community composition.

Traditional medicine based on herbal remedies has always played a key role in the health systems of many countries. In India the native people are exploiting a variety of herbals for effective curing of various ailments. The plant parts used, preparation, and administration of drugs vary from one place to other. However, the knowledge of herbal medicines is gradually perishing, although some of the traditional herbal men are still practicing the art of herbal healing effectively. These plants are frequently used by the local inhabitants of the area for treatment of various diseases. Ethno-medicinal studies have offered immense scope and opportunities for the development of new drugs. Some modern drugs have been deducted from folklore and traditional medicines. Living close to nature, traditional societies have acquired unique knowledge about the use of wild flora and fauna, most of which are unknown to the people who live away from such natural ecosystem as forests. After years of observations and analysis, trials, error, experimentation or even use of intuitive methods the innovative member of human communities have selected/identified useful and harmful members of the flora and fauna.

Such knowledge and practices/experiences were subjected to further modification or enriched with new knowledge of practice by succeeding generations and become a part of the

tradition, culture, art, belief, folklore and knowledge base of these traditional communities. The traditional knowledge, skill and practices thus developed are freely exchanged cared for and nourished as a common property of the communities (Pushpangadan and Kumar, 2005).

As far as medicinal plants are concerned, 8000 plant species have some or the other medicinal uses. About 1000 are traded and 250 are traded in large volume which are available in market. The demand for medicinal plant has increased by 5% since last year. There are many varieties of medicinal plants. More than 80% varieties are from wild and waste land. Weeds can also be used as medicinal plants, which can be a secondary source of income to the farmers. Weeds basically are medicinal plants which have lots of scope and still many species can be brought under cultivation. The value of plants in urban environment is now generally recognized both aesthetically and functionally in helping to make cities and towns conducive to live and work in. The balance of urban climate is disrupted and most of the vegetation are being altered and destroyed to make way for "urban development". With these in mind, there is also rapid spread of exotic invasive species in our environment due to their wide adaptability to different habitat situations.

Despite this importance, very little information exits on the cities flora in general, and medicinal species found within its limit in particular.

In the light of this, the current study seeks to carry out survey of plants species with an ultimate aim of providing possible conservation and management. Therefore, this study will provide baseline knowledge regarding roadside vegetation and would be helpful in future for conservation of biodiversity along the road verges of motorways and improvement

of road verges. Moreover, no survey of naturally growing medicinal plants along roadsides of Thoothukudi Township has been reported till date.

Thus, the present investigation has been undertaken to understand the diversity and distribution of weeds including medicinal plants along roadside of Thoothukudi with the following objectives:

- To conduct a detailed botanical survey for documenting the diversity of plants.
- To discover the medicinal plants, useful parts and uses
- To document traditional knowledge on the use of medicinal plants

AREA OF STUDY

known officially as Thoothukudi is a port city and a municipal corporation and an industrial city in Thoothukudi district in the Indian state of Tamil Nadu. The city lies in the Coromandel Coast of Bay of Bengal. Thoothukudi is the headquarters of Thoothukudi district. Thoothukudi is in the Gulf of Mannar about 125 km (78 mi) North of Cape Commorin and its environs form part of the coastal belt which forms a continuous stretch of the flat country relieved here and there by small rock out crops. The region, surrounding Thoothukudi is liberally dotted with rain fed tanks. Red soils found on the southern side of the Thoothukudi town are composed quartz and variable quantities of fine red dry dust. The city experiences tropical climatic conditions characterised with immensely hot summer, gentle winter and frequent rain showers. Summer extends between March and June when the climate is very humid. Thoothukudi registers the maximum temperature of 39 °C (102 °F) and the minimum temperature of 32 °C (90 °F).

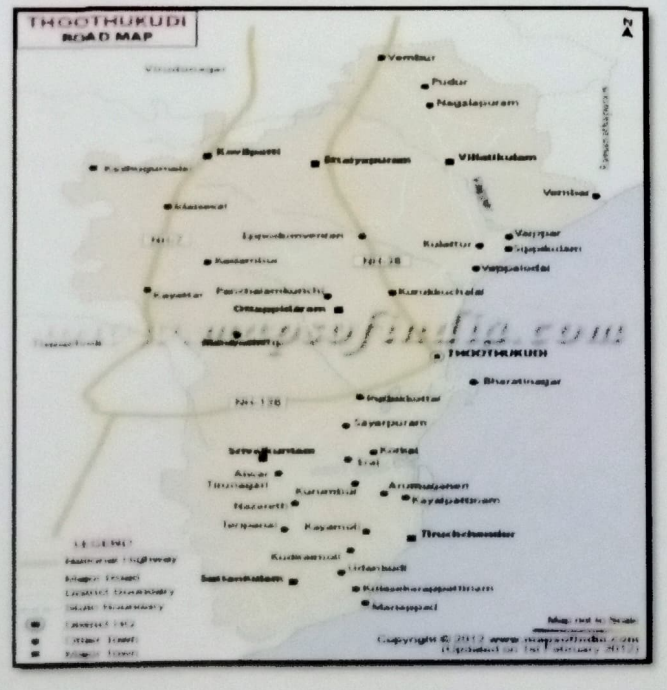


Figure 1: Map showing study area

REVIEW OF LITERATURE

In the era of the 21st century, due to the development in biotechnology and other branches of biosciences, it has become a prime need to understand the importance of plant species. The study of Plant Biodiversity of a particular region and utilization of plant species in day – to – day life of human beings of the region has become a need of the time. Biodiversity that we see around us today is the outcome of over 3.5 billion years of evolutionary development, shaped by nature. Conserving biodiversity is basic to our survival and well – being and using it sustainably forms part of the Indian culture and lifestyle. Wild plants play an important role in the national economy and well being of the occupant population in a region. The basic necessity like food, fodder, fuel, fiber, medicine, timber and many other minor produces like gum – resins, lags etc. are mostly obtained from forests, grazing lands and waste lands. Flora of a nation is the basic guide to its wealth of vegetation, as the systematic botany mostly deals with wild species. To preserve or propagate the right one at the right place, one needs to know the distribution and abundance of the vegetation. "Flora of India" is a long cherished dream of Indian taxonomists. Now many research institutions in India like, Botanical Survey of India, Forest Research Institute and National Botanical Research Institute have taken up floristic studies in a big way. Many Botanists are now actively engaged in floristic surveys and several regional floras have already come up (CBD, 2000).

Study of Floral diversity along the roadside

A study on roadside plant diversity and soil analysis in Uyo sub-urban environment was carried out by Bassey *et al.* (2017) using systematic sampling method. A quadrat of 5m x

5m was used to sample the vegetation spaced at regular intervals. The vegetation parameters determined were density and frequency. From the results, 42 plant species were found along Abak road, 38 species along Etinan road and 45 species along Idoro road.

The survey report showed that 927 medically important plant species were present at roadside of Bhilai township plant species found in following manner 53% > 27%> 12%>8% for herb , tree shrubs and climber vegetation at road side of Bhilai township. It was also noted that family Asteracae and family fabaceae shows maximum plant species in all categories. It was observed that leaves, root, stem, bark are used for various ailments (Pandey and Pandey, 2016).

Ahmad *et al.* (2013) investigated the floristic composition of roadside vegetation in Rawalpindi to Attock motorway. He collected floristic data along the motorway and different methods of quantization were applied for best interpretation. He had made an attempt to describe the vegetation types and their distribution on M-1. Two Way Indicator Species Analysis divided the whole flora into two major groups in which *Lepidium apetalum* and *Euphorbia helioscopia* were abundant among all species.

Ahmad (2007) highlighted the importance of wild medicinal plants along road side verges (M-2) Pakistan. Ahmad and Hussain (2008) conducted similar along the road verges of Kallar Kahar area of salt range and Ahmad *et al.*, (2009) along road verges of Abbottabad city.

Mallick and Acharya (2013) reported 27 roadside herbs belonging to 21 families mostly used for various diseases and disorders by the local villagers of different villages present in and around Rourkela.

The present communication deals with the medicinal weeds of Varanasi district. In all 89 species belonging to 75 genera and 43 families are enumerated along with their family, brief description and medicinal use.

Jeeva *et al.* (2006) studied medicinally important weeds frequently used by local communities of Kanyakumari district, Tamil Nadu. A total of 93 medicinal weedy species from 85 genera used in traditional medicines were identified. Majority of species are used for curing skin diseases, fever, cold and cough, etc. Of 42 families, 20 families were monospecific. Plants of family Fabaceae was largely represented (7 species) family followed by Asteraceae, Lamiaceae and Euphorbiaceae.

Previous Exploration in Thoothukudi District

Muthukumar and Samuel (2010) conducted direct interview among local communities and fishery communities Tuticorin District, Tamil Nadu and listed a total of 41 medicinal plants and their popular uses.

Muthukumar and Samuel (2011) studied Coastal sand dune flora in the Thoothukudi District, Tamil Nadu, southern India and identified total of 42 species belonging to 38 genera and 26 families at different distances from the shoreline.

An ethno-medicinal plant survey was carried out in Srivaikundam village of Tuticorin District, Tamilnadu to discover the kinds of herbal remedies used by the local populations. It resulted in about 41 medicinal plants for the treatment of several diseases either in single or in combination with some other ingredients. The information on correct botanical identities with family, local name and traditional practice of 41 plant species belonging to 28 families

are discussed for the treatment of various illnesses (Rama Rajan and Muthu Kumarasamy, 2012).

Sheela *et al.* (2016) conducted an intensive exploration in Thoothukudi district giving importance to sedges which form major constituent of wetland ecosystem. They have done floristic survey of Cyperaceae and to record field data on habit, habitat, distribution status and phenology and recorded 53 species belonging to 15 genera, of which 13 taxa are economically valuable and five are medicinally important.

MATERIALS AND METHODS

A weedy plant inventory was conducted in different areas of Thoothukudi along the roadside.. All plants were collected and identified using Gamble's Flora of presidency of Madras (1997), Matthews flora of Tamil Nadu, India. The identified specimens were described briefly. The ethnomedicinal uses of identified plants were determined using earlier publications. (Maheswerl *et al.*, 1993; Krishnan Marg, 1992; Balasingh *et al.*, 2000; Yoganarasimhan, 2000; Balasubramanian, 2013) . The ethnomedicinal uses mentioned in literature were then cross checked through interviews with local inhabitants

1. **BOTANICAL NAME** : *Abutilan indicum* G.Don.

FAMILY : Malvaceae

VERNACULAR NAME : Thuthi

DESCRIPTION:

Shrubs, Leaves cordate ,base cordate margin cunate denate ,apex – acuminate .Flowers yellow ,solitary. Fruits greenish yellow, globose schizocarps.

2. **BOTANICAL NAME** : *Acalypha indica* L.

FAMILY : Euphorbiaceae

VERNACULAR NAME : Kuppamani

DESCRIPTION:

An erect annual herb. Leaves alternate, usually serrate. Flowers usually monoecious, in auxillary or terminal spikes. Bracts folded, shortly toothed, the teeth with gland-tipped hairs, otherwise glaborus. Leaves are obovate lanceolate.

3. **BOTANICAL NAME** : *Achyranthes aspera* L.

FAMILY : Amaranthaceae

VERNACULAR NAME : Nayuruvi

DESCRIPTION:

Leaves elliptic obovate to suborbicular. Sparingly pubscent above densely so below, flowers bract broad ovte, basem truncate, apex aluminate. Entirely adnate to midrib. Base fruncate to rounded, apex acute.

4. **BOTANICAL NAME** : *Aerva lanata* Juss.

FAMILY : Amaranthceae

VERNACULAR NAME : Ceru pulai

DESCRIPTION:

Erect herbs, leaves are bifacular, obovate spikes clustered, auxillary. Flowers creamy white.

5. **BOTANICAL NAME** : *Allamanda cathartica* L.

FAMILY : Apocynaceae

VERNCULAR NAME : Golden trumpet

DESCRIPTION:

It is a handsome climbing shrub with large yellow flowers and whorled leaves. Frequently grown in gardens in the plains, and sometimes, as along back waters.

6. **BOTANICAL NAME** : *Aloe vera* L.

FAMILY : Liliaceae

VERNACULAR NAME : Kathalai

DESCRIPTION:

It have a rosette of large, thick fleshy leaves. Aloe flowers are tubular, frequently yellow, orange, pink, red are boam densely clustered and pendent at the apex of simple of leafeless stems succulent leaves.

7. **BOTANICAL NAME** : *Alpinia galanga* Sw.

FAMILY : Zingeberaceae

VERNACULAR NAME : Blue ziger

DESCRIPTION:

The plant grows from rhizomes in clumps of stiff stalks upto 2m height with abundant long leaves that bear red fruit.

8. **BOTANICAL NAME** : *Amaranthus spinosus* L.

FAMILY : Amaranthaceae

VERNACULAR NAME : Mullukeerai

DESCRIPTION:

An upright, erect annual herb, many branched. Grows up to 1m height. The stems are cylindrical and smooth, it can be green or red. A pair of stong spines, reddish and swollen, are inserted at the base of the leaf stalk. Leaves are simple and alternate.

9. BOTANICAL NAME : *Andrographis paniculata* Nees.

FAMILY : Acanthaceae

VERNACULAR NAME : Green chireta

DESCRIPTION:

An erect herb with pink corolla, darker on the lower lip, the capsule usually 12 seeded. A bitter plant, used in medicine.

10. BOTANICAL NAME : *Anisomeles malabarica* R.Br.

FAMILY : Labiatae

VERNACULAR NAME : Malabar catmint

DESCRIPTION :

It is an aromatic perennial plant with woolly stems, growing from 5-150 cm tall. The stems often woolly and persist.

11. BOTANICAL NAME : *Argemone mexicana* L.

FAMILY : Papaveraceae

VERNACULAR NAME : Mexican poppy

DESCRIPTION:

An erect prickly annual with yellow juice. Flowers bright yellow. An introduced weed found on roadsides, in waste places and fallow fields in all districts.

12. **BOTANICAL NAME** : *Blastania garcini* Cogn.

FAMILY : Cucurbitaceae

VERNACULAR NAME : Garcen's bur cucumber

DESCRIPTION:

A slender pretty annual climber with orange or red fruit.

13. **BOTANICAL NAME** : *Boerhaavia diffusa* L.

FAMILY : Nyctaginaceae

VERNACULAR NAME : Spreading hog weed

DESCRIPTION:

It is a herbaceous perennial plant with vigorous growth, many branched stems growing from a taproot up to 2 m long. The stem can be erect or procumbent.

14. **BOTANICAL NAME** : *Bogainvillaea spectabilis* Willd.

FAMILY : Nyctaginaceae

VERNACULAR NAME : Bougainvillea

DESCRIPTION:

It is an evergreen climbing shrub producing stems upto 10 meters long. The plant can support it self on other plant by means of thorns carried in the leaf axils through in cultivation. It might need trying in to pergolas

15. **BOTANICAL NAME** : *Calotropis gigantea* R.Br.

FAMILY : Asclepiadceae

VERNACULR NAME : Erukku

DESCRIPTION:

A small shrub, young parts downy tomentose, dark ash coloured leves. Flower purplish or whiten in umbellate cymes. Follicles curved, turgid, smooth. Seeds numerous broadly ovate, flat.

16. **BOTANICAL NAME** : *Cassia alata* L.

FAMILY : Leguminosae

VERNACULAR NAME : Ring worm bush

DESCRIPTION:

The plant is highly valued in many areas of the tropics for its medicinal virtues.It is commonly gathered from the wild, mainly for medicinal use but also as a food and source of material. It is often cultivated for medicinal purposes.

17. **BOTANICAL NAME** : *Cassia nigricans* Vahl.

FAMILY : Leguminosae

VERNACULAR NAME : Burkill

DESCRIPTION:

A shrub or under shrub with yellowish-red flowers, 2-5 together on rther stout pedicles some distance above the leaves.

18. **BOTANICAL NAME** : *Catharanthus roseus*

FAMILY : Apocynaceae

VERNACULAR NAME : Nithiya kalyani

DESCRIPTION:

Perennial herbs. Leaves alliptic, obovate to oblong, base acute to cuneate. Margin entire, apex obtusely apiculate. Inflorescence cymose. Flower white or pink, solitary axillary or paired. Calyx gamosepalous. Corolla coronary outgrowth. Stamens epipetalous attached near the top of the corolla. Ovary superior. Fruit linear mericarp.

19. **BOTANICAL NAME** : *Celosia cristata* L.

FAMILY : Amaranthaceae

VERNACULAR NAME : Cocks comb

DESCRIPTION:

The flower can be broken in to three parts. Their spikes plumes and crests vary from one another but have standard commonalities. They are usually brightly colored, red, pink, yellow or orange, though other colours can be present. In some instances, a variety of colours present in hybrids.

20. BOTANICAL NAME : *Cenchrus biflorus* Roxb.

FAMILY : Gramineae

VERNACULAR NAME : Indian sandbur

DESCRIPTION:

Coromandel. Not common. Spikes sometimes purple.

21. BOTANICAL NAME : *Chloris barbata* Sw.

FAMILY : Gramineae

VERNACULAR NAME : Finger grass

DESCRIPTION:

It is a widespread genus of plants in the grass family. It grows in a tropical climate. It grows up to one meter.

22. BOTANICAL NAME : *Cissus quadrangularis* L.

FAMILY : Vitaceae

VERNACULAR NAME : Devil's back bone

DESCRIPTION:

It is a evergreen climber. It prefers dry or moist soil and can tolerate drought.each leaves has a tendril emerging from the opposite side of the node. Racemes of small white, yellowish or greenish flowers.

23. BOTANICAL NAME : *Cleome viscosa* L.

FAMILY : Capparidaceae

VERNACULAR NAME : Naikkaduku

DESCRIPTION:

An annual herb having tap root system. Stem erect well branched, herbaceous, green, solid and pubescent. Leaves alternate exstipulate. Inflorescence racemose raceme.

24. **BOTANICAL NAME** : *Clitoria ternatea* L.

FAMILY : Leguminosae

VERNACULAR NAME : Blue pea

DESCRIPTION:

It is perennial herbaceous plant with elliptic, obtuse leaves. It grows as a vine or creeper doing well in moist, neutral soil.

25. **BOTANICAL NAME** : *Corchorus aestuans*

FAMILY : Tiliaceae

VERNACULAR NAME : East Indian mallow

DESCRIPTION:

Herbs or under shrub. Leaves serrate, lower pair of teeth usually prolonged into hairs. Flowers small yellow peduncles very short, auxillary or leaf-opposed, bracteate.

26. **BOTANICAL NAME** : *Corchorus tridens* L.

FAMILY : Tiliaceae

VERNCULAR NAME : Jews mallow

DESCRIPTION :

Capsule cylindric, not winged, leaves ovate, crenate-serrate.

27. **BOTANICAL NAME** : *Crotalaria retusa* L.

FAMILY : Leguminosae

VERNACULAR NAME : Rattle weed

DESCRIPTION:

An erect herbaceous under shrub reaching 3-4 ft. in height with conspicuous yellow flowers. It give a fibre.

28. BOTANICAL NAME : *Cynodon dctylon* Pers.

FAMILY : Graminae

VERNACULAR NAME : Bermuda grass

DESCRIPTION:

It is a creeping plant. It is perennial. It grows in a worm climate.

29. BOTANICAL NAME : *Datura metel* L.

FAMILY : Solanaceae

VERNACULAR NAME : Oomathai

DESCRIPTION:

Large erect and stout herb. Branched tap root system. The stem is hollow. green and herbaceous with strong odour. Leafe is simple. alternate. petiolate. Flower large. greenish white in colour and hypogynous.

30. BOTANICAL NAME : *Enicostemma littorale* Bl.

FAMILY : Gentianaceae

VERNACULAR NAME : Vellaragu

DESCRIPTION:

A perennial herb from a thick root stock. with many erect or procumbent branches. bearing narrow linear or linear oblong leaves very variable. Flowers white in dense clusters on the stem.

31. BOTANICAL NAME : *Euphorbia hirta* L.

FAMILY : Euphorbiaceae

VERNCULAR NAME : Asthma plant

DESCRIPTION:

A straggling ascending hispid herb reaching 2 ft. high. This is erect or prostate annual herb can grow upto 60 cm long with a solid, hairy stem that produces an abundant white latex. It has a white or brown taproot.

32. BOTANICAL NAME : *Euphorbia milli*

FAMILY : Euphorbiaceae

VERNACULAR NAME : Christ plant

DESCRIPTION:

It is a succulent shrub growing to 1.8 cm tall with densely spiny stems. The straight slendon spines upto 3cm long help it scramble over other plants.

33. BOTANICAL NAME : *Gomphrena globosa* L.

FAMILY : Amarantaceae

VERNACULAR NAME : Globe amaranth

DESCRIPTION:

It is a tall branched annual with large globose yellowish white or crimson heads of flowers, largely grown in gardens and often found as an escape.

34. BOTANICAL NAME : *Heliotropium curassavicum* L.

FAMILY : Boraginaceae

VERNACULAR NAME : Sea shore heliotrpe

DESCRIPTION:

It is a perennial herb which can take the form of a prostrate creeper along the ground. The stem and foliage are fleshy leaves and oval or spade shaped.

35. BOTANICAL NAME : *Ipomaea pes-caprae* Sweet.

FAMILY : Convolvulaceae

VERNACULAR NAME : Goats foot creeper

DESCRIPTION:

An extensively creeping and sand binding plant with a thick long root stock. handsome purple red flowers and curious bi lobed leaves. Very useful in checking blown sand.

36. BOTANICAL NAME : *Leucas aspera* Spr.

FAMILY : Labiatae

VERNACULAR NAME : Thumbai

DESCRIPTION:

A coarse erect diffusely branched annual herb with white flowers the stems hispid or scabrid.

37. BOTANICAL NAME : *Lippia nodiflora* Mich.

FAMILY : Verbenaceae

VERNACULAR NAME : Frog fruit

DESCRIPTION:

Frog fruit is a low growing ,herbaceous ,perennial plant that creepers along the ground ,forming roots as is spreads and often forming large mats or colonies.

38. BOTANICAL NAME : *Lycopersicum esculentum* Mill.

FAMILY : Solanaceae

VERNCULAR NAME : Tomato

DESCRIPTION:

Another particularly dreded diseases is curely top carried by the beet leaf hopper.

39. **BOTANICAL NAME** : *Mollugo lotoides* O. Kze

FAMILY : Aizoaceae

VERNCULAR NAME : Carpet weed

DESCRIPTION:

A prostate herb covered with stellate hairs. the leaves usually orbicular. the pedicles sometimes up to 75 in long. but more usually very short.

40. **BOTANICAL NAME** : *Murraya konigii* Spr.

FAMILY : Rutaceae

VERNACULAR NAME : Karivembu

DESCRIPTION:

A small tree with very aromatic leaves which are eaten in curries. Wood greyish-white. softer than that of the last.

41. **BOTANICL NAME** : *Nerium odorum* Soland

FAMILY : Apocynaceae

VERNACULAR NAME : Indian olender

DESCRIPTION:

It is a large shrub. common in rocky river beds. It is often cultivated in gardens and has pink flowers narrow linear coriaceous leaves and long follicles with brown coma to the seeds.

42. **BOTANICAL NAME** : *Ocimum sanctum* L.

FAMILY : Labiatae

VERNACULAR NAME : Holy basil

DESCRIPTION:

Holy basil is erect many branched . sub shrub 30-60cm tall with hair stems. . Leaves are Green or Purple.

43. BOTANICAL MAME : *Oldenlandia umbellta*

FAMILY : Rubiaceae

VERNACULAR NAME : Chay root

DESCRIPTION:

It is a prostrate herb .Leaves are stalkless .Flowers are borne in many flowered umbel –like cymes at branch ends and also sometimes in leaf axis .Sepals are 4,persistent about 1.5 m long ovate –tapering.

44. BOTNICAL NAME : *Pedalium murex* L.

FAMILY : Pedaliaceae

VERNACULAR NAME : Anai-nerinji

DESCRIPTION:

A succulent herb grow upto 38cm in height ,very well branched .Leaves simple opposite .Flowers bright yellow axillary and solitary.

45. BOTANICAL NAME : *Phyllanthus amarus* L.

FAMILY : Euphorbiaceae

VERNACULR NAME : Kilanelli

DESCRIPTION:

Erect herbs to 30cm tall .Leaves 6-8cm, oblong ,base unequal sides, apex obtuse to acute. Surface glacous, stipules lanceolate ,scarious.

46. BOTNICAL NAME : *Physalis minima* L.

FAMILY : Solanaceae

VERNACULAR NAME : Ground cherry

DESCRIPTION:

A herbaceous annual. The globrous form with angular fruiting calyx. It is only a weed of cultivated ground.

47. **BOTANICAL NAME** : *Physalis peruviana* L.

FAMILY : Solanaceae

VERNACULR NAME : Coat buttons

DESCRIPTION:

Sunberry is an erect ,much –branched ,annual plant growing around 50cm tall. The plant also has range of medicinal use.

48. **BOTANICAL NAME** : *Senna occidentalis* L.

FAMILY : Leguminosae

VERNACULAR NAME : Payaverai

DESCRIPTION:

An erect tropical annual herb with leathery compound leaves growing up to 6 feet tall .The seed pods re broken and curve slightly upward, the seeds are olive brown and flattened on both ends.

49. **BOTANICAL NAME** : *Setaria verticillata* Beauv.

FAMILY : Gramineae

VERNACULAR NAME : Kolukkattai pullu

DESCRIPTION:

Bristeles barbellate with descending teeth. Panicles straight or curved, continuous.cylindric, 5-8 in long; branches ending in a bristle; involucral bristels1-4, spikelets oblong ellipsoid.

50. **BOTANICAL NAME** : *Sida acuta* Burm.

FAMILY : Malvaceae

VERNACULAR NAME : Broom weed

DESCRIPTION:

Herbs or undershrubs ,pubescent with simple or stellate hairs .Leaves toothed stipules linear. Flower pedicels axillary ,solitary or clustered .Corolla small yellow or white.

51. BOTANICAL NAME : *Sida cordifolia* L.

FAMILY : Malvaceae

VERNACULAR NAME : Chittamuttie

DESCRIPTION:

A small downy erect herb or shrub,1.5 cm in height ,with long branches ,sometimes rooting at nodes .Leaves : cordate, ovate –oblong, very down on both surfaces. Flowers:small and yellow or white ,carpels 10.Fruits with a pair of awns on each carple.

52. BOTANICAL NAME : *Solanum xanthocarpum* Sch&Wendl.

FAMILY : Solanaceae

VERNACULAR NAME : Kanta kari

DESCRIPTION:

It is a semi perennial. It grows in a tropical climate .It grows up to 1.5m.

53. BOTANICAL NAME : *Sonchus oleraceus* L.

FAMILY : Asteraceae

VERNACULAR NAME : Sow thistle

DESCRIPTION:

Annual or perennial milky herbs. Leaves radical or alternate ,entire toothed or pinnate. Coralloas yellow pink or blue.

54. BOTANICAL NAME : *Spinifex squarrossus* L.

FAMILY : Graminae

VERNACULR NAME : Ravan's moustache

DESCRIPTION:

Gregarious much –branched,woody shrubs. Leves rigid ,thickly coriaceous ,involute ,spreading and recurved.

55. BOTANICAL NAME : *Suaeda maritima* (Forsk.)Dum.

FAMILY : Chenopodiaceace

VERNACULR NAME : Umari kirai

DESCRIPTION:

It is an annual herb. Stem glabrous ,wood thick branch raddish purple, leaves simple alternte ,flowers minute by sexual ,Perianth short globose five lobed ,Polyplous imbrivate,stamens 5, filamentous short ,basifixed ovary- ovoid,ovary superior seed erect.

56. BOTANICAL NAME : *Tabernemontana divaricata*

FAMILY : Apocynaceae

VERNACULAR NAME : Crepe jasmine

DESCRIPTION:

Small trees with resinous exudations at the bases of the leaves, at the bifurcations of the inflorescence and on the flower buds.

57. BOTANICAL NAME : *Tribulus terrestris* L.

FAMILY : Zygophyllaceae

VERNACULAR NAME : Palleru – mullu

DESCRIPTION:

Herbs, annual prostrate to procumbent, hairy, branches up to 1m long.

Hirsute to sericeous. Leaves 3-7 cm long; leaflets 8- 14, ovate to elliptic , oblique, 4-11x2-5mm; stipules subulate to falcate, 2-5 x 1-1.5mm.

58. BOTANICAL NAME : *Tridax procumbens* L

FAMILY : Asteraceae

VERNACULAR NAME : Coat buttons

DESCRIPTION:

A straggling hispid herb with much cut leaves, yellow flowers and achens with feathery pappus.

59. BOTANICAL NAME : *Turnera ulmifolia* L.

FAMILY : Turneraceae

VERNACULR NAME : Yellow alder

DESCRIPTION:

It grows erect with dark toothed leavesand small, yellow orange flowers and is often found as weed, growing on road side.

60. BOTANICAL NAME : *Vernonia cinerea*

FAMILY : Asterceae

VERNACULAR NAME : Naichotte Poonde

DESCRIPTION:

The stem is erect and thin with more or less vertical branching. It is usually ribbed and bears short fine hairs .The leaves are simple and alternate .and variable from 2-8cm long and 2-3cm wide. The lower leaves re ovate with entire or sub-entire margins while the upper leaves are small , linear and irregularly toothed. The fruit is an achene about 1.5mm long with white pappus.

61. BOTANICAL NAME : *Wedelia calendulaceae* Lees.

FAMILY : Asteraceae

VERNACULAR NAME : Manjalkarisalamkanni

DESCRIPTION:

It is a tender, spreading, and hairy herb, with the branches usually less than 50 cm long. The leaves are oblong to oblong-lanceolate, 2-4.5 cm in length, and narrowed at both ends. The margins are entire or obscurely toothed; and both surfaces are covered with sharp-pointed, appressed, straight, and stiff hairs. The heads are stalked, about 1 cm in diameter, and yellow. The involucral bracts are oblong-ovate. The ray flowers are 8-12, spreading, about equal to the bracts, and broad; the disk flowers number about 20, and are short, narrow, and pointed. The achenes are nearly cylindric, and hairy.

RESULT AND DISCUSSION

In the present study, a total of 61 angiospermic taxa belonging to 30 families are recorded along the roadsides of Thoothukudi. This is the first taxonomic survey in this village. All the studied species are provided with legitimate name, citation followed by family name, vernacular name and short description. Vegetation of the study area is presented in the plates I-XI. Out of 30 families, 27 families are Dicotyledons and 3 families are Monocotyledons.

The members of 38 families of dicotyledons are further systematized into 49 genera including 54 species. Similarly, members of 6 families of monocotyledons are classified into 7 genera with 7 species. The conscription of 61 angiospermic taxa from the study area proves its species richness and plurality of the ecosystem (Table 1 and Figure 2).

Generic Diversity

Table 2 shows generic diversity of 30 families studied. Dicot familes such as Amaranthaceae, Apocynaceae, Asteraceae, Leguminosae and Solanaceae have more generic diversity (4 genera) when compared to Euphorbiaceae, Lamiaceae (3 genera); Acanthaceae, Malvaceae and Nyctaginaceae (2 genera). Among monocotyledons, Poaceae represents more genera (5 genera). About 17 dicot families and 2 moncot families are monogeneric.

Species Diversity

Species diversity of the study area is recorded in table 3. 61 species belonging to 38 families have been identified and studied. When compared to other families, Solanaceae, Leguminosae and Poaceae shows more species diversity (5 species) which is

followed by Euphorbiaceae, Amaranthaceae, Apocynaceae, Asteraceae (4 species), Malvaceae, Lamiacae (3 species), Nyctaginaceae, Liliaceae and Acanthaceae (2 species). 16 dicot families and 2 monocot families are monotypic families.

Diversity of Life Forms:

Different habit of species in different families and their percentage of distribution are recorded in table 4 and figure 3. In general, vegetation is dominated by herbs both in dicotyledons and monocotyledons (67%) followed by shrubs (25%), climbers and creepers (3%) and trees (2%).

Medicinal Taxa:

Many economically important plants are recorded in the study area. Among 61 species noted, 52 species are medicinal plants (Table 5).

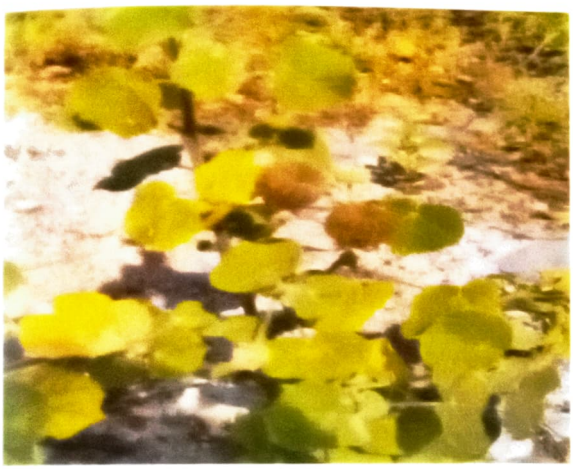
They are used to remediate variety of diseases and ailments like diabetes, skin disease, wounds, cuts, stomach pain, cough, cold, poisonous bites, body heat, body pain, bowl complaint, bronchitis, hair growth, intestinal worms, menstrual trouble, ulcer, tooth-cavities, urinary troubles, vomit, *etc.*,

Diverse parts of the medicinal plants are used based on their ability to cure disease such parts includes leaf, latex of roots, seed, fruit, flower, stem, *etc*

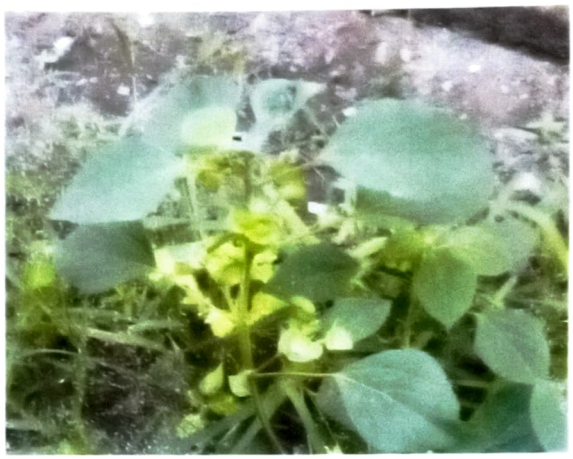
The major resources of medicines are arising from plants. The phytochemical constituents and medicinal properties of most of the medicinal plants were recorded in the last few decades by a number of workers (Nadkarni 1976; Sayed Nudrat and Usha, 2005). These medicinal plants are subjected to various processes and are then administrated to the patients. The survey and documentation of medicinal and aromatic plants in each and every place is mandatory for easy identification of local traditional

healers, conservation and sustainable utilization. The most important utilization of these plants is through medicines (Sujatha and Pushparaj, 2017). However, plants and their parts and the pattern of administration vary from person to person. Thus, there is enormous scope for local medicines based on plant products which are yet to be studied, analyzed and documented.

PLATE-I



Abutilon indicum



Acalypha indica

Achyranthes aspera

Aerva lanata

Allamanda cathartica

Aloe vera

PLATE – II

Alpinia galanga

Amaranthus spinosus

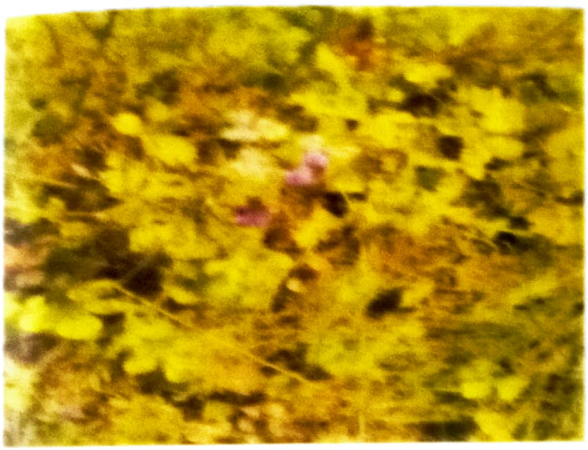
Andrographis paniculata

Anisomeles malabarica

Argemone mexicana

Blastania garcini

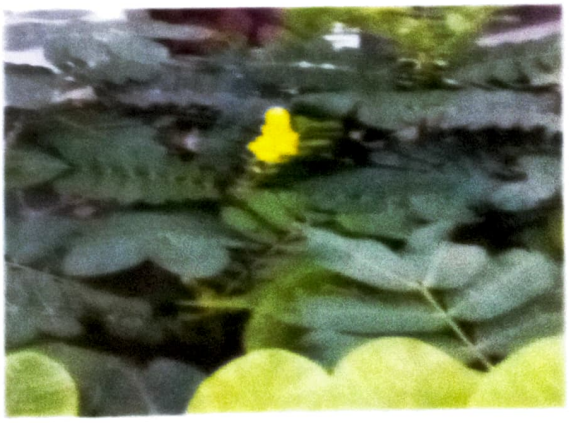
PLATE- III



Boerhaavia diffusa

Bougainvillaea spectabilis

Calotropis gigantea



Cassia alata

Cassia nigricans

Catheranthus roseus

PLATE - IV

Celosia cristata

Cenchrus biflorus

Chloris barbata

Cissus quadrangularis

Cleome viscosa

Clitoria ternatea

PLATE -V

Corchorus aestuans

Corchorus tridens

Crotalaria retusa

Cynodon dactylon

Datura metel

Enicostemma littorale

PLATE - VI

Euphorbia hirta

Euphorbia milli

Gomphrena globosa

Heliotropium curassavicum

Ipomea pescaprae

Leucas aspera

PLATE- VII

Lippia nodiflora

Lycopersicum esculuntum

Mollugo lotoides

Murraya koenigii

Nerium odorum

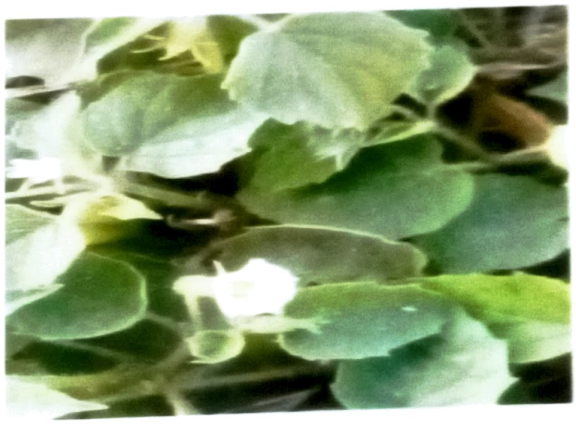
Ocimum sanctum

PLATE - VIII

Oldenlandia umbellata

Pedalium murex

Phyllanthus amarus



Physalis minima

Physalis peruviana

Setaria verticillata

PLATE – IX

Senna occidentalis

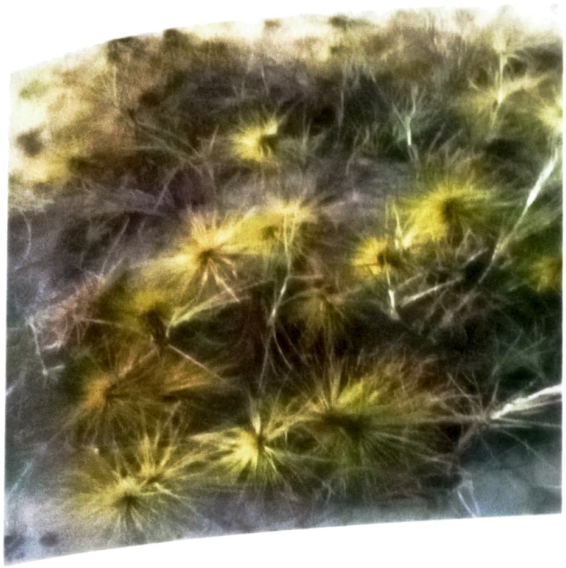
Sida acuta

Sida cordiflolia

Solanum xanthocarpum

Sonchus oleraceus

PLATE – X



Spinifex squarrossus

Suaeda maritima

Tabernaemontana divaricata

Tribulus terrestries

PLATE - XI

Turnera ulmifolia

Vernonia cinerea

Wedellia calendulacea

Table 1: Distribution of weeds in different classes of Angiosperms in the study area

Class	Families		Genera		Species	
	No	%	No	%	No	%
Dicotyledons	27	90	49	87.5	54	88.5
Monocotyledons	3	10	7	12.5	7	11.5

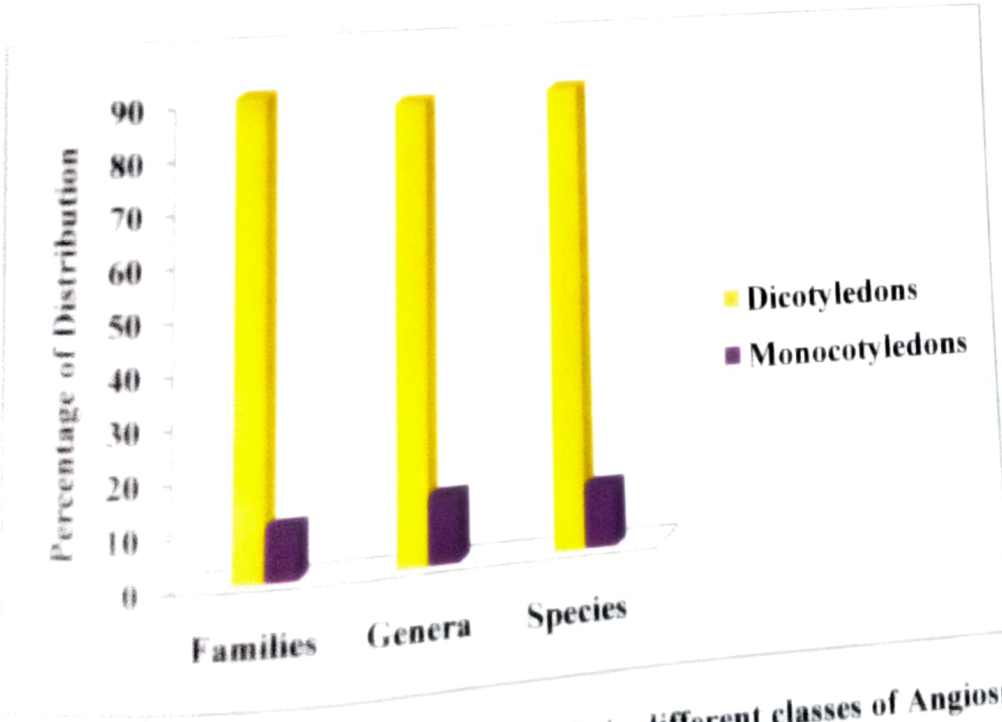


Figure 2: Percentage of Distribution of weeds in different classes of Angiosperms

Table 2: Generic diversity in different families of dicotyledons and monocotyledons in the study area

Dicotyledons	Monocotyledons	No. of genera
-	Poaceae	5
Amaranthaceae, Apocynaceae, Asteraceae, Leguminosae, Solanaceae	-	4
Euphorbiaceae, Lamiaceae	-	3
Acanthaceae, Malvaceae, Nyctaginaceae	-	2
Papaveraceae, Cucurbitaceae, Asclepiadaceae, Vitaceae, Capparidaceae, Tiliaceae, Gentianaceae, Boraginaceae, Convolvulaceae, Verbenaceae, Aizoaceae, Rutaceae, Rubiaceae, Pedaliaceae, Zygophyllaceae, Turneraceae, Chenopodiaceae	Liliaceae, Zingiberaceae	1

Table 3: Species diversity in different families of dicotyledons and monocotyledons in the study area

Dicotyledons	Monocotyledons	No. of species
Solanaceae, Leguminosae	Poaceae	5
Euphorbiaceae, Amaranthaceae, Apocynaceae, Asteraceae	-	4
Malvaceae, Lamiacae	-	3
Nyctaginaceae, Tiliaceae, Acanthaceae	-	2
Papaveraceae, Cucurbitaceae, Asclepiadaceae, Vitaceae, Capparidaceae, Gentianaceae, Boraginaceae, Convolvulaceae, Verbenaceae, Aizoaceae, Rutaceae, Rubiaceae, Pedaliaceae, Zygophyllaceae, Turneraceae, Chenopodiaceae	Liliaceae, Zingiberaceae	1

Table 4: Different life forms of species from the study area

S.No	Habit	No. of Species
1	Herb	41
2	Shrub	15
3	Tree	1
4	Climber	2
5	Creeper	2

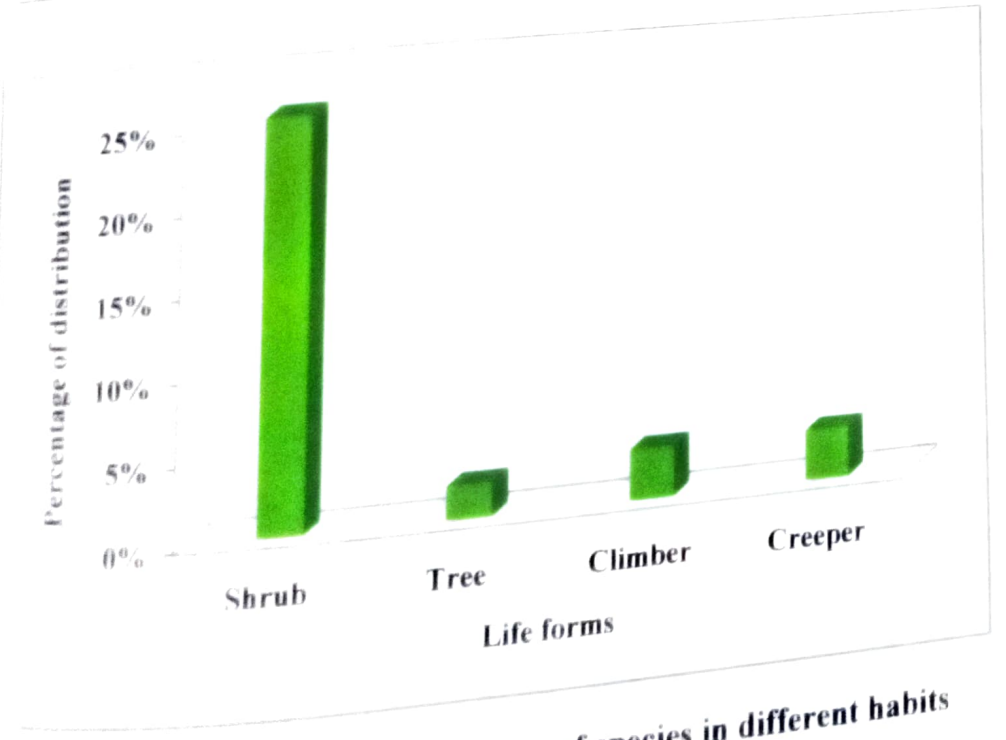


Figure 3: **Percentage of distribution of species in different habits**

Table 5: Medicinal uses of taxa of the study area

S. No	Botanical name	Family	Useful part	Uses
1	*Abutilon indicum*	Malvaceae	Root, leaf,bark and seeds	Roots are used in paralysis and fever. Leaf is used in skin problems and leaf with black pepper is used in dysentery.
2.	*Acalypha indica*	Euphorbiaceae	Leaves	Plant extract is used in muscular problems and asthma. Leaves are used in snake bite. Stem and root is used to treat constipation.
3	*Achyranthes aspera*	Amaranthaceae	Whole plant	Paste of leaves is used to cute wound and abscess.
4	*Aerva lanata*	Amaranthaceae	Whole plant	The decoction of the plant is used to dissolve kidney stones and gall bladder stones.
5.	*Aloe vera*	Liliaceae	Leaf	May improve skin and prevent wrinkles
6.	*Alpinia galanga*	Zingiberaceae	Leaves , root	It is aphrodisiac, aromatic, bitter, digestive, expectorant, pungent herb that stimulates the digestive system.

7.	*Amaranthus spinosus*	Amaranthaceae	Leaves, root, bark and seeds	Leaves and bark are used as tonic for debility after delivery. Whole plant is antidote for snake poisoning.
8.	*Anisomeles malabarica*	Lamiaceae	Whole plant	The whole plant but especially the leaves and the roots is a powerful astringent, carminative, frbrifuge nd tonic.
9.	*Antrographis paniculata*	Acanthaceae	Whole plant	The common cold , immune system support, coughs, diabetes.
10.	*Boerhaavia diffusa*	Nyctaginaceae	Leaves	It is used in the treatment of various conditions including gastric disturbances, asthma, jaundice, anemia and internal inflammation.
11.	*Calotropis gigantean*	Asclepidaceae	Whole plant	The entire plant is used as a good tonic and expectorant. The root bark is useful in treating skin disease, intestinal worms and sores.
12.	*Cassia alata*	Leguminosae	Leaves, Flowers and Fruits	The leaves, flowers and fruit are mixed in an infusion to treat stomach problem.
13.	*Cassia nigricans*	Leguminosae	Seeds	Some have toxins in their seeds are nutritional and medicinal effects.

14.	*Catheranthus roseus*	Apocynaceae	Whole plant	Plants are used in diabetes, leaf influsion used in menorrhagia, also found effective against blood cancer.
15.	*Celosia cristata*	Amaranthaceae	Flowers and Leaves	The flowers bring diarrhea under control. The leaves are used as dressings for boils and sores.
16.	*Chloris barbata*	Poaceae	Leaves	The leaves juice used in fever diarrhoea and diabietes.
17.	*Cissus quadrangularis*	Vitaceae	Stem	It is used to cure obesity, allergy, asthma, cancer and diabetes
18.	*Cleome viscose*	Capparidaceae	Leaves,fruit	The leaves are used to treat cuts and wounds. Fruit is used to treat intermittent fever.
19.	*Clitoria ternatea*	Leguminosae	Bark	The root bark is used in the treatment of snakebites. The roots are bitter, powerfully cathartic, diuretic and purgative.
20.	*Corchorus aestuans*	Tiliaceae	Seed	A good quality fibre is obtained from the bark. It is used for making string one of the jute fibre plants.

21.	*Cynadon dactylon*	Poaceae	Whole plant	It has the capacity to purify blood by eliminating toxins from the blood. It removes general weakness.
22.	*Datura metal*	Solanaceae	Leaf,flower and root	Leaf is used in fever with catarrhal and cerebral complications, antiseptic, antispasmodic, flowers in asthma, fruits in earache. Root is used to boils, pimples.
23.	*Enicostemma littorale*	Gentianaceae	Leaves	It helps in curing fever, rheumatism, skin disease.
24.	*Euphorbia hirta*	Euphorbiaceae	Stem and leaves	It is used in the treatment of cancer, diarrhea, dysentery, intestinal infections, asthma, bronchitis, fever, cough, kidney stones, and abscesses etc.
25.	*Euphorbia milli*	Euphorbiaceae	Whole plant	Euphorbia is used for breathing disorders including asthma, bronchitis and chest congestion.
26.	*Gomphrena globosa*	Amaranthaceae	Leaves and flower	The flowers are boiled to make a tea which is used for cough and diabetes.
27.	*Heliotropium curassavicum*	Boraginaceae	Root and Leaves	The dried roots are ground to powder and applied to sores and wounds. A tea is made from the dried leaves.
28.	*Ipomea percaprae*	Convolvlaceae	Seed	The seed when chewed is said to be a good remedy for stomach ache and cramp.

29.	*Leucas aspera*	Lamiaceae	Leaf and flower	Leaf is used to treat headache, malaria and sinusitis. Flower is used to treat anemia.
30.	*Lippia nodiflora*	Verbenaceae	Whole plant	It is used medicinally to treat suppuration, common colds and lithiasis .It is often grown ornamentally as a ground cover plant.
31.	*Murraya koengii*	Rutaceae	Leaves	It is used to prevent the hair graying.
32.	*Nerium odorum*	Apocynaceae	Leaves	The leaves and the flower are cardiotonic, diaphoretic, diuretic, emetic, expeclorant and sternutatory.
33.	*Ocimum sanctam*	Lamiaceae	Leaves	It is used in cough and cold
34.	*Oldenlandia umbellate*	Rubiaceae	Leaves and root	The root is used in the treatment of snake bites. The leaves are used as wash for poisonous bites.
35.	*Pedalium murex*	Pedaliaceae	Leaves and root	The leaves and roots have been used in a clinical test with patients suffering from gonorrhea.
36.	*Phyllanthus amarus*	Euphorbiaceae	Leaves	Leaf is given in allergic problems, eruptions, plant extract in jaundice.
37.	*Physalis minima*	Solanaceae	Dried fruit	The dried fruit used as antiseptic, diuretic.

38.	*Physalis peruviana*	Solanaceae	Whole plant	All parts of the plant are used as a diuretic and antipyretic. The fruit is said to be alterative appetizer, bitter, laxative and tonic.
39.	*Senna occidentalis*	Leguminaceae	Root and Flower	Paste of roots and inflorescence is given to cure irregular menstruation and other sexual diseases.
40.	*Setaria verticilate*	Poaceae	Leaves and flowers	The mainly used as a permanent pasture, but can be used for hay or silage. The difficult remove and binds nutrient.
41.	*Sida acuta*	Malvaceae	Leaves	The leaves are diuretic. An infusion used to treat dysentery.
42.	*Sida cordifolia*	Malvaceae	Whole plant	The plant is used to treat fractures, swelling, boils.
43.	*Solanum xanthocarpum*	Solanaceae	Whole plant and dry root	It is used cure cough it cures respiratory problems like Bronchitis and asthma.
44.	*Sonchus oleraceus*	Asteraceae	Leaves	The cure headaches, fever, infection liver, rheumatism.
45.	*Spinifex squarrossus*	Poaceae	Leaves	The excellent medicine in cases of diarrhea and dysentery.
46.	*Suaeda maritime*	Chenopodiaceae	Leaves	The ashes of the plant provide a soda that is used in making glass and soap.

47.	*Tabernaemontana divericata*	Apocynaceae	Leaves, root and flowers	The roots and flowers are all used in the treatment of snake and scorpion poisoning.
48.	*Tribulus terrestries*	Zygophyllaceae	Whole plant	In siddha medicine the whole plant is used in the form of decoction to treat urinary tract infections, edema, dysmenorrhea.
49.	*Tridax procumbens*	Asteraceae	Leaves	The smoke produced by part burning the plant is used to repel mosquitoes. The leaves are used as a hair restorative.
50.	*Turnera ulmifolia*	Turneraceae	Leaves	The leaves are used for treating hair loss and thrush.
51.	*Vernonia cinerea*	Asteraceae	Root and Leaves	It is used to cure fever, kidney disease and stomach discomfort.
52.	*Wedelia calendulaceae*	Asteraceae	Leaves	It is used in Snake bite

SUMMARY AND CONCLUSION

For the first time, floristic survey of weeds and their medicinal uses along the roadsides of Thoothukudi, Tamil Nadu has been analyzed through this study, since; so far there is no exhaustive inventory of flora. The present work assumes significance for floristic diversity, including medicinal flora, which will be useful for further scientific and systematic forest management programmes. We recorded 61 angiospermic taxa belonging to 30 families. Out of 61 species, 52 species are used as medicine by local people. Since the present study have provided preliminary and basic information on plant diversity and their medicinal uses, it is useful for conducting similar scientifically designed study to explore bio resources of this area.

Many parts of the country still remain unknown biogeographically. Hence, it is the need of the hour that major thrust should be given an intensive inventory and documentation of useful species, their chemical constituents, habitats and potential utilization as raw materials.

Our study reveals that, constructions, deforestation, soil erosion, pollution and drought are the major factors that affect different plants in the study area. So the necessary action should be taken to preserve the diversity of species by *in-situ* conservation method. Such steps will not only contribute to protect the habitats but also help to maintain the ecological processes. Hence, this study recommends periodic monitoring of this area to study the effect of anthropogenic activity on the floristic diversity. It is found out that, only some peoples alone have the appropriate knowledge on the plants and their medicinal properties. So, attempt should be made to initiate special programmes for raising people's awareness about conservation and utilization of plants around them.

BIBLIOGRAPHY

BIBLIOGRAPHY

Ahmad, S.S. 2007. Medicinal wild plants from Lahore Islamabad Motorway. *Pakistan Journal of Botany*. 39: 355-375.

Ahmad, S.S., Fazal, S., Waleem, E.E and Zafar,I.2009. Elevation of ecological aspects of roadside vegetation around Havailian city using multivariate techniques. *Pakistan Journal of Botany*. 41: 461-466.

Ahmad, S.S. and Hussain, S. Z. 2008. Ethno medicinal survey of plants from salt range (Kallar Kahar) of Pakistan. *Pakistan Journal of Botany*. 40: 1005-1011.

Angold, P.G. 1997. The impact of a road upon heathlands vegetation effects in plants species composition. *Journal of Applied Ecology*. 34: 409-417.

Anonymous. 1997. Amruth, August. FRLHT, Bangalore, pp. 10

Balasingh, J., Thiruchenthil Nathan, P., BeulaJerlin, S., Samuel.A. and Muthukumar. A.2000. Medicinal flora of a tropical scrub jungle. *Journal of Economic and Taxonomic Botany*.24: No. 3.

Balasubramanian. 2013. Dictionary of medicinal plants. ABS garden, Salem.

Ball, J. E., Jenks, R. and Aubourg, D. 1998. An assessment of the availability of pollutant constituents on road surfaces. *Science in the Total Environment*, 209, 243–254.

Bassey, M. E., Ogbemudia, F.O., Ezekiel, A.G. and Abdulkrareem, M. 2017. Roadside plant diversity and soil analysis in Uyo Sub-urban environment Akwa Ibom State Nigeria. *International Journal of Scientific & Engineering Research*. 8:1098-1111.

Convention on Biological Diversity (CBD). 2000. Sustaining life on Earth. Secretariat of the Convention on Biological Diversity. pp.1-20.

Dikes, C. and Geiger, W.F. 1999. Pollution retention capabilities of roadside soils. *Water Science and Technology*, 39: 201-208.

Fearnside, P. M., Graca, P. Keizer, E. Maldonado, F. D. Barbosa, R. I. and Nogueira, E. M. 2009. Modeling of Deforestation and Greenhouse-gas Emissions in the Area of Influence of the Manaus-Porto Velho highway. *Revista Brasileira Meteorologia*. 24: 208–233

Forman, R. T. and Alexander, L. E. 1998. Roads and their major ecological effects. *Annual Review of Ecological System*. 29: 207–231.

Gamble, J.S. 1997. Flora of the Presidency of Madras, Adlard and son., Ltd London.

Harper, D. 2001. Horseboating (Web). The Horseboating Society. Retrieved on April 9, 2007.

Heilman, G. E., Stritthold, J. R., Slosser, N. C. and Dellasala, D.A. 2002. Forest fragmentation of the conterminous United States: assessing forest intactness through road density and spatial characteristics. *Bioscience*. 52: 411– 422.

Jeeva, S., Kiruba, S., Mishra , B.P., Venugopal, N., Dhas , S.S.M., Regini, G.S., C Kingston, C.,Kavitha, A., Sukumaran, S., Raj, A.D.S. and Laloo, R.C. 2006. Weeds of Kanyakumari district and their value in rural life. *Indian Journal of Traditional Knowledge*. 5: 501-509

Jesse, M.K., Suzanne, J.M. and Melodie, A.M. 2008. Road verges as invasion corridors? A spatial hierarchical test in an arid ecosystem. *Landscape Ecology*, Online article.

Krishnan Marg, K.S. 1992. The useful plants of India. Publication and Information Directorate. CSIR, New Delhi. 110 012.

Larson, D. L. 2003. Native Weeds and Exotic Plants: Relationships to Disturbance in Mixed-Grass Prairie. *Plant Ecology*. 169. 31 7 – 333.

Maheswari, J. K., Kunal, G., Bhandari, M.M. and Duke, J.H. (Ed.). 1993. Ethnobotany in India. *Journal of Economic and Taxonomic Botany*.Scientific Publishers, Jodhpur.

Manikumar, K. and Samuel, A.S. 2010. Traditional herbal medicines of the coastal diversity in Tuticorin district, Tamil Nadu, India. *Journal of Phytology*. 2: 38–46.

Muthukumar, K. and Samuel, A.S. 2011. Coastal sand dune flora in the Thoothukudi District. Tamil Nadu, Southern India. *Journal of Threatened Taxa*. 3: 2211–2216.

Pandey, A.K., Patra, A.K. and Shukla, P.H. 2005. Medicinal plants in Satpura Plateau of Madhya Pradesh: Current Status and Future Prospects. Indian Forester. 200;131:857–883.

Pandey, P. and Pandey, B. 2016. Survey of medicinally important roadside vegetation of Bhilai Township. *Interntional Journl of Scientific Engineering and Research*. 4:125-128.

Posey, D. A. and Dutfield, G. 1996. Beyond Intellectual Property: Toward Traditional Resource Rights for Indigenous Peoples and Local Communities. Canada: International Development Research Centre. P. 303.

Pushpangadan, P. and Kumar, B.2005. Ethnobotany, CBD, WTO and the Biodiversity Act of India. *Ethnobotany*. 17:2–12.

Rama rajan, S. and Muthu Kumarasamy, S. 2012. Ethno medicinal plant survey of Srivaikundam village of Tuticorin district, Tamilnadu, India. *Life sciences leaflets*. 6: 47-53.

Sara, A. 2006. Plant species richness in midfield islets and road verges-The effect of landscape fragmentation. *Biological Conservation*. 127: 500-509.

Sheela, D., Uthayakumari , F. and Bharathy, V. 2016. Floristic diversity of sedges in Thoothukudi District, Tamilnadu. *Journal of Economic and Taxonomic Botany*. 36: 184-187.

Ahmad, S.S., Khan, S. and Erum, S. 2013. Multivariate analysis of roadside vegetation along motorway (m-1), Pakistan. *Pakistan Journal Botany*. 45: 49-53.

Singh, A.K., Singh, P. and Srivastava, S. 2004. Medicinal weeds of Varanasi District (Uttar Pradesh). *Journal of Economic and Taxonomic Botany*. 28:63-273.

Singh, M. 1993.Thesis (unpubl) Banares Hindu University, Varanasi. Ecological studies of some medicinal plants of Indian. 175.

Mallick, S.N. and Acharya,B.C. 2013. Phytodiversity survey of roadside herbs and their ethno medicinal values in and around Rourkela steel township, Sundargarh, Odisha. *Life Sciences Leaflets*.1:46-55

Spellerberg, I. F. 2002. Ecological Effects of Roads, Enfeld, USA: Science Publishers, Inc. 218.

Storch, H. V., Costa-Cabral, M., Hagner, C., Feser, F., Pacyna, J., Pacyna, E. and Kolb, S. 2003. Four decades of gasoline lead emissions and control policies in Europe: a retrospective assessment. *The Science of the Total Environment*. 311: 151 – 176.

ombulak, S. C. and Frissell, C. A. 2000. Review of ecological effects of roads on terrestrial and aquatic communities. *Conservation Biology*. 14: 18–30.

ison, J.B., Rapson, G.L., Sykes, M.T., Watkins, A.J. and Williams, P.A. 1992. Distribution and some climatic correlations of some exotic species along roadsides in New Zealand *Journal Biogeography*. 19: 183-194.

allarasimhan. 2000. Medicinal plants of India. Volume 2.