PHYTOCHEMICAL, ANTIOXITANT, FTIR, GC-MS ANALYSIS AND BIOLOGICAL ACTIVITIES OF TWO CASSIA SPECIES

A dissertation submitted to

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By

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CERTIFICATE

This is to certified that this dissertation entitled **PHYTOCHEMICAL, ANTIOXITANT, FTIR, GC-MS ANALYSIS AND BIOLOGICAL ACTIVITIES OF TWO CASSIA SPECIES.** Submitted by ANANTHI.S (Reg.No.18APBO01) to St. Mary's College (Autonomous),Thoothukudi- 628001 in partial fulfillment for the award of the degree of **'Master of Science in Botany'** is done by her under my supervision. It is further certified that this dissertation or any pare of this has not been submitted elsewhere for any other degree.

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I do here by declare that this dissertation entitled **PHYTOCHEMICAL, ANTIOXITANT, FTIR, GC-MS ANALYSIS AND BIOLOGICAL ACTIVITIES OF TWO CASSIA SPECIES** Submitted by me in partial fulfillment for the award of the degree of **'Master of Science in Botany'**, in the result of my original and independent work carried out under the guidance of **Dr. Mrs. B.Maria Sumathi, M.Sc., M.phil., Ph.D.** Assistant Professor, Department of Botany, St. Mary's College (Autonomous), Thoothukudi and it has not been submitted elsewhere for the award of any other degree.

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INTRODUCTION

The primary needs of every human being are food, cloth, shelter, health and education. Among them, health is of paramount importance because without health man cannot achieve the other requirements. A well-known adage says "Health is wealth". A disorder or a disease can be compared through various remedial measures like Naturopathy, Homeopathy, Ayurveda, Allopathic and Unani are some such measures. The use of natural products with therapeutic properties is as ancient as human civilization and for a long time, mineral, plant and animal products were the main source of drugs (De Pasqual 1984).

The use of plant materials to prevent and treat infectious diseases successfully over the years has attracted the attention of scientist's worldwide (Falodun *et al.,* 2006). Many investigations are being conducted on medicinal plants based on information supplied by the local populations with the object of finding out phytochemical constituents for application in the prevention and treatment of infectious diseases and other diseases of non-microbial etiology. Several studies have been conducted to provide scientific basis for the efficacy of plants in herbal medicines. The development of resistance to most of the available antimicrobial agents and the high costs of treatments consequent upon this resistance has necessitated the search for new, safe, efficient and cost effective ways for the management of infectious conditions. Akinpelu and Onakaya (2006) have warned that unless concerted efforts are made to acquire new agents, very soon the population of bacteria developing resistance will not match the arsenal to fight. The rising interests in products of natural origin in the developed economics led to the extraction and development of several drugs and chemotherapeutic agents from plants as well as from traditionally used rural herbal remedies (UNESCO, 1998). Extracts of higher plants have served as good sources of antibiotics against various bacterial and fungal pathogens (Falodun *et al*., 2006). Plant based antimicrobial compounds have great therapeutic potential as they can serve the purpose

without any side effects often associated with synthetic drugs and also little chance of development of resistance. The common view in the society and the medical community is that plant based products are healthier, safer, and more reliable than synthetic products (Benli *et al*., 2008), even though safety and efficacy data are available for only a few number of plant materials. Plants act generally to stimulate and supplement the bodies' healing forces; they are the natural foods of human beings (Ajayi *et al*., 2008). The clinical success of quinine and quinidine isolated from the Cinchona tree bark and recently artemisinin from *Artemisia annua* in the chemotherapy of malaria have rejuvenated interests in higher plants as potential sources of novel drugs (Igoli *et al.,* 2005).

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) Therefore, dietary antioxidants are needed to protect the harmful action of ROS. Well established antioxidants derived from diet are vitamins A, C, E, polyphenols and carotenoids (Pietta, 1999 & 2000). Current antioxidant research of free radicals also has confirmed that food with rich antioxidants play an essential role on the

prevention of disease caused by oxidative stress. Therefore, plant derived antioxidants now receiving a special attention (Tepe *et al*.,2005).

Secondary metabolites of medicinal plants stimulate the immune system, body's defense mechanism against bacteria and other disease-causing agents, phytochemicals also have a capacity to block the potential for carcinogens. The plants have played an important role in the drug development (Edeoga *et al* .,2005).

Of the 386 families and 2200 genera in which medicinal plants are recorded, the families Asteraceae, Euphorbiaceae, Lamiaceae, Caesalpiniaceae, Rubiaceae ,Poaceae, Acanthaceae, Rosaceae and Apiaceae share larger proportion of medicinal plants (Chandel *et al*., 1996). The Caesalpiniaceae are a family of flowering plants, which are mostly woody species occurring in tropical and subtropical trees and shrubs comprising about 150 genera and 2,200 species. The genus *Cassia* of ornamental herbs, shrubs and trees widely distributed predominantly in tropical and warm temperate regions. Genus *Cassia* consists of around 400 species and only 45 are found in India. (Kritikar and Basu, 2003). For present study, two taxa *Cassia alata* and *Cassia glauca* are selected.

Cassia alata is a shrub, widely distributed in the tropical countries. It is native to South America, but has been planted widely for medicinal and ornamental purposes and is now pantropical (Rahman *et al.*, 2008). In many countries, including most countries of tropical Africa, it has become naturalized and is often considered a weed. *Cassia alata* commonly known as semaiagathi in Tamil is well known for its various medicinal properties in Indian systems of medicine. Various parts of this plant are used as vermicide, astringent, purgative, expectorant and to treat skin diseases. Plant is used to treat urinary tract infections, bronchitis and asthma (Rao *et al*., 1973)

Cassia glauca is a glabrous tree found throughout India, tropical Asia and Australia. *Cassia glauca* are used for the treatment of diabetes and gonorrhea. In Ayurvedic

systems of medicine, herbal extracts but not purified compounds have been used from centuries because of many constituents are considered to be beneficial. It is also used to reduce fever and sore throat pain. The macerated juices of the young fresh leaves are used to treat eye infections and parasitic diseases.

Scope and Objectives

The aspiration of current study was to assess the biochemistry and bioactivities of the plant extracts (*Cassia alata* and *Cassia glauca*). In this work the following objectives are focused.

Collection of leaves and flowers of *Cassia alata* and *Cassia glauca* for extract preparation.

- To qualitatively screen the presence phytochemicals by using different solvents (acetone, methanol, ethanol, petroleum ether) and aqueous extracts of leaves and flower of *Cassia alata* and *Cassia glauca.*
- To quantitatively analyses and compare the total phenolics, flavonoids, vitamin C, Tannin and vitamin E of leaves and flower of *Cassia alata* and *Cassia glauca.*
- To identify and compare the functional group of leaves and flower of *Cassia alata* and *Cassia glauca* by Fourier transform infrared spectroscopy (FTIR) analysis.
- To identify the bioactive compounds of the *Cassia alata* leaf extract using GC-MS analysis.
- To assess the antioxidant potential of *Cassia alata* and *Cassia glauca* using aqueous extract against DDPH radical scavenging activity .
- To evaluate the anti-bacterial potential of acetone, methanol, ethanol and aqueous extracts of leaves and flower of *Cassia alata* and *Cassia glauca.*
- To investigate the anthelmintic activity of methanol and aqueous extracts of leaves and flower of *Cassia alata* and *Cassia glauca*

LITERATURE REVIEW

Phytochemicals are chemical compounds formed during the plants' normal metabolic processes. These chemicals are often referred to as *"*Secondary metabolites*"* of which there are several classes including alkaloids, flavonoids, coumarins, glycosides, polysaccharides, phenols, tannins, terpenes and terpenoids (Okwu, 2004). In addition to these substances, plants contain other chemical compounds. These can act as agents to prevent unconsiderable side effects of the main active substances or to assist in the assimilation of the main substances. Many herbaceous and medicinal plants contain important photochemical and vitamins such as alkaloids, flavonoids, tannins, cyanogenic glycosides, phenolic compounds, saponins, lignins, vitamin C, vitamin E and carotenoids, which are utilized both by humans and animals as important components of diets [Hussain *et al.,* 2011]. The medicinal effects of plants are considered to be due to metabolites, especially secondary compounds, produced by plant species. Phytochemical analysis suggests that the presence of various biologically active compounds [alkaloids, phenols, flavanoids, proteins-lectin, carbohydrates, indigo, steroids etc.] and could be correlated to various therapeutic purposes [Vinoth *et al.,* 2011]. Plants have an almost limitless ability to synthesize aromatic substances, mainly secondary metabolites of which 12,000 have been isolated, a number estimated to be less than 10% of the total (Mallikharjuna *et al.,*2007).

The phytochemical screening of different parts of the *Jatropha curcus* revealed the presence of tannins, saponins, carbohydrates, sterols, diterpenes, alkaloids, flavanoids and various enzymes. Root contains di-terpenoid, Jatrophol and Jatropholones A and B, taraxerol b-sito-sterol. The bark contains tannins, resins, saponins, reducing sugar and traces of a volatile oil. Leaves contain Steroid, alkaloids triterpene [Rajore and Batra, 2004]. Musa *et al*., (2000) studied the phytochemistry of powdered leaves of *Acalypha recemosa*

(Euphorbiaceae). This study revealed the presence of alkaloid, tannin, flavanoid and terpenes.

Sivaraj *et al.*, (2011) conducted preliminary phytochemical screening using five different solvents extracts of *Aegle marmelos, Ruta graveolens, Opuntia dillenii, Euphorbia royleana* and *Euphorbia antiquorum*. Phytochemical profiling of *Mimosa pudica* was carried out by Sriram *et al.*, (2011). Sukumaran *et al*., (2011) identified the phytochemical constituents of methanol extract of flower of *Peltophorum pterocarpum*. Phytoconstituents found in *Tridax procumbens* were isolated and characterized by Surendra and Talele (2011).

Nwokocha *et al.,* [2011] studied the comparative phytochemical screening of *Jatropha curcus*, *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.

Vindhya K *et al*.,(2014) conducted the preliminary phytochemical study in *Gardenia latifolia* and *Gardenia gummifera* , using different solvents . The petroleum ether extract of both the plants were found to contain glycosides, phytosterols, fats and oils, resins, phenols and triterpenes. Flavonoid was found to be present in *Gardenia latifolia* and not in *Gardenia gummifera*. Alkoloids, carbohydrates, saponins, tannins, proteins, amino acids and diterpenes were absent in both the plants. Ethyl acetate extracts of the plant was found to contain glycosides, phytosterols, resins, phenols, flavonoids and triterpenes. Alkoloids, carbohydrates, saponins, fats, oils, tannins, proteins, amino acids and diterpenes were absent in both the plants.

Ved Prakash *et al.,* (2015) investigated phytochemical screening and antioxidant activity of *Adina cordifolia* leaf. The plant extracts were screened for presence of

flavonoids, carbohydrate, alkaloid, saponin, phenol, tannins, phlobatannins, terpenoids, and cardiac glycosides. Total flavonoid content, phenols content was estimated. Antioxidant activity was determined using nitric oxide scavenging assay, DPPH assay, hydrogen peroxide scavenging and ferric reducing methods, also MIC was calculated against a set of bacteria (*S. aureus, B. subtilis, E. coli, K. pneumonia*). Ravindranath [2003] has been isolated a novel macrocyclic diterpene–Jatrophenone from the whole plant of *Jatropha gossypifolia*. This compound possesses significant antibacterial activity.

FTIR

A large number of medicinal plants are used as alternate medicine for diseases of man and other animal since most of them are without side effects when compared with synthetic drugs. Identification of the chemical nature of phytochemical compounds present in the medicinal plant will provide some information on the different functional groups responsible for their medicinal properties. Iqbal Ahamed *et al*., (2006) detected major groups of compounds as the most active fraction of four plants extract by infrared spectroscopy.

Ramamoorthi and Kannan (2007) screened the bioactive group of chemicals in the dry leaf powder of *Calotropis gigantea* by FTIR analysis .Kareru *et al.*, (2008) detected saponins in crude dry powder of 11 plants using FTIR spectroscopy.

Muruganantham *et al.*, (2009) carried out the FTIR spectroscopic analysis in the powder samples of leaf , stem and root of *Eclipta alba* and *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), analyzed the ethanolic extracts of *Ichnocarpus frutescens*, by FTIR, revealed the presence of functional group components of amino acids, amides, amines, carboxylic acid, carbonyl compounds, organic hydrocarbons and halogens. Parag A. Petnekar and Bhanu Raman (2013) carried out the FTIR spectroscopic analysis of methanolic leaf extract of *Ampelocissus lantifolia* for antimicrobial compounds.

FTIR analysis for five selected green leafy vegetables(GLVs) viz., *Hibiscus cannabinus* , *H. sabdariffa* , *Basella alba* , *B .rubra* L. and *Rumex vesicarius* confirmed the presence of free alcohol, intermolecular bonded alcohol, intramolecular bonded alcohol, alkane, aromatic compounds, imine or oxime or ketone or alkene, phenol and amine stretching (Sravan Kumar and Manoj., 2015).

The functional group identification is made by FTIR analysis and the active components based on the peak value in the region of infrared radiation. The ethanolic flower extract of *Erythrina variegata* L. is passed into the FTIR spectroscopy and the functional groups of the components are separated based on the peak ratio. The results of FTIR analysis confirm the presence of functional groups such as non-bonded, O-H stretch, carboxylic group, acidic, H bonded, C-H stretch, asymmetric stretching of –CH (CH2) vibration, C=N (stretch), carbon-carbon triple bond, multiple bonding, carbonyl compound frequency, C=O stretch, C=C stretch, O-H bend, alcoholic group, C-N stretch, C-O stretch, PO3 stretch, =C-H bending and C-Cl (Priyanga S *et al.,* (2017).

GC-MS

The chemical composition of the essential oils from leaves and wood of *Ocotea brenesii* growing wild in Costa Rica was determined by capillary GC/FID and GC-MS. From the leaves, 64 compounds were identified, corresponding to 85.9% of the oil, and from the wood 57 compounds were identified corresponding to 69.0% of the oil (Carlos and Jose,

2005). The chemical compositions of the essential oils of *Ocimum basilicum* L. *cv*. purple and *Ocimum basilicum* L. *cv*. green cultivated in Iran were investigated by GC-MS (Seyed, 2006).

GC-MS analysis of *Jatropha curcas* leaves revealed the presence of 16 compounds. The most abundant components were 22, 23-dihydro-stigmasterol (16.14%) alpha-tocopherol (15.18%), beta amylin (7.73%) and dotriacontanol (7.02%) The content of gamma tocopherol reached 2.88% and Vitamin E reached 18.06% in the extract (Wang *et al*., 2009). The GC-MS analysis of *Strobilanthes crispus* oil revealed the presence of 28 components. The main constituents were found to be phytol, α-cadinol, Megastigmatrienone, 2,3-dihydrobenzofuran and eugenol (Asmah *et al*., 2006).

Nithya Narayanaswamy and Balakrishnan (2011) evaluated the antioxidant properties of 13 important medicinal plants and it showed that *Ocimum basilicum* leaf, *Alpinia calcarata* leaf, *Jatropha mulitifida* flower, *Hyptis suaveolens* leaf, *Solanum indicum* leaf and *Clitoria ternatea* leaf and flower possessed higher DPPH scavenging activity. Moussa *et al*., (2011). The aqueous leaf extracts of 124 Egyptian plant species belonging to 56 families were investigated and compared for their antioxidant activity by DPPH scavenging assay. Safi *et al*. (2012) studied the biological activities of aqueous extract of the root of *Jatropha curcas* like antimicrobial and free radical scavenging activities. In the evaluation of DPPH free radical scavenging activity. Olabinri *et al.,* (2013) investigated *in vitro* antioxidant and nitric oxide radical scavenging capabilities of *Jatropha gossypifolia* extract.

Sermakkani M. And V. Thangapandian (2012) evaluated GC-MS analysis of *C. italica* leaves revealed the presence of seventeen compounds. The identified compounds possess many biological properties. For instance, 9,12,15-Octadecatrienoic acid, (Z,Z,Z)- Linolenic acid (R/T 20.06) possesses anti-inflammatory, insectifuge, hypocholesterolemic,

cancer preventive, nematicide, hepatoprotective, antihistaminic, antieczemic, antiacne, 5-alpha reductase inhibitor, antiandrogenic, antiarthritic and anticoronary properties. n-Hexadecanoic acid - palmitic acid (R/T 17.25) can be an antioxidant, hypocholesterolemic, nematicide, pesticide, lubricant activities.

Fenghuan Wei *et al*., (2015) identified thirty compounds in *Jasminum grandiflorum* by using GCMS. The major volatile components of the flower were 3,7,11,15-tetramethyl-2-hexadecen-1-o（phytol） (25.77 %), 3,7,11- trimethyldodeca -1,6,10-trien-3-ol (12.54 %) and 3,7,11,15- tetramethyl -1-Hexadecen-3-ol (12.42 %). The results show that phytol is the major volatile component of *Jasminum grandiflorum.*

Praveen Kumar P *et al.,* (2018) studied the identification of bioactive compounds from the Neem sap by Gas chromatography and Mass spectroscopy (GC-MS). The GC-MS analysis of the Neem sap revealed the presence of 30 volatile compounds. Among the 30 compounds, the most predominant compounds are fatty acids like Hexadecanoic acid and Pentadecanoic acid. Hence, this current attempt forms a basis for the biological characterization and importance of the compounds which could be exploited for future development of drugs.

Seventy six kinds of chemical compounds were found in methanol extract of *E.cephalotes* including aldehydes (7.9%), phenols (7.5%), fatty acids (5.8%) and furfural (5.4%) and 86 kinds of chemical compounds found in *M.anisodan* extract. Furfural, steroids, vitamin B and flavonoids are the main compounds of *M.anisodan* by S. Mohammadi *et al*., (2019).

Antioxidant activity

Antioxidant compounds in food play an important role as a health protecting factor. Primary sources of naturally occurring antioxidants are whole grains, fruits and vegetables.

Natural antioxidants can also be replaced by commercially available, synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), which are quite unsafe to use and is restricted due to their carcinogenic effect (Velioglu *et al*., 1998). Natural antioxidants or phytochemical antioxidants are the secondary metabolites of plants (Walton and Brown, 1999). Carotenoids, flavonoids, cinnamic acids, folic acid, ascorbic acid, tocopherols, tocotrienols *etc*., are some of the antioxidants produced by this plant for their sustenance. Beta-carotene, ascorbic acid and alpha tocopherol are the widely used as antioxidants (McCall and Frei, 1999).

Flavonoids are polyphenolic compounds, which are ingredients of many vegetables and fruits. They are classified into flavanols, flavanones, flavones, iso-flavones, catechins, anthocyanins, proanthocyanidins, etc. [Huy *et al*., 2008]. They are among the most bioactive plant secondary metabolites which outperform well-known antioxidants.

Natural antioxidants are known to exhibit a wide range of biological effects including antibacterial, antiviral, anti-inflammatory, anti-allergic, anti-thrombic and vasodilatory activities. Antioxidant activity gives rise to anti-carcinogenicity, anti-immunogenicity and anti-aging activity [Gulcin *et al*., 2010].

Flavonoids serve as ROS scavengers by locating and neutralizing radicals [Gill and Tuteja, 2010]. Bioactive properties such as free radical scavenging, inhibition of hydrolytic and oxidative enzymes and anti-inflammatory action of flavonoids is known [Njoku *et al*., 2011]. The antioxidant activity of the dietary phenolics considered to be superior to that of the essential vitamins and is ascribed to their high redox potential, which allows them to interrupt free radical mediated reactions by donating hydrogen from the phenolic hydroxyl groups [Beevi *et al.*, 2010].

Phenolics are secondary metabolities that behave as antioxidants due to the reactivity of the phenol moiety (hydroxyl substituent on the aromatic ring). The antioxidant activities of phenolic compounds are also attributed to their ability to chelate transition metal ions, such as those of iron and copper, which have been proposed as the catalyst for the initial formation of ROS [Knezevic *et al*., 2011].

Ascorbic acid (vitamin C) is a vital component in human diet with the highest concentrations in animal organs like the liver, leukocytes, and anterior pituitary. It is used for its antioxidant effect [Ensafi *et al*.,2010]. Vitamin C is a major ubiquitous non-enzymatic, water soluble antioxidant [Ueta *et al*., 2003]. It acts as ROS scavenger, thus potentially protecting cells from harmful oxidative products [Fossati *et al*., 2011]. Vitamin C functions in enzyme activation, oxidative stress reduction, and immune function. There is considerable evidence that vitamin C protects against respiratory tract infections and reduces risk for cardiovascular disease and some cancers [Schlueter and Johnston, 2011].

Tannins are group of polymeric phenolic substances. Consumption of tannin containing beverages, especially green teas and red wines can cure or prevent a variety of illness including heart related diseases (Van-Burden and Robinson, 1981).

Swamy *et al*.,(2004) tested the leaf extracts of medicinal plant, *Leptadenia reticulata* for AgNPs production and antioxidant activity studies. He observed that, 500 µg/ml of green synthesized silver nanoparticles showed maximum (64.81 %) radical scavenging activity. The silver nanoparticles were synthesized using aqueous *Piper longum* fruit extract and the aqueous *P. longum* fruit extract and the green synthesized silver nanoparticles showed powerful antioxidant properties *in vitro* antioxidant assays. Haes *et al*., (2002).

Pourmorad *et al*.,(2006) carried out a comparative study on the antioxidant

potentials of some selected Iranian medicinal plant extracts. The antioxidant properties of 25 edible tropical plants were studied by Wong *et al.*, (2006). Badami and Channabasavaraj (2007) studied the *in vitro* antioxidant activities of thirteen medicinal plants collected from Western Ghats of India.

Ademiluyi and Oboh (2008) studied the antioxidant activity of methanol leaf extract of *Viscum album* by using linolenic acid peroxidation and DPPH methods. Effat *et al.*, (2008) screened thirteen medicinal plant extracts for antioxidant activity. MoniRani *et al.,*(2008) evaluated antioxidant activities of methanol extract of *Ixora coccinea* by DPPH free radical scavenging activity, reducing power and total antioxidant activity assays.

Gayatri *et al*., (2011) observed that the piperine, an alkaloid found naturally in *Piper nigrum* and *Piper cubeba*. It is widely used in various herbal cough syrups and anti-inflammatory, antimalarial, anti-leukemia treatement. Ethanol extract of *Piper cubeba* showed high antioxidant activity.

Inbathamizh *et al.,* (2013) studied in vitro evaluation of antioxidant and anticancer potential of *Morinda pubescens* synthesized silver nanoparticles. The decolorization from purple DPPH radical to yellow DPPH molecule by the sample in a dose-dependent manner with an IC50 value of 84±0.25 µg/ml indicated the sample's high radical scavenging activity, which was closer to that of the standard whose IC50 value was found to be 80±0.69 µg/ml.

Niraimathi *et al.,* (2013) investigated on biosynthesis of silver nanoparticles using *Alternanthera sessilis* (Linn.) leaf extract and determined antioxidant activities. Free radical scavenging activity of the AgNPs on DPPH radical was found to increase with increase in concentration, showing a maximum of 62% at 500 µg/ml. The standard gallic acid, however, at this concentration exhibited 80% inhibition. The IC50 value was found to be 300.6 µg/ml.

The silver nitrate extract of *Annona squamosa* and *Sapium macrocarpum* showed two times more DPPH scavenging activity than the commercial antioxidant butylated hydroxyl anisole. (Ruiz *et al.*, 2008). The silver nitrate extracts of *Melissa officinalis*, *Matricaria recuttia* and *Cymbopogan citratus* were found to possess DPPH scavenging activity. (Pereira *et al*., (2009). Sowndharajan *et al*., (2010) studied the antioxidant capacity and total phenolic contents present in the silver nitrate extracts of leaves, stem, and roots of *Melothria maderaspatana* were evaluated. Sathisha *et al*., (2011) determined antioxidant potentials in silver nitrate extract of some plants, *Curcuma longa, Coffea Arabica*, *Tribulus terrestris*, *Bacopa monnieri* and *Trigonella foenumgraceum* using various *in vitro* assays.

Iwalewa *et al*., (2005) studied the pro and antioxidant effects of silver nitrate extracts of nine edible vegetables in southwest Nigeria using 1, 1-diphenyl-2-picrylhydrazyl free radical assay. The silver nitrate extract of *Helichrysum plicatum* had been reported to have antioxidant activity using two *in vitro* methods, namely DPPH and -carotene linoleic acid assays . (Tepe *et al*.,(2005)

The silver nitrate extracts of *Chlorophytum borivilianum* had been shown to scavenge DPPH radical and decrease TBRAS (Thiobarbituric Acid Reactive Substances), revealing that it is a promising anti-stress agent as well as a potential antioxidant. (Kenjale *et al*., 2007).

Antibacterial Activity

Musa *et al*., (2000) studied the phytochemistry of powdered leaves of *Acalypha recemosa* (Euphorbiaceae). This study revealed the presence of alkaloid, tannin, flavanoid and terpenes. Antimicrobial activities of cold water, hot water and methanolic extracts were studies against *Staphylococcus aureus* was more than *Escherichia coli* but *Candida albicans* was completely resistant to the extracts. The cold water extracts showed activity with MIC range from 3.0 mg/ml (against *S. aureus*) to 4.0 mg/ml *Escherichia coli* for cold water and

7.0 mg/ml for the two isolates (methanolic extract). The MBC of cold water extract (6.0 mg/ml) was able cause 2 log cycle reduction of cell population in 90 minutes. Prema (2004) studied the antibacterial activity in eleven medicinal plants. The acetone extract of *Acalypha indica* was more effective against *Staphylococcus aureus*. Ethanol extract of *A. indica* and *Eucalyptus globulus* were highly sensitive to *S. aureus* and *P. Aeruginosa*.

Poonkothai *et al*., (2005) worked on antibacterial activity of chloroform, ethanol and aqueous extracts of the leaves of *Gymnema sylvestre* on *Bacillus subtilis, Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa, Escherichia coli* and *Salmonella typhi* on Muller Hindon agar plates. Commercially available chloramphenicol disc (30 mg) was used as control and discs impregnated with DMSO were also used in this technique. *Klebsiella pneumoniae* was resistant to both chloroform and ethanol extracts exhibiting a zone of inhibition of 12 and 11 mm respectively. *Pseudomonas aeruginosa* (16 and 21mm) and *Salmonella typhi* (17 and 19mm) were found to be sensitive to both the extracts. This indicates that gymnemic acid, an active component of *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 17mm. *N. laevis* varies between 10 and 23 mm.

Dhale and Birari (2010) studied the antimicrobial effect of *Jatropha gossypifolia* leaf extracts on gram positive species *Staphylococcus spp.* and *Bacillus spp*. and gram negative species like *Escherichia spp*. and *Pseudomonas spp.,* in solvents like petroleum

ether, alcohol and chloroform. The method employed was disc diffusion method, standard was Amphicillin, the alcoholic extract of leaves showed maximum antibacterial activity.

Dipankar Choudhury *et al*.,(2011) studied phytochemical screening and antimicrobial activity of extracts from leaves and stem of *Ecbolium linnean* .The bacterial pathogens were strongly inhibited by leaf extracts but acetone extracts of stem have failed to inhibit the growth of *Staphylococcus aureus* and *Pseudomonas aeruginosa* even at the highest concentration. The results revealed that leaf extracts were found to be more effective than stem extracts. *E. linneanum* possesses antimicrobial activity against most commonly encountered human pathogens.

Yusha'u, *et al*., (2011) studied antibacterial activites of ethanolic extracts of *Annona squamosal (L.)* leaves were studied against clinical respiratory tract isolates of *Klebsiella pnemoniae, Proteus species, Pseudomonas species, Staphylococcus aureus, Streptococcus pnemoniae*and α- haemolytic *Streptococci* using disc diffusion and micro broth dilution techniques. Sensitively test results showed that water fraction of the plant was active on *Stephylococcus aures and Streptococcus pnemoniae* (10mm) at 50µg/disc concentration while ethanolic extract of the plant was active , *Streptococcus pnemoniae* and *Proteus species* at 200µg/disc concentration with zone diameter formed by *Klebsiella pnemoniae* (11mm) being wider than that formed in response to standard Augmentin disc (06mm).

Nidhi uttam kumar and sumit kumar (2013) evaluated antibacterial activity of rhizome of *Barleria prionitis* . The methanol extract showed antibacterial activity against two Gram's positive *(S. aureus and B. cereus*) and two Gram's negative (*E. coli and S. typhi*) bacteria. The antibacterial potential was measured by agar disc plate method. The active phytocomponents of *Barleria prionitis* were revealed using Gas chromatography with

mass spectrophotometric detector and 27 constituents identified, Phthalazine was the most abundant phytocompound in methanol extract. All the results supported that the extract can be used to prevention of bacterial infection and may have role in pharmaceutical medicine evolution.

Nayan R.Bhalodia and V.J.Shukla (2014) reported extracts obtained from *Cassia fistula* show strong activity against most of the tested bacterial and fungal strains. The results were compared with standard antibiotic drugs. The results show that the activity of hydroalcohol extracts of *Cassia fistula* shows significant antibacterial and antifungal activities.

Nivedita patel *et al*.,(2014) reported phytochemical analysis and antibacterial activity of *Moringa oleifera*. The result showed that the plant leaves are very good nutrient supplement for malnutrition and also used as an antibiotic. To evaluate the antibacterial activity of *Moringa oleifera* leaf extracts, *Escherichia coli, Pseudomonas aeroginosa, Staphylococcus aureus, Proteus vulgaris, Streptococcus mutans, Bacillus subtilus,* and *Staphylococcus epidermidis bacteria* were used. Phytochemical analysis of the leaf in solvents of varying polarity; viz., aqueous, ethanol were also carried out. The phytochemical screening indicated the presence of flavonoids, tannins, steroid, alkaloid, saponins etc., in the both extracts. Well diffusion method was used to assess the antibacterial effect of the extracts on micro-organisms. The ethanolic and aqueous extract were active against all strains but the ethanol leaf extract showed maximum activity against *Streptococcus mutant* and aqueous extract shows maximum activity against *Proteus vulgaris*.

Hassan Waseem *et al*., *(*2016) detected the antimicrobial activity of *C. tamala* against a number of organisms. The Plant extract from *C.tamala* was found to have antimicrobial activity against only one tested bacterium, *S.aureus* (ATCC 25293). They found different degrees of antimicrobial activity against all tested gram positive and gram negative bacteria contrary to our result where only *S.aureus* was found to be effective.

Anthelmitic activity

Anthelmintic effects of various extracts of the whole plant of *Enicostemma littorale*, resulted helminthiasis of the worm in following order, ethanol > ethyl acetate > chloroform > hexane. In particular the ethanol extract exhibited an increased paralytic as well as anthelmintic effect over albendazole. This may be due to the increased level of extraction of tannins in ethanol followed by ethyacetate > water > chloroform > hexane extracts (Vidyadhaer *et al*., 2010).

Seema and Amrish (2012) studied anthelmintic activity of colloidal solution of silver nanoparticles using *Saraca indica* leaves extract. Anthelmintic activity of colloidal solution of silver nanoparticles was more than the aqueous extract. Overall the anthelmintic activity revealed the concentration-dependent nature of the aqueous extracts and colloidal solution of silver nanoparticles. Different concentration of aqueous extracts and colloidal solution of silver nanoparticles were tested against adult Indian earthworms (*Pheretima posthuma*) as test worms. The bioassay involved determination of the time of paralysis and time of death control. Piperazine citrate 23 (10 mg/ml) was used as a standard reference drug. Normal saline was used as a control.

Anthelmintic activity of various concentrations (25-500mg/ml) of hot and cold hydroalcohoic extracts of *Gymnema sylvestre* were evaluated that involving determination of time of paralysis (p) and time of death (D) of the worms. Albendazole was used as standard anthelmintic drug and distilled water was used as control. The hydro alcoholic extracts significantly exhibited the paralysis in worm and also caused death of worms in dose dependent manner, among which hot maceration extract showing more significant results when compared with the cold maceration extract (Reddy *et al*., 2013).

Anthelmintic activity of the alcoholic and aqueous bark extracts of the *Holarrhena antidysentrica* was assessed by Satpute *et al*.,(2014). The effect was dose dependent and

shortest time taken for paralysis and observed in case of alcohol extract at 40mg/ml concentration with potent activity against Indian adult earthworms (*Pheretima poshuma*).

Dora Babu *et al*.,(2018) evaluates anthelmintic activity of methanol extract of *Buchanania axillaris* Desr on Indian adult earthworms, *Pheretima posthuma* (annelid).Bark was extracted by using soxhlet apparatus. Phytochemical screening of crude extracts showed the presence of steroids, alkaloids, tannins, flavonoids, carbohydrates and glycosides. Various concentrations (25, 50, 100mg/ml) of crude extracts were tested for Anthelmintic activity.The activity was compared with standard piperazine citrate. The methanolic extract shows significant activity when compared to the standard piperazine citrate. The paralysis and death time is 50, 31, 17 and 76, 52, 34 minutes respectively at concentrations 25, 50 and 100mg/ml. whereas these are 31, 18, 10 and 63, 41, 22 minutes for piperazine citrate.

Narasimha Rao, Y *et al* ., (2018) investigated anthelmintics activity of ethanolic extract of *Potrulaca quadrifida* whole plant using earthworms (*Pheretima posthuma*), various concentrations (50 and 100 mg/ml) of plant extract were tested .Piperzine citrate (10 mg/ml) was used as reference standard drug whereas distilled water as control. Determination of paralysis time and death time of the worms were recorded. Extract exhibited significant anthelmintics activity at the concentration of 100 mg/ml. The result shows that aqueous extract possesses vermicidal activity and found to be effective as anthelmintics.

Somnath De *et al*.,(2019) studied anthelmintic activity of methanol and aqueous extract of *Calotropis gigantea*. Both are showed different paralysis and death time at similar concentrations. Albendazole was used as reference standard drug. As expected control (0.5% CMC) does not show any positive results. But standard drug (albendazole 20mg/ml), methanol and aqueous plant extract (50mg/ml and 100mg/ml) showed significant results of paralysis and death time of each worms.

Ruby Philip *et al*., (2019) was investigated for anthelmintic potential from extracts of *Jasminum sessiliflorum* using earthworms, *Pheretima posthuma*. Different concentration of plant extracts were used for the evaluation. Albendazole (10 mg/ml) was used as reference standard drug. The method employs the determination of paralysis time and death time of the worms and these results were recorded. Extracts showed significant activity. The ethanolic extract was found to be most efficient.

MATERIALS AND METHODS

Collection and identification of plant materials

The fresh plant materials of *Cassia alata* L. and *Cassia glauca* Lam. are collected from Thalamuthu nagar, Thoothukudi. The collected samples were cut into small fragments and shade dried until the fracture is uniform and smooth. The dried plant material was granulated or powdered by using a blender, and sieved to get uniform particles by using sieve No. 60. The final uniform powder was used for extraction of active constituents of the plant materials.

Qualitative analysis:

WATER SOLUBLE EXTRACTIVE

Two gram of the shade dried powder of *Cassia alata* leaf, flower and and *Cassia glauca* leaf and flower was macerated with 50 ml water in a closed flask for 24 hours. Shaking frequently during first 6 hours and allowed to stand for 18 hours. It was filtered using a muslin cloth and used for phytochemical analysis.

METHANOL SOLUBLE EXTRACTIVE

Two gram of the shade dried powder of *Cassia alata* leaf, flower and and *Cassia glauca* leaf, flower was macerated with 50 ml methanol in a closed flask for 24 hours. Shaking frequently during first 6 hours and allowed to stand for 18 hours. It was filtered using a muslin cloth and used for phytochemical analysis.

ACETONE SOLUBLE EXTRACTIVE

Two gram of the shade dried powder of *Cassia alata* leaf, flower and *Cassia glauca* leaf, flower was macerated with 50 ml acetone in a closed flask for 24 hours. Shaking frequently

during first 6 hours and allowed to stand for 18 hours. It was filtered using a muslin cloth and used for phytochemical analysis.

ETHANOL SOLUBLE EXTRACTIVE

Two gram of the shade dried powder of *Cassia alata* leaf, flower and *Cassia glauca* leaf, flower was macerated with 50 ml ethanol in a closed flask for 24 hours. Shaking frequently during first 6 hours and allowed to stand for 18 hours. It was filtered using a muslin cloth and used for phytochemical analysis.

PETROLEUM ETHER SOLUBLE EXTRACTIVE

Two gram of the shade dried powder of *Cassia alata* leaf, flower and *Cassia glauca* leaf, flower was macerated with 50 ml petroleum ether in a closed flask for 24 hours. Shaking frequently during first 6 hours and allowed to stand for 18 hours. It was filtered using a muslin cloth and used for phytochemical analysis.

Test for tannins (Ciulei I.)

To 1 ml of the extract, 2 ml of 5% $FeCl_3$ was added. A dark blue or green -black indicates the presence of tannins.

Test for saponins (Harbrone jb)

Foam test

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

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

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

Test for Coumarins (Harbrone JB)

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

Test for Terpenoids (Harbrone JB)

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

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

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

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

Wagner's test

A fraction of extract was treated with Wagner's reagent (1.27 g of iodine and 2 g ofpotassium iodide in 100 mL water) and observed for the formation of reddish brown colour precipitate. There was a formation of reddish brown colour confirming the presence of alkaloid.

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

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

Test for Carbohydrate (Harbrone JB)

Fehling's test

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

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

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

Test for Protein(Harbrone JB)

Ninhydrin test:

0.5 mg of extract was taken and 2 drops of freshly prepared 0.2% ninhydrin reagent was added and heated. The appearance of pink or purple colour indicates the presence of proteins, peptides or amino acids.

Test for phenol(Harbrone JB)

To 1 ml of the extract, 2 ml of distilled water was added and followed by few drops of 10% aqueous ferric chloride. Appearance of blue or green colour indicates the presence of phenols.

Quantitative analysis of antioxidant

Total phenolic content:(Duan *et al*.,2006)

Reagents

- 50%Folin – ciocalteau reagent
- 20%sodium – carbonate
- Gallic acid – standard

Procedure

100mg of samples was homogenate with 10 ml of distilled water and filtered through a muslin cloth. 1ml of the filtrate was added to 1.5 ml of deionized water and 0.5 ml of 50% folinciocalteau reagent and the contents were mixed thoroughly. After 1min, 1 ml of 20% sodium carbonate solution was added mixed the control contained all the reagents except the sample. After 30 minutes of incubation at 37^{o}c, the absorbance was measured at 750nm. Total phenolics were calculated as Gallic acid equivalent (GAE) per gram fresh weight.

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

Reagents

- 5% sodium nitrate (NaNo2)
- 10% Aluminium chloride (Alcl3,H2O)
- 1N sodium hydroxide (NaoH)
- Quercetin standard

Procedure

100mg of plant material was homogenized with 10ml of distilled water and filtered through a muslin cloth. 0.5 ml of the extract was added with 2.5 ml distilled water and mixed. After 6 minutes 0.15 ml NaNO, was added and again after 6min 0.3 ml of 10% $Alcl^{3}$ was added. After 5 minutes 1ml of 1M NaNH and 0.5 ml of water were added. Following through mixing of the solution the absorbance against blank were recorded at 510nm. Quercetin was used as standard and the results were expressed as my quercetin equivalents (QE) 1g fresh weight.

Vitamin C [Ascorbic acid] (Baker and Frank, 1968)

Reagents

- 5% of TCA
- Indophenol reagent
- 20mg of dichlorophenol indophenols was dissolved in 10ml of warm distilled water
- DT reagent 2g of 2, 4 dinitraphenyl hydrazine and 1g of thiourea were dissolved.
- 85% sulphuric acid
- L-ascorbic acid - standard

Procedure

100 mg of plant material was homogenized with 10ml of 5% Trichloro acetic acid (TCA). The homogenate was centrifuged. To 2 ml of indophenols reagent and 0.5ml of DT reagent was added and incubated at 10c for 1hour and then cooled in ice bath and 2.5 ml of 85% sulphuric acid was added and shaken well for 30 minutes (until) red colour appeared. The absorbance was measured at 540nm. 1-ascorbic acid was used as standard and the results were expressed as mg/1g/FW.

Estimation of Tannin (Julkunen-Titto, 1985)

Procedure

100 mg of sample homogenized with 10 ml of distilled water and filtrated through a muslin cloth. 1ml of aliquot of aqueous extract was mixed with 1.5ml of 4% vanillin (prepared with methanol) and 750 µl of concentrated HCL was added the solution was

shaken vigorously and left to stand at room temperature for 20 minutes in darkness the absorbance against blank was read at 500nm using UV-Visible spectrophotometer. Results were expressed as mg catechin equivalent (CE) 1g tissue.

Vitamin E (Tocopherol): Rosenberg, 1992

Procedure

The plant sample (2.5g) was homogenized in 50ml of 0.1 N sulphuric acid and allowed to stand overnight the content in the flask was shaken vigorously and filtered through what man No.1 filter paper. Aliquots of the filtrate were used for estimation.

In stoppered centrifuge tubes 3ml of extract and 3ml of water were pipette out separately. To both the tubes, 3ml of ethanol and 3ml of xylene were added, mixed well and centrifuged. Xylene (2.0ml) layer was transferred into another stoppered tube. To each tube, 2.0 ml of dipyridyl reagent was added and mixed well, the mixture (3ml) was pipette out into a cuvette and the extinction was read at 460nm. Ferric chloride solution (0.66 ml) was added to all the tubes and mixed well. The red colour developed was read exactly after 15min at 520nm. Tocopherol was used as standard.

FT-IR analysis

A little powder of plant specimen was mixed with 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).

GC-MS Analysis:

Extract Preparation

The 50g tuber powder of *Cassia alata* was serially extracted with 250 ml of Methanol with the help of Soxhlet apparatus. The extraction procedures were continued for 3-4 hours at 60°C -80°C[15]. These extracts were concentration under reduced pressure evaporator and stored in air tight vials at 4°C for further study.

Phytochemical analysis by GC-MS

Gas chromatography-Mass spectrometry (GC-MS) analysis of the methanolic extracts was performed by using a GC-MS (Model; QP 2010 series, Shimadzu, Tokyo, Japan) equipped with a VF-5ms fused silica capillary column of 30 m length, 0.25 mm dia. and 0.25µm film thickness. For GC-MS detection, an electron ionization system with ionization energy of 70 eV was used. Helium gas (99.99%) was used as a carrier gas at a constant flow rate of 1.51 ml/min. Injector and mass transfer line temperature was set at 200 and 240°C respectively. The oven temperature was programmed from 70 to 220°C at 10°C/min, held isothermal for l min and finally raised to 300°C at 10°C/min. 2 µl of respective diluted samples was manually injected in the split less mode, with split ratio of 1:40 and with mass 18 scan of 50-600 amu. Total running time of GC-MS is 35min. The relative percentage of the each extract constituents was expressed as percentage with peak area normalization.

Identification of phytochemical components

The identity of the components in the extracts was assigned by the comparison of their retention indices and mass spectra fragmentation patterns with those stored on the computer library and also with published literatures. NIST08s.LIB and WILEY8. LIB library sources were used for matching the identified components from the plant material.

ANTIOXIDENT ACTIVITY

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

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

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

DPPH scavenging activity (%)

A control –A test / A control * 100

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

Antibacterial studies

Extraction of plant materials

The plant powder was extracted with methanol, acetone and water.25 gms of plant powder was extracted with methanol, acetone and water solution individually in soxhelt apparatus continuously for about 4-6 hours, which was again concentrated till it

become semisolid. It was evaporated to dryness and stored at 0 C, until the time of the experiment.

Bacterial strains used

The test organisms were obtained from the Department of Microbiology;St. Mary's College (Autonomous), Thoothukudi. The one gram positive bacteria viz; *Bacillus subtilis* G-ve MTCC 1133 and four gram negative bacteria *Escherichia coli*, G-ve, MTCC 50, *Salmonella typhi* G-ve, 1357. *Proteus vulgaris* and *Klebsiella pneumonia* G-ve, MTCC 3384 were used in the present study.

Broth Medium:

- Nutrient broth Himedia MOO1
- Nutrient broth 1.3 gm
- Distilled water 100 ml

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

Agar medium:

- Nutrient broth Himedia MOO1
- Nutrient broth 1.3 gm
- Distilled water 100 ml

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

Disc diffusion method

Anti- bacterial activity was evaluated by agar disc diffusion method (Kirby – Bauer *et al*., 1986) . Test solution were prepared with known weight of methanol, acetone and water extracts dissolved in 5% dimethyl sulphoxide (DMSO). What man No.1 filter paper disc (5mm) were impregnated with 20 0f these extracts and allowed to dry at room temperature. The spread plates were prepared by proper concentration of inoculate. Each sample loaded discs was placed in the seeded agar plate. 24-48 hours of + 37^0c incubation, the diameter of the inhibition zone was for positive control, streptomycin discs (100g/ml) was used, whereas for negative control; respective solvents loaded on the sterile discs.

Anthelmintic activity (Kumar *et al*., 2010)

Preparation of extract

Powered dry materials (50gm) were extracted with 100 ml of water and methanol for 24 hours. The extracts were filtered through filter paper (whatman No.1). The filtrate was collected and concentration till a syrupy mass was obtained and dried at room temperature. The dried extracts were dissolved in normal saline and used for anthelmintic activity.

Experimental animal

Anthelmintic activity was performed on adult earthworms *Phertima postuma* due to its anatomical and physiological resemblance with the intestinal parasite of human beings. The Indian adult earthworms were collected from moist soil of field and washed with normal water and saline solution to remove soil and faecal matter. Earthworms were identified from Department of Zoology, St .Mary's College (Autonomous), Thoothukudi. The earthworms of 4-8 cm in length and 0.22 -0.3 cm width were used for all experimental parameters.

Experimental design

In the present investigation of earthworms were divided in to the following 7 groups. Each group consists of 6 earthworms.

Group 1 Earthworms were placed in normal saline and served as control.

Group 2 Earthworms were placed in aqueous extracts of *Cassia alata* and *Cassia glauca* (leaf and flower) at the dose of 1.25 mg/ml.

Group 3 Earthworms were placed in aqueous extracts of *Cassia alata* and *Cassia glauca* (leaf and flower) at the dose of 2.50 mg/ml.

Group 4 Earthworms were placed in methanol extracts of *Cassia alata* and *Cassia glauca* (leaf and flower) at the dose of 1.25 mg/ml.

Group 5 Earthworms were placed in methanol extracts of *Cassia alata* and *Cassia glauca* (leaf and flower) at the dose of 2.50 mg/ml.

Group 6 Earthworms were placed in standard drug albendazole of *Cassia alata* and *Cassia glauca* (leaf and flower) at the dose of 1.25 mg/ml served as standard.

Group 7 Earthworms were placed in standard drug albendazole of *Cassia alata* and *Cassia glauca* (leaf and flower) at the dose of 2.50 mg/ml served as standard.

RESULT AND DISCUSSION

Plant have been major sources of bioactive principle employed in drug formulations both modern and traditional medicine. According to World Health Organization 80% of the people living in rural areas depend on medicinal herbs as primary health care system. (Sakarkar and Deshmukh , 2011).

Cassia alata and *Cassia glauca* are the important medicinal plants in Caesalpiniaceae. 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 leaf and flower of *Cassia alata* and *Cassia glauca* showed different results. The alkaloids, phenols, tannin, saponins, glycosides, quinones, flavonoids, terpenoids and coumarins were predominantly present in different parts of different solvent extracts. Table: (1&2).

Johnson *et al*.,(2012) reported the methnolic extracts of some medicinal plants contain tannin, saponin, flavonoid, phenol, betacyanin and coumarin. Sukumaran *et al*.,(2011) reported the presence of alkaloids, flavonoids, tannins, saponins, phenol and terpenoids in *Peltrophorum pterocarpum* flowers.

QUANTITATIVE ANALYSIS

The total phenol, flavonoid, tannin, vitamin C and vitamin E were analysed in leaf and flower extract of *Cassia alata* and *Cassia glauca* belonging to the family Caesalpiniaceae. Table (5-9)

TOTAL PHENOL

Phenolics are the most wide spread secondary metabolites and are believed to be responsible for antioxidant activity. The total phenol contents of leaf (2.088 mg GAE/g) were higher than that flower (1.753 mg GAE/g) in *C. alata*. (Table -5) Similarly , the total phenol contents of leaf (2.057 mg GAE/g) were higher than that of flower (1.824mg GAE/g) in *C. glauca* (Fig:1) . Phenolic compounds are as class of antioxidant agents act as free Terminators (Shahidi and Wanasundara, 1992). Phenolic compounds have a variety of beneficial activities. They have potential antioxidants and free radical scavenger. (Meenakshi et al., 2012). The antimicrobials (most of the phenolics) may provide a microbe-free environment with in the body.

TOTAL FLAVANOID

Flavonoids are secondary metabolites and has responsible for antioxidant activity in medicinal field. The total flavonoids contents of leaf (7.763 mg QE/g) were higher than that flower (5.2703 mg QE/g) in *C. alata* . (Table -6) Similarly , the total flavonoids contents of leaf (6.4793 mg QE/g) were higher than that of flower (5.193 mg QE/g) in *C. glauca* (Fig -2). Flavonoids are potent antioxidants and epidemic Studies indicate that high flavonoids in take is correlated with decreased risk of lifestyle diseases like diabetes and cardiovascular diseases (Kaur *et al*.,2008). Flavonoids are potent water-soluble antioxidants and free radical scavengers, which prevent oxidative cell damage and have strong anti- cancer activity (Havsteen, 2002).

TOTAL VITAMIN -C

Table -7 shows *C. alata* leaf (14.85 mg/g) and flower (8.05 mg/g) contain significant amount of vitamin C. Total vitamin C content in *C. glauca* leaf (9.772mg/g) and flower (6.57mg/g)(Figure-3).Vitamin C is a vital component in human diet with the

highest concentrations in animal organs. Vitamin-C is a non-enzymatic, water soluble antioxidant (Ueta *et al*., 2003).Vitamin C functions in enzyme activation, oxidative stress reduction, and immune function. It is protects against respiratory tract infection and reduces risk for cardiovascular disease and some cancer.

TOTAL TANNINS

(Table -8) *C. alata* leaf (4.65mg CE/g) contain significant amount of tannin, while the flower of *C. alata* contain the lowest amount of tannin (2.564mg CE/g) (Table -8). Tannin content in *C. glauca* of leaf (1.763mg CE/g) were higher than that of flower (0.733mg CE/g)(Figure-4). Tannins are present primarily in the leaves of trees growing in stress conditions. They are accumulated in the vacuoles, especially those of the epidermal layer and the palised mesophyll. Tannins are useful in treating inflammation, ulcers, and remarkable activity in cancer prevention and anticancer activities (Li *et al*., 2003; Akinpelu *et al*., 2009).

TOTAL VITAMIN –E

Total vitamin E content in *C. alata* leaf (6.662mg/g) highest and *C. alata* flower(4.653 mg/g) lowest(Table-9). Total vitamin E content in *C. glauca* of leaf (7.825 mg/g) were higher than that of *C. glauca* flower (4.982 mg/g)(Figure-5). Vitamin E is a fat-soluble nutrient found in many foods (Jacob, 1995). In the body, it acts as an antioxidant, helping to protect cells from the damage caused by free radicals. Free radicals are compounds formed when our bodies convert the food we eat into energy (Havsteen, 1983).

FTIR

Fourier Transform Infrared spectroscopy was used to analyse the fnctional group present in the leaf and flower of the *Cassia alata* and *Cassia glauca.*

The FTIR spectroscopy analysis of *Cassia alata* leaf obtained peaks at 3858.36 cm^{-1}, 2918.48 cm^{-1}, 1717.09 cm^{-1},1515.11 cm^{-1}, 1450.30 cm^{-1},1241.09 cm^{-1}. These absorption peaks are known to be associated with the stretching vibration for N-H in Aromatic amines, C-H in Alkane, C=O in Carbonyl, CH_3 in ketone, C=N in Guanidine,C – O in Ester groups. Fig: 6, Table: (10).

The FTIR spectroscopy analysis of *Cassia alata* flower obtained peaks a 3847.09cm^{-1}, 2618.98 cm^{-1}, 1638.42 cm^{-1},1409.30 cm^{-1}, 1071.48 cm^{-1}. These absorption peaks are known to be associated with the stretching vibration for N-H in Aromatic amines, C-H in Alkane, C=C in Aromatic,C=N in Guanidine, C –O ibn Ether groups. Fig : 7, Table: (11).

The FTIR spectroscopy analysis of *Cassia glauca* leaf obtained peaks at 3809.09 cm^{-1}, 2931.33 cm^{-1}, 1725.56 cm^{-1},1411.57 cm^{-1}, 1386.74 cm^{-1}, 1073.02. cm^{-1}. These absorption peaks are known to be associated with the stretching vibration for N-H in Aromatic amines, C-H in Alkane, N=O in Nitro group,C=O in Carbonyl,C=C in Aromatic, C–O in Ether groups. Fig : 8, Table: (12).

The FTIR spectroscopy analysis of *Cassia glauca* flower obtained peaks at 3847.65 cm^{-1}, 2941.43 cm^{-1}, 1727.46 cm^{-1},1453.65 cm^{-1}, 1158.09 cm^{-1}, 1109.12 cm^{-1}. These absorption peaks are known to be associated with the stretching vibration for N-H in Aromatic amines, C-H in Alkane, N=O in Nitro group,C=N in Guanidine,S=O in Sulphinic acid, C–O in Ester groups. Fig : 9, Table: (13).

From the spectral data presence of C=O, C-H, N-H, S=O,N=O, C-O, C=C were identified. These bonding are responsible for the presence of alkyl group, aldehyde group, nitro group, sulphinic acid, alcohol, ether, carboxylic group, aliphatic group and iodo group. Carboxylic acid present in the medicinal plant serves as main pharmaceutical product in curing ulcer,

jaundice, head ache, stomatitis, hemicranias, fever, pain in lever, treatment of edema and rheumatic joint pain. Amides, amine and amino acid are the main groups which are involved in protein synthesis. The study revealed that the whole plant of *Cassia alata* and *Cassia glauca* contain a considerable amount of secondary metabolites and it may considered in future to be used human disease management.

GC-MS Analysis

The GC-MS analysis of methanolic leaf extract of *Cassia alata* was confirmed the presence of 22 compounds with retention time. Interpretation of mass spectrum of GC-MS was conducted using the database of NIST and WILEY libraries. Out of this 22 compounds 7 compounds were majority present in the leaf extract of *Cassia alata* respectively 9-Octadecenoicacid,(E)-(23.04%),Octadecenoicacid(Z)-,2,3-dihydroxypropylester(11.54%) ,15Hydroxypentadecanoicacid(2.18%),Oxacyclododecan2one(7.33%),OleicAcid(7.33%),Hexadecanoicacid,2-hydroxy1-(hydroxyl methyl) ethyl ester (2.18%), and cis-13-Octadecenoic acid (2.02%).

The fifteen minor compounds such as cis-9-Hexadecenal (1.90%), cis-Vaccenic acid (1.86%),Oleoylchloride(1.86%),6Octadecenoicacid-Z(1.86%), Silane, dimethyl (3phenylpro2enyloxy)silylox) (3phenylpro2enyloxy) (1.71%), 4(3-4 Dihydroxy2-oxobuty lamino)benzonitrile(1.38%),Cinnamylcinnamate(1.38%),5,5-Dimethyl-1,3-dioxane-2etha nol,tertbutyldimethyl-silylether(1.38%),Quinoline,1,2,3,4tetrahydro1 (2phenylcycloproyl) sulfonyltrans (1.04), Phenyltrans2phenyl-1cyclopropane sulfonate (1.04%), Heptadecanoi cacid(0.93%), n-Hexadecanoic acid(0.46%) and Hexadecanoic acid, methyl ester (0.35%) were also reported from the methanolic leaf extract of *C. alata*. The chemical constituent's analysis results of *C. alata* leaf were reported in Table- 15 and their GC-MS chromatogram is presented in Fig:10 , Table-14&15.

The first compound identified with less retention (11.315 min.) was Hexadecanoic acid, methyl ester whereas 6-Octadecenoic acid and Oleoyl chloride was the last compound which took longest retention time (18.265 min.) to identify. At (13.38 min) retention time Octadecenoicacid,(E)-compound was found to be high (23.04%) and the lowest percentage (0.35%) was found to be Hexadecanoic acid, methyl ester. The above mentioned isolated compounds from the methanolic extract of *C. alata* leaf have a medicinal important. Hexadecanoic acid or Palmitic acid is a type of saturated fatty acid that is found in leaf methanolic extract of *Cassia alata* is a main antimicrobial compound (Ibibia *et al*., 2016) . n-Hexadecanoic acid was confirmed to be present in other plants exhibiting anti-inflammatory (Sermakkani and Thangapandian, 2012) antioxidant, nematicide, pesticide, larvicidal effects (Thomas *et al*., 2013).

Oleic acid identified in the leaf methanolic extracts of *Cassia alata* is a main antioxidants compound that help remove harmful toxins and free radicals in the body. It helps to prevent premature ageing in cells and increases immune system functioning. (http: // www. natural wellbeing. com/ learning-center/Oleic Acid). The presence of the identified bioactive components present in *Cassia alata* leaf extract could be responsible for the antioxidant and antimicrobial effects of the plants leaves. Identification of these compounds in the plant serves as the basis in determining the possible health benefits of the plant leading to further biological and pharmacological studies.

ANTIOXIDANT ACTIVITY

An antioxidant is a molecule capable of showing or preventing the oxidation of other molecules. In a biological system, they protect cells from the damage caused by unstable molecules known as free radicals. Antioxidants terminate the chain reactions by removing free radical intermediates, and inhabit other oxidation reactions by being oxidized

themselves. They are believed to play a role in preventing the development of chronic diseases like cancer, heart disease, storke, AD, RA and cataracts (chakraborty *et al.,* 2010).

Antioxidant chemicals found in nature inhibit or prevent oxidation of substrate leading to the formation of reactive oxygen species and reactive nitrogen species and thus protect the biological system (Hwang *et al.,*2007).Fruits and vegetables are endowed with antioxidants and consumption of these, prevent and protect from oxidative stress related diseases, inflammatory diseases viz., arthritis, autoimmune disease, carcinogenesis, neurodegenerative diseases, inflammatory diseases, cardiovascular disorders etc. Several food industries use butyated hydrooxyanol, butylated hydroxyl toluene and tertiary butyl hydroquinone, the common synthetic antioxidants for preventing lipid oxidation in food products while processing and storage. These synthetic antioxidants have been suspected to be carcinogenic and hence their use as food ingredients has been prohibited (hung and Wang 2004). Natural antioxidants comprised non-detrimental chemical combinations are considered to be rather safer for use in food products. Further, uncared wastes are if exploited as resource of antioxidants, will be more beneficial to human kind and protecting the environment. Flavonoids are water soluble polyphenolic molecules with antioxidant activity which has many beneficial effects on the cardiovascular system (Evans, 1989). Vitamin C acts as ROS scavenger, thus potentially protecting cells from harmful oxidative products (Fossati *et al.,*). Vitamin E supplement elevates the activities of antioxidant enzymes (Kiron *et al.,* 2004).

DPPH FREE RADICAL SCAVENGING ACTIVITY

The DPPH is a stable free radical and is widely used to assess the radical scavenging activity of a specific compound or plant extracts (Wei et al., 2012). DPPH solution hows a strong absorption band at 517nm appearing as a deep violet colour. The absorption vanishes and the resulting decolourization is stoichiometric with respect to

degree of reduction. The leaf and flower extracts of *Cassia alata* and *Cassia glauca* was able to reduce stable DPPH radical to yellow colour diphenyl picrylhydrazine. The degree of reduction in absorbance is the reflection of radical scavenging power of the compound.

The antioxidant activity of aqueous extract using leaf and flower of *Cassia alata* and *Cassia glauca* plants was evaluated by using DPPH scavenging assay Fig(11). Aqueous extract using *Cassia alata* leaf has higher scavenging activity (82.06%) followed by flower (73.56), as shown Figure (11) and Table(16). In aqueous extracs of *Cassia glauca* leaf has higher scavenging activities (77.14) and followed by flower (61.86)as shown in Figure(11) and Table(16).

This result indicated aqueous extract using leaf and flower of both plants shows higher scavenging activities. It has been reported that the antioxitant activity of aqueous extract using leaf and flower of *Cassia alata* and *Cassia glauca* was due to presence of phenolics and it is responsible for redox properties, which allow them to act reducing agent, hydron donors and singlet oxygen quenchers. (Arasali and Kadimi 2009).

ANTIBACTERIAL ACTIVITY

In the present study, antibacterial activity of different solvents (acetone , methanol and water) using *Cassia alata* and *Cassia glauca* were tested against five human pathogenic bacteria (*Bacillus substilis, Escherichia coli, Ptoteus vulgaris, Salmonella typhi , Klebseiella pneumonia*) presented in Table (17&18). The diameter of the inhibition zones against these spceies ranged from (3 to 12).

The different solvents (acetone, methanol and water) extracts of leaf and flower of *Cassia alata* exhibited maximum activity against different bacterial species, *E.coli* (3-6mm)&(4- 7mm), *Bacillus substilis* (3-11mm) & (5-10mm), *Ptoteus vulgaris* (3-

10mm) & (4-8mm), *Salmonella typhi* (5-8mm) &(4-8mm) and *Klebseiella pneumonia* (3-9mm)&(3-6mm) inhibition zone. (Plate:3 and 4)

The different solvents (acetone, methanol and water) extracts of leaf and flower of *Cassia glauca* exhibited maximum activity against different bacterial species, *E.coli* (3-7mm)&(5- 7mm), *Bacillus substilis* (4-12mm) & (3-11mm), *Ptoteus vulgaris* (5-10mm) & (6-8mm), *Salmonella typhi* (3-8mm) &(5-8mm) and *Klebseiella pneumonia* (3-8mm)&(5-9mm) inhibition zone. (Plate:5 and 6).

The maximum activity was found to be 11mm zone of inhibition obtained by methanol extract of *C.alata* leaf against *Bacillus substilis*. The methanol extract of *C.alata* flower exhibited high antibacterial activity against *Bacillus substilis*, the diameter of inhibition zone was 10mm. The methanol extract of *C.alata* leaf exhibited more or less same zone of inhibition compared to standard antibiotics streptomycin. Maximum bacterial effect was found in *Bacillus substilis* for methanol extracts of leaf and flower of *Cassia alata.*

The maximum activity was found to be 12mm zone of inhibition obtained by acetone extract of *C.glauca* leaf against *Bacillus substilis*. The acetone extract of *C. glauca* flower exhibited high antibacterial activity against *Bacillus substilis*, the diameter of inhibition zone was 11mm. The methanol extract of *C.glauca* leaf exhibited more or less same zone of inhibition compared to standard antibiotics streptomycin. Maximum bacterial effect was found in *Bacillus substilis* for acetone extracts of leaf and flower of *Cassia glauca.*

The antibacterial activity of methanol and acetone leaf of both the taxa were compared with of standard antibiotics like streptomycin (10mm).The antibacterial activity of leaf extract of *C.alata* and *C. glauca* were nearly similar to streptomycin. Maximum bacterial effect were found in *Bacillus substilis* for leaf extract of both plants. The effect

were significant in *Cassia alata* and *Cassia glauca*. The antibacterial activities of *Cassia alata* and *Cassia glauca* 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 *Cassia alata* and *Cassia glauca* showed the extracellular of saponins. The antimicrobial activity of these plants may be due to the presence of saponons. The presences of tannins were also reported in *Cassia alata* and *Cassia glauca*.

The antibacterial activity of tannins may due to their intercalation with enzymes, cell envelope transport proteins and also complex with cell wall polysaccharides (*Ya et al*., 1998). Hence these plants stand as a potential candidate as a source of ingredients in drug formulation for the treatment of bacterial infection.

ANTHELMINTIC ACTIVITY

Table (19-22) and plate (7-10) showed anthelmintic activity of methanol and aqueous extract of leaf and flower of *Cassia alata* and *Cassia glauca*. It was evident that methanol, aqueous extract of *Cassia alata* and *Cassia glauca* exhibited anthelmintic activity in dose dependent manner giving shortest time of paralysis and death with 2.50 mg/ml concentration.

From the table (19), it was concluded that methanol extract of *Cassia alata* leaf exhibited highest anthelmintic activity at a concentration of 2.50 mg/ml whereas the aqueous extract showed the marked degree of anthelmintic activity at similar concentration. Methanol and aqueous extracts of leaf and flower of *Cassia alata* exhibited less anthelmintic activity at a concentration of 1.25 mg/ml. The anthelmintic effect of methanol and aqueous

extract of *Cassia alata* comparable with that of the effect produced by standard drug albendozol. Plate (7).

Table (20), illustrated anthelmintic activities of methanol and aqueous extracts of *Cassia alata* flower. Methanol extract of *Cassia alata* flower showed highest anthelmintic activity in dose dependent manner giving shortest time of paralysis and death with 2.50mg/ml concentration followed by aqueous extract showed considerable level of anthelmintic activity at same concentration in dose dependent manner. The effect of anthelmintic activity of methanol extract of *Cassia alata* flower was higher than aqueous extract of flower and standard drug albendozol. Plate (8).

Table (21), it was concluded that methanol extract of *Cassia glauca* leaf exhibited highest anthelmintic activity at a concentration of 2.50 mg/ml whereas the aqueous extract showed the marked degree of anthelmintic activity at similar concentration. Methanol and aqueous extracts of leaf and flower of *Cassia glauca* exhibited less anthelmintic activity at a concentration of 1.25 mg/ml. The anthelmintic effect of methanol and aqueous extract of *Cassia glauca* comparable with that of the effect produced by standard drug albendozol. Plate(9).

Table (22) ,it was concluded that methanol extract of *Cassia glauca* flower exhibited highest anthelmintic activity at a concentration of 2.50 mg/ml whereas the aqueous extract showed the marked degree of anthelmintic activity at similar concentration. Methanol and aqueous extracts of leaf and flower of *Cassia glauca* exhibited less anthelmintic activity at a concentration of 1.25 mg/ml. The anthelmintic effect of methanol and aqueous extract of *Cassia glauca* flower comparable with that of the effect produced by standard drug albendozol. Plate(10).

The different part *Cassia alata* and *Cassia glauca* showed anthelmintic activity in descending order of *Cassia alata* leaf > *Cassia glauca* leaf > *Cassia alata* flower >*Cassia glauca* flower. The anthelmintic activity of methanol and aqueous extracts of *Cassia alata* was higher than *Cassia glauca.*

Chemically flavonoids are polyphenolic compound and they interfere with the energy generation by uncoupling the oxidative phosphorylation which interfere with the glycoprotein of cell surface leads to parasite death.(Vaishalio *et al*., 2009). Tannins ae the secondary metabolite, occur in several plants have reported to show anthelmintic property by several investigators (Athanasidau *et al*., 2001; Waller.,1999). Tannins the polyphenolic compounds, are shown to interfere with energy generation in anthelmintic parasities by uncoupling oxidative phosphorylation or, binds to the glycoprotein on the cutiecle of parasities (Thompson and Geary, 1995) and cause death. Coming to the chemistry to nematode surface, it is a collagen rich extracellular matrix (ECM) providing cuticle that forms exoskeleton, and is critical for viability, the collagen is the class of protein that are modified by a range and copost translational modification prior for to assembly into higher order complexes (or) ECMS (Page and Winter 2003). The mammalian skin also consists of largely collogen in the form of fibrous bundles. This result in the loss of flexibility in the collogen matrix and gain of mechanical property with improved resistance to thermal or microbial or enzymatic attack. Similar kind of reaction is expected to take place between the nematode cuticle (earth worm) and the tannin of *Cassia alata* and *Cassia glauca*, possibly by linking through bonding a proposed in this study. This form of reactivity bring toughness in the skin and hence the worms become immobile and non-functional leading to paralysis followed by death. Thus, this study exposed that the efficacy of *Cassia alata* and *Cassia glauca* against human intestinal parasites and it may consider on future to be used as herbal source of anthelmintic agent.

SUMMARY AND CONCLUSION

Plants have been an important source of medicine for thousands of year. Medicinal plants are a source of great economic value. The leaf and flower of *Cassia alata* and *Cassia glauca* were collected near Thalamuthunagar, Thoothukudi,Tamil Nadu for current study. *Cassia alata* and *Cassia glauca* are known plants of family Caesalpiniaceae. The shrub *Cassia alata* leaves and flowers are used by practitioners of herbal medicines to treat burns, skin and wound infections, diarrhoeal diseases, gastrointestinal and upper respiratory tract infections. *Cassia glauca* are used for the treatment of diabetes and gonorrhea. In Ayurvedic systems of medicine, herbal extracts but not purified compounds have been used from centuries because of many constituents are considered to be beneficial. It is also used to reduce fever and sore throat pain. The macerated juices of the young fresh leaves are used to treat eye infections and parasitic diseases (Dalziel, 1937). The medicinal effects of plant are considered to be due to metabolites, especially secondary compounds, produced by plants. The phytochemical study revealed the presence of steroids, flavonoids, alkaloids, saponoins, terpenoids, phenol and tannins. The preliminary phytochemical tests are helpful in finding chemical constituents in the 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 genuinity of the drug.

In this study, total phenol, flavonoid, tannin, vitamin C and vitamin E content were quantitatively analysed in different parts of *Cassia alata* and *Cassia glauca* using spectrophotometric methods. The result of this study showed that the leaf of *Cassia alata* and *Cassia glauca* have significant amount of phenol, flavonoids, tannins, vitamin C and vitamin E and ascorbic acids compared to flower.

The FTIR spectrum of *Cassia alata* showed strong IR bands characteristics of Alkane (2918.48 cm^{-1}), Carbonyl (1717.09 cm^{-1}), Guanidine (1515.11cm^{-1}), Ester (1154.72 cm^{-1}),Amides (836.65 cm^{-1}) functional groups and in *Cassia glauca* showed strong IR bands characteristics of Aromatic (3809.09 cm^{-1}), Alkane (2931.33 cm^{-1}), Cabonyl(1725.56 cm^{-1}), Nitro group (1386.74 cm^{-1}), Amide (647.03 cm^{-1}) functional group. From the spectral data, presence of C=O, C-H, O-H, C=C, N=O,S=O were identified. These bonding are responsible for the presence of alkyl group, aldehyde group, nitro group, sulphinic acid, alcohol, ether, carboxylic group, aliphatic group and iodo group. Carboxylic acid present in the medicinal plant serves as main pharmaceutical product in curing ulcer, jaundice, head ache, stomatitis, hemicranias, fever, pain in lever, treatment of edema and rheumatic joint pain. Amides, amine and amino acid are the main groups which are involved in protein synthesis.

The GC-MS analysis of methanolic leaf extract of *Cassia alata* was confirmed the presence of 22 compounds with retention time. Out of this 22 compounds 7 compounds were majority and fifteen minor compound present in the leaf extract of *Cassia alata.* The above mentioned isolated compound from the methanolic extract of *C.alata* leaf have a medicinal important. Hexadecanoic acid or Palmitic acid is a type of saturated fatty acid that is found in leaf methanolic extract of *Cassia alata* is a main antimicrobial compound (Ibibia *et al*., 2016) . n-Hexadecanoic acid was confirmed to be present in other plants exhibiting anti-inflammatory (Sermakkani and Thangapandian, 2012).Oleic acid identified in the leaf methanolic extracts of *Cassia alata* is a main antioxidants compound that help remove harmful toxins and free radicals in the body. It helps to prevent premature ageing in cells and increases immune system functioning. The presence of the identified bioactive components present in *Cassia alata* leaf extract could be responsible for the antioxidant and antimicrobial effects of the plants leaves.

The antioxidant or free radical scavenging activity of leaf and flower extracts of these selected medicinal plants are investigated by using methods like DPPH scavenging activity. The leaf and flower extracts of *Cassia alata* and *Cassia glauca* show maximum antioxidant activity and these extracts are further subjected for antimicrobial studies.

The different solvent extracts of leaf and flower of *Cassia alata* , *Cassia glauca* and streptomycin were used for antibacterial studies against human pathogenic bacteria, *Bacillus substilis, Escherichia coli, Ptoteus vulgaris, Salmonella typhi , Klebseiella pneumonia.* These extracts showed ranging degree of antibacterial activity. The maximum activity was found to be 11mm zone of inhibition obtained by acetone extract of *Cassia alata* leaf against *Bacillus substilis.* The maximum activity was found to be 12mm zone of inhibition obtained by acetone extract of *Cassia glauca* leaf against *Bacillus substilis.* The acetone extract of *Cassia alata* and *Cassia glauca* leaves exhibited more or less same zone of inhibition compared to standard antibiotics streptomycin. The effect were significant in *Cassia alata* than *Cassia glauca*. The antibacterial activity of various phytochemicals which are known to be synthesized by plants in response to microbial infection.

Existence of various phytochemical made *Cassia alata* and *Cassia glauca* a potent anthelmintic agent. Methanol and aqueous of these plants showed dose dependent anthelmintic activity against earthworms. Methanol exhibited maximum effect giving shortest time of paralysis and death of earthworms whereas the aqueous extracts also offered satisfactory effect against earthworms. Hence, the anthelmintic activity of both the plants were comparable with standard drug albendazole.

Thus, the current study leads to conclusion that *Cassia alata* and *Cassia glauca* can replace the conventionally used synthetic drugs which produce side effects. The anthelmintic agent from *Cassia alata* and *Cassia glauca* will be cheaper and provide lesser

side effects on the host organism. Hence, further study must be carried out to isolate using in *vivo* models in order to develop safe anthelmintic drug.

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PRELIMINARY PHARMACOGNOSTICAL, ANTIOXIDANT AND ANTIMICROBIAL EVALUATION OF SELECTED MEDICINAL HERBS

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EXAMINER

DECLARATION

I do here by declare that this dissertation entitled"**Preliminary Pharmacognostical, Antioxidant And Antimicrobial Evaluation Of Selected Medicinal Herbs"**Submitted by me in partial fulfilment for the award of the degree of '**Master of Science in Botany**'in the result of my original and independent work carried out under theguidance of **Dr.Mrs. S. Beulah Jerlin M.Sc., M.Phil., Ph.D.,**Assistant Professor, Department ofBotany, St.Mary's College (Autonomous),Thoothukudiand it has not been submittedelsewhere for the award of any other degree.

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INTRODUCTION

INTRODUCTION

Traditional knowledge of medicine has long been used since ages for curing various human aliments. About 60-80% of world populations still rely on plant based medicines (Santhi,*et al.*,2011). Though the traditional Indian system of medicine has a long history of use, yet they lack adequate scientific documentation, particularly in light of modern scientific knowledge (Srivastava and Leelavathi.2010). The medicinal value of the bioactive phytochemical constituents of the plant shows various physiological effects on human body (Nilofer *et al.*, 2013).

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).

Pharmaceutical and cosmetic industries are increasingly using plant resources from rural or un polluted areas. *Plectranthus amboinicus* (Lour). Spreng, is a large succulent herb. The leaves are extensively used in traditional medicine for the

treatments of cough, sore throat and nasal congestion, but also for a range of other problems such an infection, rheumatism and flatulence. The plant is cultivated in home garden throughout India for use in traditional medicine, being used to treat malarial fever, hepatopathy; renal and vesical calculi, cough, chronic asthma, hiccough, bronchitis, helminthiasis, colic convulsion and epilepsy. (Kaliappan., *et al.*,2008).

Since antioxidants are capable of preventing oxidative damage, the wide use of natural antioxidants as a replacement of conventional synthetic antioxidants in food and food supplements has been employed, owing to the fact that natural products are considered to be a promising and safe source (Mandal *et al.*, 2011). Moreover, these natural antioxidants have easy and unlimited access to metabolic processes in the body, and produce virtually none of the side effects associated with synthetic antioxidants (Beevi *et al.*, 2010). The most commonly used antioxidants at present are Butylated Hydroxy Anisole (BHA), butylate dehydroxy toluene (BHT), propyl gallate (PG) and tera-butyl hydroquinone (TBHQ). However, they are suspected of being responsible for liver damage and acting as carcinogens in laboratory animals. Therefore, the development and utilization of more effective antioxidants of natural origin is desirable (Raja and Pugalendi 2009).

Alkaloids are major secondary metabolite produced by plants and are utilized for the defensive mechanism in the treatment of diseases. Alkaloids usually have pharmacological effects and are used in medicines or as recreational drugs. Thus they are very useful pharmaceutical agents because of their biological activities such as antimicrobial, antioxidant, analgesic potential and anti-inflammatory activities. *Eclipta alba* (Linn.) Hassk., is commonly known as Bhringraj, commonly used for the

treatment of gastrointestinal disorders, respiratory tract disorders (including asthma), fever, hair loss and greying of hair, liver disorders (including jaundice), skin disorders, spleen enlargement, and cuts and wounds (Satish *et al.*, 2013).

Pharmacological activities of plant extracts have revealed anticancer, hepatoprotective, anti-inflammatory, and antimicrobial properties. *Boerhaavia diffusa* L. (Nyctaginaceae) fresh or dried is the source of the drug punarnava which is official in Indian Pharmacopoeia as a diuretic. The plant is bitter, astringent, cooling, anthelmintic, diuretic, aphrodisiac, cardiac stimulant, diaphoretic, emetic, expectorant, anti-inflammatory, febrifuge and laxative besides being an active ingredient as a tonic. It is useful in all types of inflammation, strangury, leucorrhoea, lumbago, myalgia, cardiac disorders, jaundice, anaemia, dyspepsia, constipation, cough, bronchitis and general debility, dyspepsia, oedema, jaundice, cough, haemorrhoids, pulmonary cavitations, anaemia, enlargement of spleen, abdominal pain, abdominal tumours, cancers and acts as an anti stress agent. (Maya Verma and Ashwani Kumar 2017).

Major sources of traditional medicines are plants with large variety of bioactive constituents, which are effective against different diseases. The significant biological activities of the plants are due to these bioactive constituents. Rich sources of antibacterial and antifungal agents are medicinal plants used in many countries as sources for potent and beneficial drugs (Mahesh, 2008).

Through powder microscopy process of standardization, we can use different techniques and methodology to achieve our goal in the stepwise manner e.g. pharmacognostic and phytochemical studies. These steps and processes are helpful in identification and standardization of the plant material. Correct characterization and

quality assurance of starting material is an essential step to ensure reproducible quality of herbal medicine which will help us to justify its safety and efficacy.

Medicinal plants are an important source for the therapeutic remedies of various ailments. Phytochemical are basically divided into two groups that are primary and secondary metabolites based on the function in plant metabolism. The major constituents are consists of carbohydrates, amino acid, protein and chorophyll while secondary metabolites of alkaloids, saponins, steroids, flavonoids, tannins and so on. Nature is the source of medicinal agent for thousands years and an impressive number of modern drug have been isolated from natural sources, many of these isolated where based on the uses of the agents in the traditional medicine (Thaku Hemant kumar 2016).

The medicinal plants continue to offer valuable therapeutic value for both modern and traditional medicine in resolving several health problems (Kshirsagar *et al*, 2010). The dependence of man on plant resources is as old as the various human civilizations. People throughout the world use medicinal plants for the treatment of various human as well as animal diseases. Since time immemorial, People have been using various medicinal plants for curing varieties of ailments (Bhattachariya 1998). With the associated side effects of modern medicines are gaining importance and now are being studied extensively to unravel the scientific basis of their therapeutic actions (Gupta and and chandra, 1995). Plant based medicines are safe and effective for the treatment of many ailments. Phytochemical screening is helpful to detect the various important compounds which could be used as the base of modern drugs for curing various diseases keeping this in view the plants *Abutilon indicum* G.Don. *Achyranthus aspera* L., *Boerhaavia diffusa* L. and *Bryophyllum calcycinum* Kurz and *Centella asiatica* Urh. has been taken for this present study.

SCOPE AND OBJECTIVE

SCOPE AND OBJECTIVES

Medicinal plants have been widely used for thousand years for the treatment of many diseases. Most of the medicinal plants are allelopathic in nature has been used a popular folk and an orient medicines treats against many diseases. The aim of the study was to evaluate the pharmaceutical activity of *Abutilon indicum* G .Don. *Achyranthus aspera* L. *Boerhaavia diffusa* L. and *Bryophyllum calcycinum* Kurz and *Centella asiatica* Urh. used in traditional herbal medicine. The plants were selected on the basis of their reported ethno botanical uses.

- To perform qualitative analysis of phytochemical in various leaf extracts of *Abutilon indicum* G. Don. *Achyranthus aspera* L. *Boerhaavia diffusa* L. and *Bryophyllum calcycinum* Kurz and *Centella asiatica* Urh.
- To estimate quantitatively the percentage of elements and the various secondary metabolites present in *Abutilon indicum* G. Don. *Achyranthus aspera* L. *Boerhaavia diffusa* L. and *Bryophyllum calcycinum* Kurz and *Centella asiatica* Urh.
- To study the Pharmacognostic evaluation of *Abutilon indicum* G. Don. *Achyranthus aspera* L. *Boerhaavia diffusa* L. and *Bryophyllum calcycinum* Kurz and *Centella asiatica* Urh.
- To perform the FT-IR studies of leave powder of *Abutilon indicum* G. Don. *Achyranthus aspera* L. *Boerhaavia diffusa* L. and *Bryophyllum calcycinum* Kurz and *Centella asiatica* Urh.
- To investigate the in vitro antibacterial activity of leaf of *Abutilon indicum* G. Don. *Achyranthus aspera* L. *Boerhaavia diffusa* L. and *Bryophyllum calcycinum* Kurz and *Centella asiatica* Urh.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

A large number of medicinal plant are used as alternate medicine disease for man and animals. Since most of them are without side effects when compared to synthetic drug. Identification of the chemical nature of phytochemical compounds present in the plants will provide some information on the different functional groups responsible for their medicinal properties.

Jasmeet Kaur Abat *et al.*,2017 The World Health Organization has also recognized the benefits of drugs developed from natural products. *Abutilon indicum, Hibiscus sabdariffa, Sida acuta and Sida rhombifolia* 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.

Quality can be defined as the status of a drug that is determined by identity, purity, content and other chemical, physical or biological properties or by the manufacturing processes. For the quality control of a traditional medicine, the traditional methods are followed to get traditional information about the plants. The identity and quality assessment are interpreted in terms of modern assessment (Vikrant *et al.*,2012).

Phytochemicals are knows as secondary plant metabolites and have biological properties such as antioxidant activity antimicrobial effect, modulation of detoxification enzymes, stimulation of immune system, decrease of platelet aggregation, modulation of hormone metabolism and anticancer property. There are more than thousand known and unknown phytochemicals. It is well known that plants

produce these to protect themselves, but recent researches demonstrate that many phytochemicals can also protect human against diseatses (Rao, 2003). In addition to these substances, plants contain other chemical compounds. These can act as agents to prevent inconsiderable side effects of the main active substances or to assist in the assimilation of the main substances. Plants have an almost limitless ability to synthesize aromatic substances, mainly secondary metabolites of which 12,000 have been isolated, a number estimated to be less than 10% of the total (ahmed *et al.*, 2007).

Pandey *et al.*, 2011 observed a chemical compound, β-sitosterol, which has been identified as the active ingredient in many medicinal plants, is present in *Abutilon indicum* and a petroleum ether extract provided larvicidal properties against the mosquito larvae *Culex quinquefasciatus*. The investigation deals with the isolation and identification of D-Glucosyloxybenzoic acid, Hydroxybenzoic,

Vikrant *et al.*,2012 worked at a preliminary phytochemical analysis of *Psidium* leaves. The qualitative phytochemical analysis was done in rat tubers of six species *Dioscorea* found Meghalaya. The test confirmed the presence of various phytochemicals like flavonoids, saponins, steroids, cardiac, glycosides and terpenoids in two aqueous extract of methanol and ethyl acetate.

Oancea *et al.*, 2013 observed the phytochemical screening of the bio active compounds in the most wide spread medicinal plants from Calarasi country. The following categories were identified during the study: aminoacids, protein, polysaccharides, vitamin A and E.

Rajalaxmi Nayak *et al.*, 2013 Phytochemical fingerprinting was done with TLC studies where methanolic plant extract of *Abutilon indicum* and *Paederia*

foetida were loaded along with standard antioxidant compounds. The result of above studies was backed up by UV, FTIR and NMR studies. The screening confirmed that both the plants have secondary metabolites contributing to their antioxidant potentiality. Estimation of total flavonoid content shows *Abutilon indicum* was having higher phenolics and lower flavonoid content whereas *Paederia foetida* has almost equal phenolic with high flavonoid content.

Sharmila Banu and Ramar Ramar, 2018 studied the phytochemical screening of successive extracts showed a positive reaction for Alkaloid, Phenols, Glycoside, Saponin, Terpenoids, Tannin, Flavanoids, Glycoside, Anthraquinones. The UV profile showed different peak ranging from 200-1100 nm with different absorption respectively. The FT-IR spectrum confirmed the presence of Alcohol, Carboxylic acid, Alkynes, Aromatic ring, Aliphatic, Amide, Carbolic acid salts, Sulfonic chlorides, Hydrocarbons, Organophosphorus compounds.

Deepti Malhotra *et al.*, 2013 conducted the phytochemical analysis and evaluation of the antimicrobial activity of the aqueous and methanolic extract of the roots of *Boerhaavia diffusa.* The result revealed the presence of alkaloid, glycoside, saponins, flavonoids, polysaccharides, steroid and tannin in both the root extracts. *B. diffusa* root extract possesses antimicrobial activity as the zone of inhibition was observed for both gram positive as well as gram negative bacterial strains.

Boerhaavia diffusa root is mainly used to treat gonorrhea, internal inflammation of all kinds, dyspepsia, edema, jaundice, menstrual disorders, anemia, liver, gall bladder and kidney disorders, enlargement of spleen, abdominal pain, abdominal tumours, and cancers, diuretic documented inIndian Pharmacopoeia, digestive aid, laxative and a menstrual promoter (Ramachandra *et al.*, 2012).*B. diffusa*

and to evaluatethe antimicrobial activity of the aqueous and methanolic extract of the roots of *B. diffusa*.

Natural antioxidants are known to exhibit a wide range of biological effects including antibacterial, antiviral, anti- inflammatory, antiallergic, antithrombic and vasodilatory activities. Antioxidant activity gives rise to anti carcinogenicity, ant immunogenicity and antiaging activity (Gulcin *et al.*,2010).

Phenolics are secondary metabolities that behave as antioxidants due to the reactivity of the phenol moiety (hydroxyl substituent on the aromatic ring). The antioxidant activities of phenolic compounds are also attributed to their ability to chelate transition metal ions, such as those of iron and copper, which have been proposed as the catalyst for the initial formation of ROS (Knezevic *et al.*, 2011). The antioxidant activity of the dietary phenolics is considered to be superior to that of the essential vitamins and is described to their high redox potential, which allows them to interrupt free radical mediated reactions by donating hydrogen from the phenolic hydroxyl groups (Beevi *et al.*,2010).

Flavonoids are polyphenolic compounds, which are ingredients of many vegetables and fruits. They are classified into flavanols, flavanones, flavones, isoflavones, catechins, anthocyanins, proanthocyanidins, etc. They are among the most bioactive plant secondary metabolites which outperform well-known antioxidants. Flavonoids serve as ROS scavengers by locating and neutralizing radicals (Gill and Tuteja, 2010). Bioactive properties such as free radical scavenging, inhibition of hydrolytic and oxidative enzymes and anti-inflammatory action of flavonoids is known (Njoku *etal.*, 2011).

Ascorbic acid (vitamin C) is a vital component in human diet with the highest concentrations in animal organs like the liver, leukocytes and anterior pituitary. It is used for its antioxidant effect (Ensafi *et al*.,2010), protecting cells from harmful oxidative products (Fossati *et al*., 2011). Vitamin C functions in enzyme activation, oxidative stress reduction, and immune function. There is considerable evidence that vitamin C protects against respiratory tract infections and reduces risk for cardiovascular disease and some cancers (Schlueter and Johnston, 2011).

The antioxidant capacity and total phenolic contents present in the acetone and methanolic extracts of leaves, stem, fruits and roots of *Melothria maderaspatana* were evaluated by (Sowndharajan *et al*., 2010).

Igbinosa *et al*., 2011 assessed the polyphenolic contents and antioxidant potential of the aqueous ethanol and methanol stem bark extracts of *Jatropha curcas*. There was correlation between total phenol, total flavonoids, total flavonol and total proanthocyanidins (r=0.996, 0.978, 0.908, and 0.985) respectively. This study also indicated that *Jatropha curcas* is a potential source of natural antioxidants and may be a good candidate for pharmaceutical plant basedproduct.

Omoregie and Osagie 2012 studied the antioxidant properties of methanolic extracts of six locally consumed plants in Nigeria. Among the six plants, the leaf extracts of *Jatrop tanjorensis* phenolic content and flavonoid content is significantly high ($p>0.05$). These result also suggested that the plant leaves possess varied degrees of antioxidant activity.

Jain *et al*. 2013 studied *in vitro* free radical scavenging activity of *Jatropha gossypifolia* containing phenolic compounds. Ethanolic extract of *Jatropha gossypifolia* showed 58.7 ± 0.62% inhibition in superoxide scavenging model.

Aqueous extract also showed almost similar activity (54.9 ± 0.53%). *Jatropha gossypifolia* had the highest total phenolic content (42.60 mg tannic acid equivalent (TAE) /100 g fresh weight). Total phenolic content had positive correlation with antioxidant potential. This shows that the plants, especially *J. gossypifolia*, may be potent source of natural antioxidants.

Olabinri *et al.*, 2013 investigated *in vitro* antioxidant and nitric oxide radical scavenging capabilities of *Jatropha gossypfolia* extract. The result shows that the nitric oxide scavenging activity of the aqueous extract of *Jatropha gossypifolia* stem bark in the dry season was significantly higher than the antioxidant activity of the aqueous leaf extract of the plant.

Safi *et al.*, 2012 reported the biological activities of methanol extract of the root of *Jatropha curcas* like antimicrobial and free radical scavenging activities. In the evolution of DPPH free radical scavenging activity, methanolic crude extract and chloroform soluble fraction showed strong antioxidant activity with IC50 value of 35, 62 ug/ml and 43.81 ug/ml respectively where the standard antioxidant Butylated Hydroxy Toluene (BHT) showed the IC50 value of 18.31 ug/ml.

Father and Iqbal, 2011 observed the phytochemical screening of some Pakistanian medicinal plants.

Murugesh and Vino 2017 tested the antioxidant potential of the extracts of *Pisonia grandis* and was assessed by employing different assays such as DDPH and ABTS. The screening tests also were performed for the presence of secondary metabolites such as alkaloids, flavonoids, terpenoids, phenols, steroids, saponins and tannins in the extracts.

Ullah Naveed *et al.*,2010 worked on the aqueous and ethanolic extract derived from the leaves of an indigenous medicinal plant *Mirabilis jalapa* L (white, cream, yellow and pink, flowered plants) and were screened for antibacterial activities against *Staphylococcus aureus.* Aqueous extracts did not display any inhibition to the tested bacteria. The growth inhibitions (%) were calculated with reference to the activities of tetracycline which was taken as a standard (100%). The research clear cut indicates the effectiveness of white flowered plant of *M. jalapa* against *S. aureus*. This is responsible for causing diseases like skin infections, pneumonia, and food poisoning etc.

Navaneethakrishnan *et al.,* 2011 carried out to evaluate the antimicrobial property of *Sida spinosa* Linn (Malvaceae) leaves against certain bacterial strains causing microbial infection using cup plate method. The test organism were *Staphylococcus aureus, Bacillus subsitlis, Pseudomonasaeroginosa, Escherichia coli, Candida albicans, Aspergillus niger*. All the different concentration of ethanolic leaf extracts of *sida spinosa* exhibited the concentration dependant significant antimicrobial activity comparable with ciprofloxacin 5µg/disc and amphotericin B 30µg/disc were used respectively as standard for bacteria and fungi stains.

Ayeni *et al.*, 2017 evaluated *Daucus carota* Linn. leaf to establish some pharmacognostic standards for its further investigation. The plant material was prepared and evaluated according to standard methods of assessing crude drugs. Findings from the study shows the leaf has alternate covering base, tri-pinnate leaf arrangement, parallel venations, finely divided uniform division, acute leaf shape and also serrated leaf blade.

Simran Aneja *et al.*, 2011 worked on the study deals with the pharmacognostic evaluation including examinations of morphological and microscopic characters, powder analysis, extractive values, moisture content and fluorescence analysis. Preliminary phytochemical screening was also carried out leaf powder showed the presence of cork cells, cortex, fibers, xylem and phloem.

Prasanth *et al.*,2017 worked on *Gomphrena serrata* (Amaranthaceae) and recorded that it has been utilized for many ailments in the conventional system ethno medicinally, most significantly against bronchial asthma, diarrhoea, heavy fever, pains, tonic, carminative, diabetes, dermatitis, and piles. The key challenge experienced in the standardization of herbal drugs is the correct identification of the plant source. Pharmacognostic and phytochemical analysis which assures the purity, safety, and efficiency of *G. serrata* is necessary. The current research was conducted to assess the pharmacognostic characteristics including macroscopic, microscopic, phytochemical and physicochemical parameters of the root of *G. serrate.*

Praveen Kumar *et al.*,2017 studied the retention factor for Boeravinone in *B. diffusa* extract was found to be 0.461. Presence of functional groups such as phenyl groups at 1450.47 cm-1 , 1591.27 cm-1 , C=C (Carbon skeleton) at 1618.28 cm-1 and Isoflavonoid structure at 1649.14 cm-1 of absorption spectra of isolated Boeravinone were characterized by FTIR spectroscopy through recording absorption spectra.

Fourier transform infrared spectroscopy is a physicochemical analytical technique which provides a clear picture of the metabolic composition of leaves at a given time (Bobby *et al.*, 2012). It is possible to detect the minor changes in the primary and secondary metabolites in leaves by observing the IR spectra (Surewicz *et al.*, 1993).

Ranjana Singh *et al.*, 2015 studied the phytochemical profiling of leaf aqueous extract and the total phenolic compounds, flavonoids were determined

spectrophotometrically following the standard methods. FTIR and UV-Vis profiles showed the presence of phenolic compound and flavonoids in leaf extract. The peaks at 304.0 nm and 278.0 nm in UV- spectrum confirmed the flavonoids and their derivatives.

Murugesh and Vino, 2017 stated that FT-IR spectrum was used to identify the functional groups of the active components present in extract based on the peaks values in the region of IR radiation. When the extract was passed into the FT-IR, the functional groups of the components were separated based on its peaks ratio. The results of FTIR analysis confirmed the presence of alcohols, alkenes, Phenols, aromatic carboxylic acid, esters, aliphatic amines, and primary secondary amines.

Shanmugapriya *et al.*, 2017 tested to identify the functional groups present in the ethanolic combination of *Tridax procumbens* (leaves) and *Boerhavia diffusa* (leaves) ethanolic extracts through UV and FT-IR spectroscopy. It is one of the most widely used methods to determine and elucidate the structure of the chemical constituents. The results of FT-IR analysis confirmed the presence of phenols, alkanes, aldehydes, alkynes, alkenes, aromatics, aromatic amines, alkyl halides, and aliphatic amines which showed major peaks at 3363.97, 2975.61, 2542.73 and 1230.63, respectively.

Sravan Kumar *et al.*,2015 studied the FTIR analysis for five selected green leafy vegetables (GLVs) viz., *Hibiscus cannabinus* L., (kenaf), H. *sabdariffa* L., (roselle), *Basella alba* L., (vine spinach), B. *rubra* L., (malabar spinach) and *Rumex vesicarius* L., (sorrel) confirmed the presence of free alcohol, inter molecular bonded alcohol, intramolecular bonded alcohol, alkane, aromatic compounds, imine or oxime or ketone or alkene, phenol and amine group.

Ranjana Singh *et al.*,2015 studied the phytochemical profiling of leaf aqueous extract

was carried out and the total phenolic compounds and flavonoids were determined spectrophotometrically following the standard methods. FTIR peaks obtained at the presence of alcohol, phenol, alkanes, amino acids, aldehyde, aromatic compound, secondary alcohol, sulfur compounds, carboxylic acid, and amide groups in the extract.

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. Parag Petnekar and Bhanu Raman,2013 carried out the FTIR spectroscopic analysis of methanolic leaf extract of *Ampelocissu lantifolia* for antimicrobial compounds.

Yusuf Nasution *et al.*, 2018 observed the ethanol, aqueous and chloroform extracts of leaf and root of *Centella asiatica* against six bacteria namely E. coli , *Staphylococcus aureus*, *Staphylococcus albus*, *Streptococcus pyogenes*, *Psedomonas aeruginosa*, *Sreptococcus pneumonia* and three *fungi: Aspergillus niger*, *Aspergillus flavus*, *Microsporium boulardii* and one yeast *Candida albicans* were determined using agar well diffusion and paper disk methods.

Saravanan *et al.*, 2016 stated that the allopathic drugs are relatively more toxic and explored to produce various side effects. Herbal drugs have some advantages regarding toxic effects over allopathic drugs. Present study is to evaluate antibiotic activity of ethanolic extracts of the leaves and roots of Abutilon indium Linn. against various harmful bacteria. *Abutilon indicum* Linn.

Sujatha Edupuganti *et al.*,2015 conducted a study to determine the anti

microbial activity of different plant leaf extracts with different solvent viz; ethanol against Gram positive bacteria (*Staphylococcus aureus*), Gram negative bacteria (*E. coli*) and Fungal (*Aspergillus niger & Candida parapsilosis*). The disc diffusion method was used to test the antimicrobial activity. The result shows that more antimicrobial activity shown for *E. coli, Staphylococcus aureus* by *Abutilon indicum* at low concentration of 5µg/ml. The result shows that more antimicrobial activity shown for by *Abutilon indicum* at low concentration of 5µg/ml.

Shweta Majgaine *et al.,* 2017 studied the effect of plant extracts on bacteria and fungi have been studied by a very large number of researchers in different parts of the world as well as in India. Punarnava is an important rejuvenating drug used in Ayurveda.

Farhana Nazira Idris *et al.,* 2017 studied various antibiotics and antimicrobial medicine that have been developed over the years to improve human quality of life. Beside drug resistance, undesirable side effect of certain antibiotics encourage the use of plants extract as antimicrobial agents.

Maximum percentage of alkaloids are shown by *Amaranthus tricolor* L., *Aamaranthus viridis* L. and *Amaranthus caudatus* L. (8- 8.8%) and the least were shown by *Amaranthus spinosus* L. and *Amaranthus dubius* Mart. (5.8-6%). Antibacterial and antifungal studies were carried out using the precipitated alkaloid, which showed zone of inhibition ranges from 6 to 11mm. Thus the present study showed that isolated alkaloids can show antimicrobial activity and used for the formulation of drugs or antiseptics (Pinkie Cherian and Sheela, 2016)

Maya verma & Ashwani kumar.,2017 studied the antimicrobial efficacy of methanol extracts of some medicinal plants by agar well diffusion method against

selected pathogenic bacterial strains. Gram +ve strains (*S. aureus, B. subtilis*) were tested and Gram-ve strains tested were (*E. coli, S. typhii and K.pneumoniae*). Antifungal activity against was tested.

Bio monitored study of extracts of *Alternanthera brasiliana*, which is used in Brazilian folk medicine as bactericidal, analgesic and anti-inflammatory (Luciane *et al.*, 2011). The work led to the isolation of two substances in the ethyl acetate extract, identified as quercetin and sitosterol glycoside and the antibacterial evaluation of these substances demonstrated that the flavonoid presented antibacterial action against *S. aureus*. Further studies intend to identify new substances with antibacterial activity in extracts of *A. brasiliana*.

Thakur Hemantkumar, 2016 studied the extracts of *Celosia argentea* viz. petroleum ether, chloroform, acetone, ethanol and aqueous extracts were carried out. A total of 5 microorganisms (4 bacteria and 2 fungal strains) were used for antimicrobial activity. The extracts were screened for the presence of Phytochemicals and their effect on various microbes especially fungi such as *Candida albicans* (only single strain) and bacteria such as *E.coli, Staphylococcus aurus, Klebsiella pneumoniae, Salmonella tophi*, etc. Aqueous extract showed moderate inhibitory activity against *Staphylococcus aureus* (14mm).

Sumaira Sarwar *et al.*, 2016 studied about two strains of gram positive bacteria, *Staphylococcus aureus* and *Bacillus subtilis, whereas Proteus vulgaris, Pseudomonas picketii, Klebsiella pneumoniae* and *Escherichia coli* were gram negative. All dilutions were made in DMSO which has no inhibitory effect on growth of bacteria. Five concentrations (max. 25 mg/mL and min. 5mg/mL) of this plants extract was tested against five bacterial strains. Zones of inhibition were measured finally by MIC test.

Sileshi Woldeyes *et al.*, 2012 results showed that antibacterial activities were comparable to each other. But their activities were relatively weaker as compared to that of the reference compound (ciprofloxacin). Among the three crude extracts, the chloroform extract was subjected to column chromatographic separation that led to isolation of SRL-1, SRL-2 and SRL-3.The observed antibacterial activities of the crude extracts and the isolated compounds could justify the traditional use of the plant for the treatment of different bacterial infections.

Eseoghene Okpako *et al.*, 2015 results showed the compounds such as alkaloids, flavonoids, tannins saponins and terpenes were detected in *Celosia argentea* L. The leaves, stem and root extract elicited antibacterial activity against all the strains tested by zones of inhibition ranging between 7 mm to 26 mm. Bactericidal and fungicidal effects of the leaves extract were same, with no cidal effect against *Aspergillus niger* and *Escherichia coli*. The stem extract had inhibitory effects at 25µg/ml and no bactericidal effect. The root extract was bacteriostatic and bactericidal at the same concentration for all bacterial strains with the exception of Escherichia coli that showed MIC of 50µg/ml.

Sharmila Banu and Ramar, 2018 studied the medicines traditionally used for treating skin burning (dermatological infections) and skeleton muscular disorders. *Hibiscus micranthus* also use for burn-in Negelle-Borona, Ethiopia. (Begashaw *et al.*, 2017). Human population in countries around the world has been using plants for thousands of years for treating various ailments of humans and animals. Hibiscus micranthus is a shrubby, erect, branched, slender and stellately hairy plant. (Ashok Kumar *et al.*, 2010).

Shanmugapriya *et al.*, 2017 studied the combination of *Tridax procumbens* (leaves) and *Boerhavia diffusa* (root) ethanolic extracts through UV and FT-IR

spectroscopy. The results of FT-IR analysis confirmed the presence of phenols, alkanes, aldehydes, alkynes, alkenes, aromatics, aromatic amines, alkaloides, and aliphatic amines.

Mohideen *et al.*,2002 studied the characteristic of stellate and glandular trichomes, druses of calcium oxalate crystals and siphonostelic vasculature in the petiole and the presence of three layered palisade and mucilage cells, druses in the spongy tissue, anisocytic (Cruciferous) type of stomata in the leaf are the salient features of diagnostic value in the pharmacognostic determination of the drug.

Bhavsar Shruti *et al.*,2018 studied extracts of *Kalanchoe pinnata* (Lam.) Pers. were investigated for its macroscopical, microscopical, physicochemical and phytochemical properties and the comparative statistical analysis was done. All the extracts showed significant results for their phytochemical study, and Extract 1 showed highest diversity of compounds which places it as best extracts. This study provides important information for the selection of best extract as folkloric preparation.

MATERIALS AND METHODS

MATERIALS

Sample 1

Botanical Name : *Abutilon indicum G. Don*

Family : Malvaceae

Common Name : Indian mallow

Tamil name : paniyaratutti

Abutilon indicum is a herbaceous or shrubby softly tomentose plant, stem is round often tinged with purple colour. The leaves are petiolate, ovate to orbicular cordate ,acuminate and toothed .Flowers are borne solitary in long jointed and axillary pedicels. Calyx lobes divided in the middle ovate and apiculate .Corolla is yellow or orange yellow and opens in the evening. Carpels are 15-20 in number. Fruits are hispid, scarcely longer than the calyx and the awns are erect seeds are three to five kidney shaped dark brown of black tubercled or with minutely stellate hairs (plate 1).

Sample 2

Botanical Name :*Achyranthes aspera*

Family : Amaranthaceae

Common Name : Pricky chaff flower

Tamil name : Naiyurivi

Achyranthes aspera locally is one of the most important trationally used anti-fertility plants in the indigenous health care delivery system of Ethiopia.It is a stiff erect perennial herb of 1 to 3 feet with simple eliptic leaves. Flowering plant is in summer. The stems are square, leaves elliptic ovate or broadly rhombate. The inflorescences are 8-30cm long, with many single, white or red flowers (plate 1).

Plate I

Abutilon indicum G. Don

Achyranthes aspera Linn.

Boerhaavia diffusa Linn.

Bryophyllum calcycinum Kurz

Centella asiatica Urh

Sample 3

Botanical Name : *Boerhaavia diffusa* Linn.

Family : Nyctaginaceae

Common Name :Punarnava

Tamil name :Mookiratti

Boerhaavia diffusa is a prostrate herb with very diffuse inflorescences.It is awed found throughout India. Leaves are unequal, ovate, blunt, wavy, along margins, flat to somewhat eart shaped at base, leaf stalk is 1cm long. Inflorescence occur at the end if branches , are forked about 3-6 times, Occasionally with sticky intermodal bands, Branches are divergent , terminating in compact subumbellate or capitates 2-5 flowers clusters. Flowers have stalk shorter than 0.5. Bract at base of flower tube quickly deciduous, lancelike,0.8-1mm flowers are purplish red to reddish pink or nearly white, bell-shaped beyond the constriction,1-1.5mm stamens 2-3, are inside the flower or protruding (plate 1)

Sample 4

Botanical name :*Bryophyllum calcycinum*

Famil :Crassulaceae

Common name : Cathedral bells

Tamil name : Rannakalli

Bryophyllum calycinum is used as medicinal plant, ornamental crassulecent herb. It is cultivated in houses, herbal garden, and field. The plant grows all over the India in hot, humid and moist areas. The plant height is about 1-1.5m in long and it consist of opposite leaves and 10-20cm long glabrous leaves. The lower leaf is usually simple and upper one 3-7 foliate and are long petioled. They are freshly dark green color and trimmed in reddish purple and leaf blade are pinnately

compound with 3-7 leaflets. The flowers are membranous follicle enclosd in persistent papery calyx and corolla, seed smooth and ellipsoid (plate1).

Sample 5

Botanical Name : *Centella asiatica*

Family : Apiaceae

Common name : Brahmi

Tamil name : Vallarai

Centella grows in temperate and tropical swampy areas in many regions of the world. The stems are slender, creeping,stolons, green to reddish green in colour ,connecting plants to each other. It has long stalked, green, rounded apices which have smooth texture with palmately netted veins. The leaves are borne on pricladial petiols, around 2 cm .The root stock consists of rhizomes, growing vertically down. They are creamish in colour and covered with root hairs. The flowers are white or pinkish to red in colour, born in small, rounded bunches near the surface of soil. Each flower is partly enclosed in two green bracts (plate1).

METHODOLOGY

Collection and processing

The whole plant samples *Abutilon indicum* G.Don. *Achyranthusaspera* L. *Boerhaavia diffusa* L. *Bryophyllum calcycinum* Kurz and *Centella asiatica* Urh was collected from the house garden. The collected samples were cut into small fragments and shade dried until the fracture was 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 leaf.

Preparation of extracts for phytochemical screening

Preparation of extracts Cold maceration method

The coarse powder of sample was extracted successively with acetone, benzene, chloroform, ethanol, methanol, petroleum ether. All the extracts were filtered through whatman no.41 filter paper. All the extracts (acetone, benzene, chloroform, ethanol, methanol, petroleum ether) were subjected to qualitative tests for the identification of various phytochemical constituents as per standard procedures.

Hot maceration method using Soxhelet apparatus

The coarse powder (100 g) was extracted successively with chloroform, ethanol, methanol, and petroleum ether each 500 ml in a Soxhelet apparatus for 24 hrs. All the extracts were filtered through whatman No.41 filter paper. All the extracts were subjected to qualitative tests for the identification of various phytochemical constituents as per standard procedure (Brinda *et al.*,1993 and Lala,1993).

Qualitative phytochemical analysis of different extract:

The chemical test for various phytochemical in the extracts were carried out as described below:

Test of alkaloids

Mayer test:

To the powder 2 ml of mayer reagent was added, a dull white precipitate reveals the presence of alkaloids

Test for Terpnoids

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.

Test for steroids:

The powder was dissolved in two ml of chloroform in a dry test tube ten drops of concentrated sulphuric acid were added. The solution became red, then blue and finally bluish green indicates the presence of steroids.

Test for Coumarins:

To 1 ml of extract 1 ml of 10% Sodium hydroxide was added. The presence of coumarins is indicated by the formation of yellow colour.

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 Saponins:

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

Test for Flavones: (Shinadow test)

To a few mg of the powder, magnesium turnings and 1 or 2 drops of concentrated hydrochloric acid (HCl) were added formation of blue colour indicates the presence of flavonoides.

Test for phenols:

To 1 ml of the extract 2ml of distilled water was added followed of 5% aqueous ferric chloride. Appearance of blue or green colour indicates the presence of phenols.

Test for Protein: (Biuret test)

To 1ml of the extract, one drop of 2 CuSo4 and 1 ml of 95% ethanol add KOH pellets. Appearance of pink colour indicates presence of protein.

Test for Carbohydrates: (Benedict's test)

To 0.5 ml of extract (500 µl) add 0.5 ml of Benedict's reagent, Incubate in boiling water bath, Appearance of brick red precipitate indicates presence of sugar.

Test for Quionones:

To 1ml of extract and 1 ml of conc. sulphuric acid was added. Appearence of red indicates presence of quionone.

Test for Gums:

1 ml of extract was mixed with water. Tickening of substance shows the presence of Gum.

Quantitative analysis of antioxidant

Total phenolic content: (Duan *et al.*, 2006)

Reagents

- 50% Folin - ciocalteaureagent
- 20% sodium -carbonate
- Gallic acid –standard

Procedure

100 mg of sample was homogenized with 10 ml of distilled water and filtered

through a muslin cloth 1ml of the filtrate was added to 1.5ml of deionized water and0.5 ml of 50% folinciocalteau reagent and the contents were mixed thoroughly. After 1min, 1ml of 20% sodium carbonate solution was added mixed the control contained all the reagents except the sample. After 30 minutes of incubation at 37c, the absorbance was measured at 750 nm. Total phenolics were calculated as Gallic acid equivalent (GAE) per gram fresh weight.

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

Reagents

- 5% sodium nitrate ($NaNo_2$)
- 10% Aluminium chloride ($Alcl_3,H_2o$)
- 1N sodium hydroxide (NaoH)
- Quercetin standard

Procedure

100 mg of plant material was homogenized with 10 ml of distilled water and filtered through a muslin cloth. 0.5 ml of the extract was added with 2.5 ml of distilled water and mixed. After 6 minutes 0.15 ml $NaNO_2$, was added and again after 6 min 0.3 ml of 10% $AlCl_3$ was added. After 5 min 1 ml of 1M NaOH and 0.5ml of water were added. Following through mixing of the solution the absorbance against blank was recorded at 510 nm. Quercetin was used as standard and the results were expressed as my quercetin equivalents (QE) 1g fresh weight.

Vitamin C [Ascorbic acid] (Barker and Frank, 1998)

Reagents

5%TCA

- Indophenol reagent
- 20 mg of dichlorophenol indophenols was dissolved in 10 ml of warm

distilled water.

- DT reagent 2 g of 2, 4 dinitraphenyl hydrazine and 1 g of thiourea were dissolved.
- 85% sulphuricacid
- L - ascorbic acid –standard

Procedure

100 mg of plant material was homogenized with 10 ml 0f 5% Trichloro acetic acid (TCA). The homogenate was centrifuged. To 2 ml of indophenols reagent and 0.5ml of DT reagent was added and incubated at 10^0 C for 1 hour and then cooled in ice bath and 2.5 ml of 85% sulphuricacid was added and shaken well for 30 mins (until) red colour appeared. The absorbance was measured at 540 nm. L–ascorbic acid was used as standard and the results were expressed as mg/lg/Fw.

Estimation of Tannin (JulkunenTitto, 1985)

Procedure

100 mg of sample was homogenized with 10 ml of distilled water and filtered through a muslin cloth. 1ml of aliquot of aqueous extract was mixed with 1.5 ml of 4% vanillin (Prepared with methanol) and 750 µl of concentrated HCl was added the solution was shaken vigorously and left to stand at room temperature for 20 mins in darkness the absorbance against blank was read at 500 nm using uv-visible spectrophotometer. Results were expressed as g catechin equivalent (CE) 1g tissue.

Vitamin E (Tocopherol): Rosenberg,1992

Procedure

The plant sample (2.5 g) was homogenized in 50 ml of 0.1 N sulphuric acid and

allowed to stand overnight the content in the flask was shaken vigorously and filtered through what man No: 1 filter paper. Aliquots of the filtrated were used for estimation. In stopper centrifuge tubes 3 ml of extract and 3 ml of water were pipette out separately. To both the tubes, 3 ml of ethanol and 3 ml of xylene were added, mixed well and centrifuged. Xylene (2.0 ml) layer was transferred into another stopper tube. To each tube 2.0ml of dipyridyl reagent was added and mixed well, the mixture (3 ml) was pipette out into a cuvette and the extinction was read at 460 nm. Ferric chloride solution (0.66 ml) was added to all the tubes and mixed well. The red colour developed was read exactly after 15 min at 520nm. Tocopherol was used as standard.

Powder microscopy: Shehla *et al*., (2014)

Shade dried leaves, stem, fruit and roots were finely powdered and studied under microscope. Small quantity of different plant parts powder was placed separately on slides and each slide was mounted 2-3 drops of chloral hydrate and each slide was covered with cover slip then examined under microscope. Different cell components i.e. cork cells, sieve tubes fibers, lignified fibers, cortex cells, calcium oxalate crystals, mesocarp, endocarp and stomatal cells were noted and photography was done by using digital camera.

FT-IR Spectroscopy Analysis

A little powder of plant specimen was mixed with KBr (Potassium bromide) salt, using a mortar and pestle, and compressed into a thin pellet. Infrared spectra were recorded as a KBr pellets on a Thermo scientific Nicot is 5 iDL transmissions, between 4000-400 cm (Kareru *et al*., 2018).

Anti-bacterial activity

Extraction of plant materials

The plant powder was extracted different solvents. 5 mg of plant powder was extracted with different solvents of (acetone, methanol, water).

Bacterial Strains

The test organisms were obtained from the department of Botany, St. Mary's College (Autonomous), Thoothukudi. *Proteus vulgaris, Salmonella typhi, E.coli , Bacillus substill, Klebsilla pneumonia* were used in the present study.

Broth medium

- Nutrient broth Himedia MOOI
- Nutrient broth 1.3 g
- 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 loop full bacterial colony was transferred to the broth medium. This microbe culture was incubated at room temperature for 24 hours.

Agar medium

- Nutrient ager Himedia MOOI
- Nutrient agar 2.8 g
- 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. The solutions were prepared with known weight of extracts dissolved in 5%

dimethyl sulphoxide (DMSO). What man No.1 filter paper discs (5mm) were impregnated with 20 0f these extracts and allowed to dry at room temperature. The spread plates were prepared by proper concentration of inoculation. Each sample loaded discs was placed in the seeded agar plate. After 24- 48 hours of + 37^0c incubation, the diameter of the inhibition zone was measured for positive control, streptomycin discs (100 mg/ml) was used , whereas for negative control; respective solvents loaded on the sterile disc.

RESULTS AND DISCUSSION

RESULTS AND DISCUSSION

The term 'phytochemical' is reserved for those plant chemicals that have a beneficial effect on human-health but are not essential from the point of view of nutrition. A medicinal herb is considered to be a chemical factory as it contains a multitude of chemical compounds like alkaloids, glycosides, Saponins, resins, oleoresins, sesquiterpene lactones and oils (essential and fixed). Antifungal activity of medicinal plants is mainly due to the presence of Phytochemicals like alkaloids, glycosides, phenols, tannins and flavonoid (Sarojini *et al.*, 2011). Moreover, phytochemical screening of the drug is significant for proper identification, which further exerts importance on therapeutic activity of the medicinal plant.

Qualitative Analysis

The current study was attempted to find out the presence of preliminary Phytochemicals I in acetone, benzene, chloroform, ethanol, methanol, petroleum ether extract of leaf of selected medicinal plants such as *Abutilon indicum* G.Don. *Achyranthus aspera* L. *Boerhaavia diffusa* L. *Bryophyllum calcycinum* Kurz and *Centella asiatica* Urh. Presence or absence of certain important compounds in an extract is determined by colour reactions of the compounds with specific chemicals which act as dyes. This procedure is a simple preliminary pre-requisite before going for detailed phytochemical investigation. Different chemical compounds were detected in different extracts of leaf of *Abutilon indicum* G.Don. *Achyranthusaspera* L. *Boerhaavia diffusa* L. *Bryophyllum calcycinum* Kurz and *Centella asiatica* Urh. and were presented in **Table 1-23.** Here after test samples will be referred as A, B, C, D and E respectively.

Table-1 Alkaloids in the leaf cold extracts of selected plant samples

S.No	Solvent	Plant-A	Plant-B	Plant-C	Plant-D	Plant-E
1	Acetone	–	–	–	–	–
2	Benzene	–	–	–	–	–
3	Chloroform	–	–	–	–	–
4	Ethanol	–	–	–	–	–
5	Methanol	–	–	–	–	–
6	Petroleum Ether	–	–	–	–	–

Table-2 Terpenoids in the leaf cold extracts of selected plant samples

S.No	Solvent	Plant-A	Plant-B	Plant-C	Plant-D	Plant-E
1	Acetone	–	–	–	–	–
2	Benzene	–	–	–	–	–
3	Chloroform	–	–	–	–	–
4	Ethanol	–	–	–	–	–
5	Methanol	–	–	–	–	–
6	Petroleum Ether	–	–	–	–	–

Table-3 Steroids in the leaf cold extracts of selected plant samples

S.No	Solvent	Plant-A	Plant-B	Plant-C	Plant-D	Plant-E
1	Acetone	–	–	–	–	–
2	Benzene	–	–	–	–	–
3	Chloroform	–	–	–	–	–
4	Ethanol	–	–	–	–	–
5	Methanol	–	–	–	+	+
6	Petroleum Ether	–	–	–	–	–

Table-4 Coumarins in the leaf cold extracts of selected plant samples

S.No	Solvent	Plant-A	Plant-B	Plant-C	Plant-D	Plant-E
1	Acetone	–	–	–	–	+
2	Benzene	–	–	–	–	–
3	Chloroform	–	–	–	–	–
4	Ethanol	–	–	–	–	–
5	Methanol	–	–	–	+	+
6	Petroleum Ether	+	+	+	+	–

Table-5 Tannin in the leaf cold extracts of selected plant samples

S.No	Solvent	Plant-A	Plant-B	Plant-C	Plant-D	Plant-E
1	Acetone	+	–	–	–	+
2	Benzene	+	+	+	+	–
3	Chloroform	+	–	–	–	–
4	Ethanol	–	–	–	–	–
5	Methanol	–	–	–	+	+
6	Petroleum Ether	+	+	+	+	–

Table-6 Saponin in the leaf cold extracts of selected plant samples

S.No	Solvent	Plant-A	Plant-B	Plant-C	Plant-D	Plant-E
1	Acetone	–	+	+	–	–
2	Benzene	–	+	+	–	–
3	Chloroform	–	–	–	–	–
4	Ethanol	–	–	–	+	–
5	Methanol	–	–	–	–	+
6	Petroleum Ether	+	+	+	+	–

Table-7 Flavanoids in the leaf cold extracts of selected plant samples

S.No	Solvent	Plant-A	Plant-B	Plant-C	Plant-D	Plant-E
1	Acetone	–	–	–	–	–
2	Benzene	–	–	–	–	–
3	Chloroform	–	–	–	–	+
4	Ethanol	–	–	–	–	–
5	Methanol	–	–	–	+	+
6	Petroleum Ether	+	+	+	+	+

Table-8 Phenols in the leaf cold extracts of selected plant samples

S.No	Solvent	Plant-A	Plant-B	Plant-C	Plant-D	Plant-E
1	Acetone	–	–	–	–	–
2	Benzene	+	–	+	–	–
3	Chloroform	+	–	+	+	–
4	Ethanol	–	–	–	–	–
5	Methanol	–	–	–	+	+
6	Petroleum Ether	+	+	–	–	–

Table-9 Protein in the leaf cold extracts of selected plant samples

S.No	Solvent	Plant-A	Plant-B	Plant-C	Plant-D	Plant-E
1	Acetone	–	–	–	–	–
2	Benzene	–	–	–	–	–
3	Chloroform	–	–	–	–	–
4	Ethanol	–	–	–	–	–
5	Methanol	–	–	–	–	–
6	Petroleum Ether	–	–	–	–	–

Table-10 Carbohydrate in the leaf cold extracts of selected plant samples

S.No	Solvent	Plant-A	Plant-B	Plant-C	Plant-D	Plant-E
1	**Acetone**	–	–	–	–	–
2	**Benzene**	–	–	–	–	–
3	**Chloroform**	–	–	–	–	–
4	**Ethanol**	–	–	–	–	–
5	**Methanol**	–	–	–	+	–
6	**Petroleum Ether**	–	–	–	–	–

Table-11 Quinones in the leaf cold extracts of selected plant samples

S.No	Solvent	Plant-A	Plant-B	Plant-C	Plant-D	Plant-E
1	Acetone	–	–	–	–	–
2	Benzene	–	–	–	–	–
3	Chloroform	–	–	–	–	–
4	Ethanol	–	–	–	–	–
5	Methanol	–	–	–	–	–
6	Petroleum Ether	–	–	–	–	–

Table-12 Gum in the leaf cold extracts of selected plant samples

S.No	Solvent	Plant-A	Plant-B	Plant-C	Plant-D	Plant-E
1	Acetone	–	–	–	–	–
2	Benzene	+	–	+	–	–
3	Chloroform	–	–	–	+	–
4	Ethanol	–	–	–	–	–
5	Methanol	–	–	–	–	–
6	Petroleum Ether	+	–	+	+	–

Table-13 Alkaloids in the leaf hot extracts of selected plant samples

S.No	Solvent	Plant-A	Plant-B	Plant-C	Plant-D	Plant-E
1	Acetone	–	+	+	–	–
2	Chloroform	–	+	+	–	–
3	Methanol	–	+	+	–	–
4	Petroleum Ether	–	+	+	–	–

Table-14 Terpenoids in the hot leaf extracts of selected plant samples

S.No	Solvent	Plant-A	Plant-B	Plant-C	Plant-D	Plant-E
1	Acetone	–	+	+	–	–
2	Chloroform	–	+	+	–	–
3	Methanol	–	+	+	–	–
4	Petroleum Ether	–	+	+	–	–

Table-15 Steroids in the leaf hot extracts of selected plant samples

S.No	Solvent	Plant-A	Plant-B	Plant-C	Plant-D	Plant-E
1	Acetone	–	+	+	–	–
2	Chloroform	+	+	+	–	–
3	Methanol	–	–	–	–	+
4	Petroleum Ether	–	+	+	–	–

Table-16 Coumarin in the leaf hot extracts of selected plant samples

S. No	Solvent	Plant-A	Plant-B	Plant-C	Plant-D	Plant-E
1	Acetone	+	+	+	–	+
2	Chloroform	+	+	+	–	–
3	Methanol	–	+	+	–	–
4	Petroleum Ether	+	–	–	–	–

Table-17 Tannin in the leaf hot extracts of selected plant samples

S.No	Solvent	Plant-A	Plant-B	Plant-C	Plant-D	Plant-E
1	**Acetone**	+	+	+	+	–
2	**Chloroform**	+	+	+	+	–
3	**Methanol**	–	–	–	–	+
4	**Petroleum Ether**	+	–	–	+	–

Table-18 Saponin in the leaf hot extracts of selected plant samples

S.No	Solvent	Plant-A	Plant-B	Plant-C	Plant-D	Plant-E
1	Acetone	–	–	–	–	–
2	Chloroform	–	–	–	–	–
3	Methanol	–	–	+	–	+
4	Petroleum Ether	–	–	–	–	–

The extracts were prepared by using different solvents like Acetone, benzene, chloroform, ethanol, methanol, petroleum ether.

Alkaloids were absent in all the cold extracts of all the plants. In hot extract it is present in plant B and C.

Terpenoids were absent in cold extracts of all the plants and in hot extract, it is present in plant B and C. Steroids were present in cold methanol extract of plant D and E. In hot extract of acetone, chloroform and petroleum ether showed positive results in plant B and C.

Coumarin is present in all the extracts of plant D, cold extract of petroleum ether and benzene shows the positive result in plant B, C, D. Hot extract of acetone, chloroform, methanol and petroleum ether of plant A and C.

Tannin of plant B, D, E shows positive result in petroleum ether. In hot extract tannin is present in acetone extract of plant A and D and petroleum ether of C and D.

In plant C saponin is present in all the extract except in methanol and petroleum ether. Petroleum ether extract of plant A and C shows positive result. In hot extract it is present only in the methanol extract of plant C and E in others it is absent. Petroleum ether extracts of plant A, B, C and E shows positive result in hot extract. It was absent in plant D and E. Chloroform and methanol extract showed positive result in plant B and C. Chloroform extracts of plant A, B, C, and D show positive results of phenols but it is absent in hot extract.

Protein is absent in all the extracts except petroleum ether of plant E. Carbohydrates are absent in all the extract except methanol extract of plant D. Gum is absent in the entire sample.

Table-19 Flavones in the leaf hot extracts of selected plant samples

S.No	Solvent	Plant-A	Plant-B	Plant-C	Plant-D	Plant-E
1	Acetone	+	–	–	+	–
2	Chloroform	+	+	+	–	–
3	Methanol	+	+	+	–	–
4	Petroleum Ether	+	–	–	–	–

Table-20 Phenols in the leaf hot extracts of selected plant samples

S.No	Solvent	Plant-A	Plant-B	Plant-C	Plant-D	Plant-E
1	Acetone	–	–	–	+	–
2	Chloroform	–	–	–	–	–
3	Methanol	–	–	+	–	–
4	Petroleum Ether	–	–	–	–	–

Table-21 Protein in the leaf hot extracts of selected plant samples

S.No	Solvent	Plant-A	Plant-B	Plant-C	Plant-D	Plant-E
1	Acetone	–	–	–	–	+
2	Chloroform	–	–	–	–	–
3	Methanol	–	–	–	–	–
4	Petroleum Ether	–	–	–	–	+

Table-22 Carbohydrate in the leaf hot extracts of selected plant samples

S.No	Solvent	Plant-A	Plant-B	Plant-C	Plant-D	Plant-E
1	Acetone	–	–	–	–	–
2	Chloroform	–	–	–	–	–
3	Methanol	–	–	–	+	–
4	Petroleum Ether	–	–	–	–	–

Table-23 Gram in the leaf hot extracts of selected plant samples

S.No	Solvent	Plant-A	Plant-B	Plant-C	Plant-D	Plant-E
1	Acetone	+	–	–	–	–
2	Chloroform	–	–	–	–	–
3	Methanol	+	–	–	–	–
4	Petroleum Ether	–	–	–	–	–

The Phytochemical screening of plant A,B,C,D,E are consist with the result found in Alaghazeer and El-Saltani (2012) and Partial agreement with previous studies of Ramawat and Dass (2009) and Yadav and Kumar (2010). It is difficult to compare the data with the literature because several variables influence the results. According to some authors the quantity and the composition of bioactive compounds present in plant are influenced by the genotype, extraction, procedure, geographic and climatic conditions and the growth phase of the plants (Ciulei and Istodor 1995). These phytochemicals have been shown to possess an algesic, anathatic, allergenic, and anti-bacterial, anti-inflammatry, antioxidant, antipytric, antiseptic, sedative, anticancer, hypocholestrolemic, nematicide, anticornary, antiarthritic and hepatoprotective, activities Sakthivel *et al.,*(2006).

The therapeutic benefits of secondary metabolism of plant origin have been researched in several recent studies, Nayak, *et.,al*(2006).The phytochemical screening results of plant A,B,C,D and E are consistent with the results found in Alghzeer and Saltani,(2012).Where authors mentioned the presence of tannins, alkaloids, saponin and terpenoids in this plant.

Similar analysis were conducted in areas that have a long tradition in the cultivation and utilization of medicinal plants, such as Pakistan Dai and Munper. Phytochemical screening results can be found in a database with the most important medicinal and aromatic plants in Calarasi-Silistra region.

Quantitative Analysis

Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties (Kumar *et al.*, 2009). Many herbaceous and medicinal plant contain important phytochemicals and vitamins such as alkaloids, flavonoids,

Table-24Quantitative estimation of antioxidants of leaf of selected medicinal herbs

S. No	Antioxidant	Plant-A	Plant-B	Plant-C	Plant-D	Plant-E
1	Phenol (mg (GAE)/ g)	2.374±0.097	2.083±0.083	2.601±0.042	2.462±0.070	2.397±0.070
2	Flavonoid (mg (QE)/ g)	0.205±0.029	0.203±0.044	0.323±0.012	0.264±0.021	0.525±0.015
3	Vitamin C (mg / g)	1.657±0.463	0.897±0.03	1.763±0.532	1.863±0.672	1.562±0.328
4	Tannin (mg (CE)/ g)	7.272±0.363	5.861±0.358	4.363±0.182	2.863±0.357	7.483±0.139
5	Vitamin E (mg / g)	6.662±2.046	4.982±1.789	7.825±2.389	4.852±1.658	4.653±1.752

Figure:1Total phenol content of *Abu... ...spera , Boerhaaviadiffusa , Bryophyllum... ...atica .*

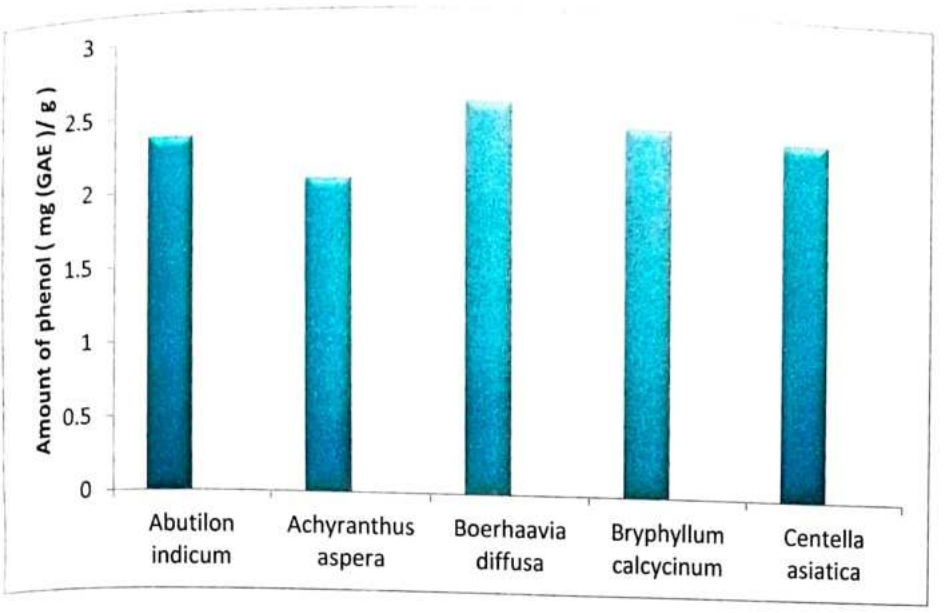


Figure:2 Total Flavonoid content of *Abutilon indicum , Boerhaaviadiffusa, Centellaasiatica, Achyranthusaspera and Bryophyllumcalcycinum.*

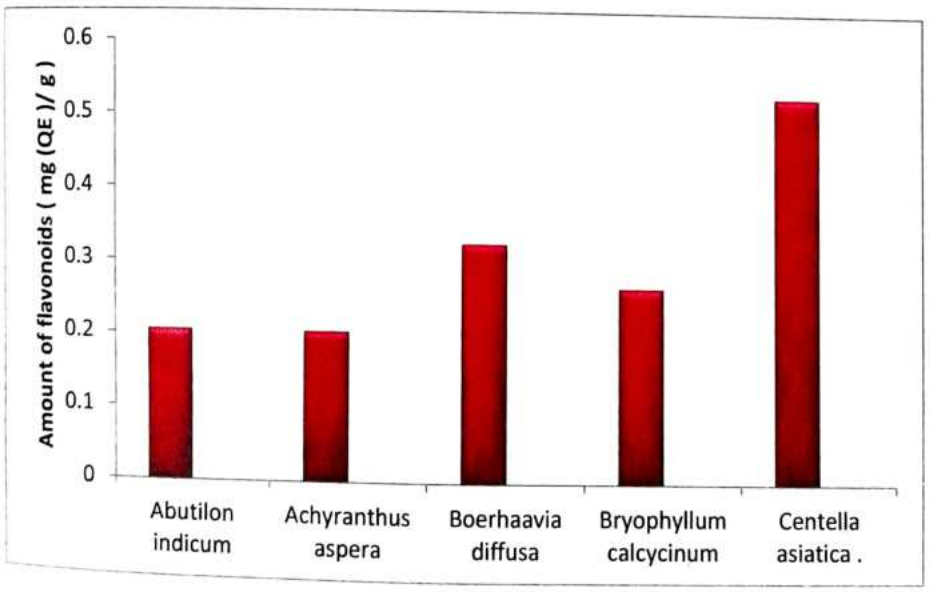


Figure:3 Total Vitamin-C content of ... *...nthusaspera*, *Boerhaaviadiffusa*, *Bryophyllum...* ...*siatica*.

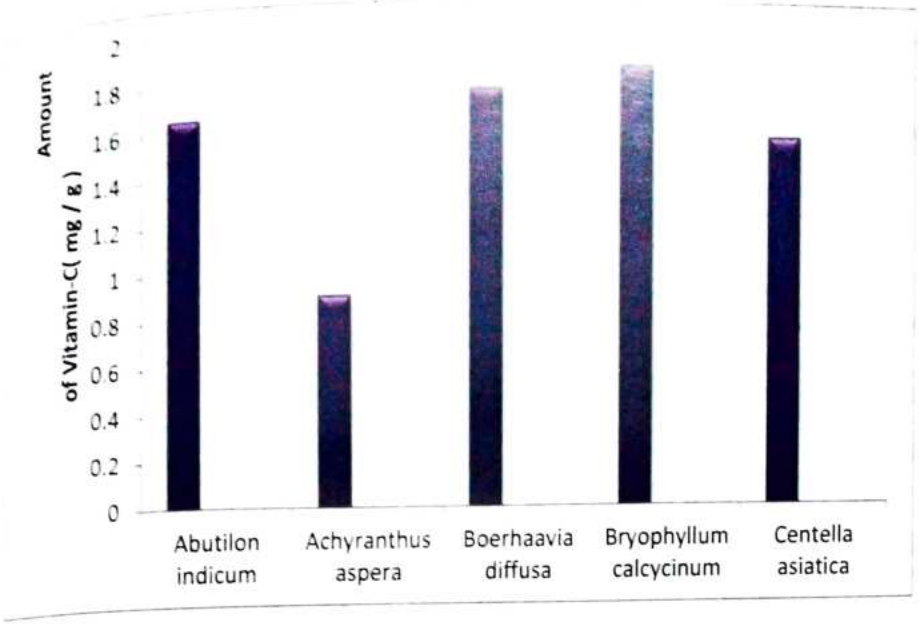


Figure:4 Total Tannin content of *Abutilon indicum* ,*Achyranthusaspera* , *Boerhaaviadiffusa*, *Bryophyllumcalcycinum*and*Centellaasiatica*

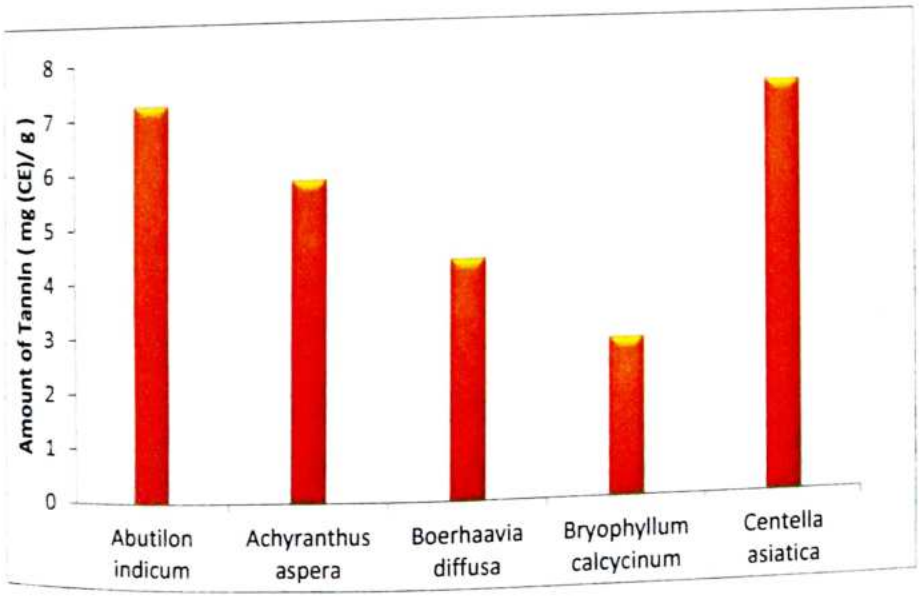
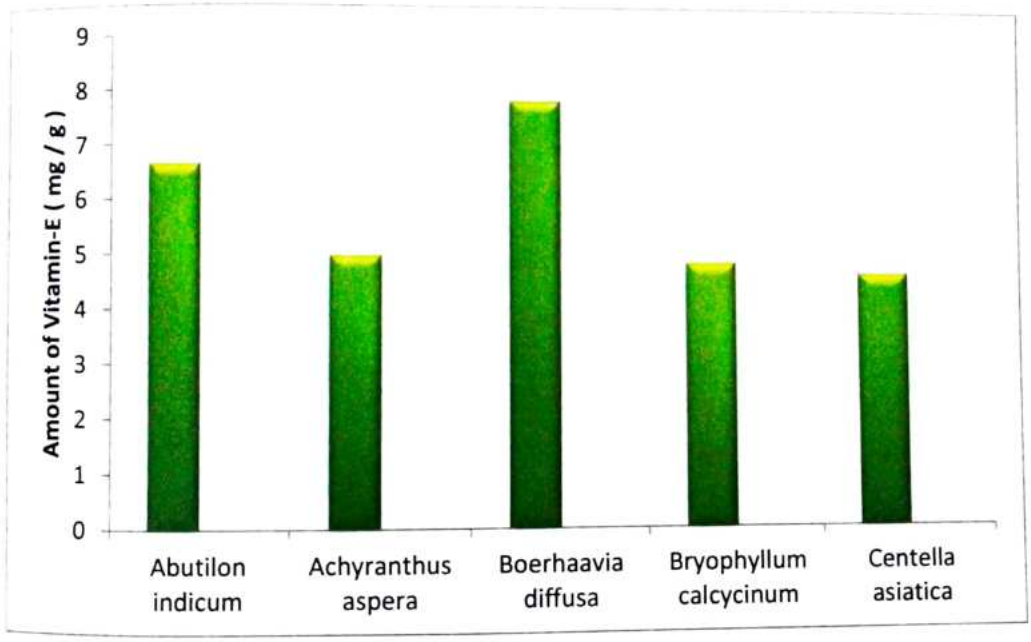


Figure:5Total Vitamin-E content of *Abutilon indicum* ,*Achyranthusaspera* , *Boerhaaviadiffusa*, *Bryophyllumcalcycinum*and*Centellaasiatica*.



amines, 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 antioxidant activity such as phenol, flavonoids, tannin and vitamin were analysed in different plants of leaf *Abutilon indicum*, *Boerhaavia diffusa*, *Centella asiatica*, *Achyranthes aspera* and *Bryophyllum calycinum*. Table-24 and Figure.1 shows the total phenol contents of leaves of selected plants A,B, C,D, E. The total phenolic content of Plant C is higher than that of all other plants. Phenolic compounds are a class of antioxidant agents act as free terminators. Phenolic compounds have a variety of beneficial activities. They have potential antioxidants and free radical scavenger (Meenakshi *et al.*,2012).

Table-24 and Figure.2 shows the total flavonoids contents of leaves of selected plants A,B,C,D,E. Plant E shows the highest flavanoid content. Flavonoids are potent water soluble antioxidants and free radical scavengers, which prevent oxidative cell damage and have strong anti-cancer activity (Havsteen,2002).

Table-24 and Figure.3 shows the amount of vitamin-C contents of leaves of selected plants A,B,C,D,E. Plant D shows higher Vitamin C content than that of all other plants. Vitamin C is a vital component in human diet. Vitamin C is a non-enzymatic, water soluble antioxidant. Vitamin C functions in enzyme activation, oxidative stress reduction and immune function. It protects against respiratory tract infection and reduces risk for cardiovascular disease and some cancer (Vieta *et al.*, 2003).

Table-24 and Figure.4 shows the total amount of tannin contents of leaves of selected plants A,B,C,D,E. The tannin content of Plant E is higher when compared to

other plants. Tannins are present primarily in the leaves of trees growing in stress conditions. They are accumulated in the vacuoles, especially those of the epidermal layer and the palisade mesophyll. Tannins are useful in treating inflammation, ulcers, and remarkable activity in cancer prevention and anticancer activities (Li *et al.*, 2003; Akinpelu *et al.*,2009).

Table-24 Figure:5 shows the total vitamin-E of leaves of selected plants A,B,C,D,E. Plant C shows higher amount of vitamin-E than other plants. Vitamin E is a fat-soluble nutrient found in many foods (Jacob *et al.*, , 1995). In the body, it acts as an antioxidant, helping to protect cells from the damage caused by free radicals. Free radicals are compounds formed when our bodies convert the food we eat into energy.

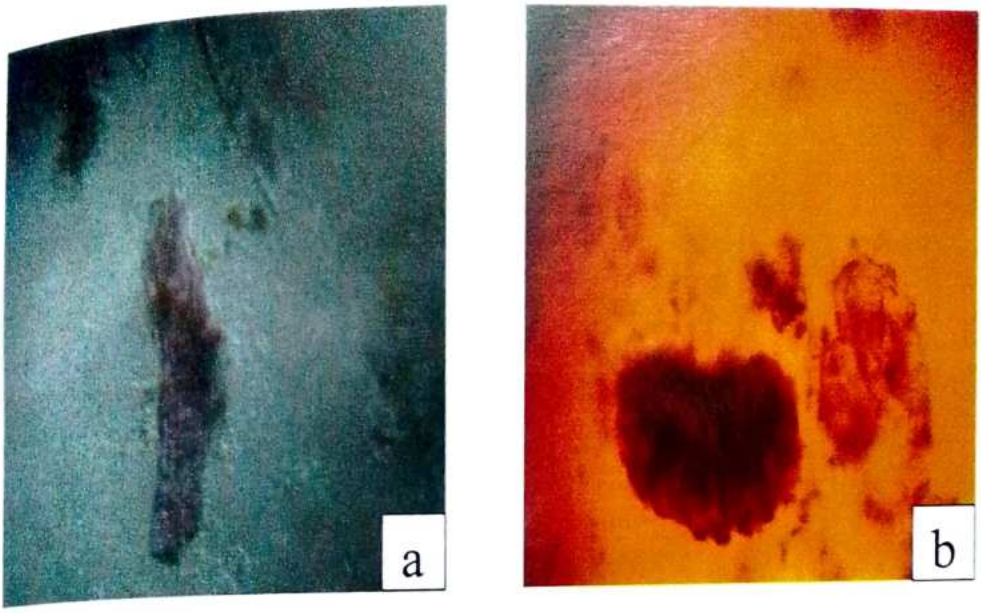
Powder microscopy

Evaluation of the crude drug is an integral part of establishing correct identity of the drug. Pharmacognostical parameters are necessary for the confirmation of the identity of the crude drug. The powder microscopic evaluation of leaf of *Abutilon indicum ,Boerhaavia diffusa, Centella asiatica, Achyranthus aspera , Bryophyllum calcycinum* sample is useful for setting standards for identification and authentication of the drug not only in crude form but also in finished products.

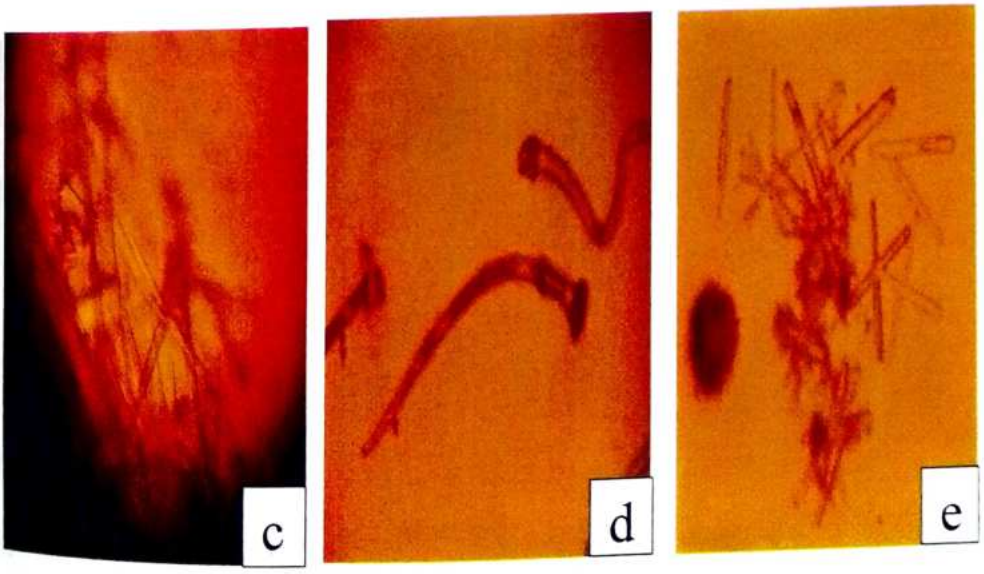
Under microscopical examination, the leaf of *Abutilon indicum ,Boerhaavia diffusa, Centella asiatica, Achyranthus aspera , Bryophyllum calcycinum* leaf powder shows the fibers, cortical cells, xylem vessels, tracheid cells, trichomes and stellate hairs. The photographs were taken and shown Plate (2 & 3). These results were supported by the work done by Maria Sumathi, 2014 and Sheela, 2014

Plate 2

Abutilon indicum **leaf powder shows (a) xylary fibers and (b) cortical cells.**



Achyranthes aspera **leaf powder shows (c, d) long multicellular warty trichomes and (e) Stellate hair**



Boerhaavia diffusa **leaf powder shows (f) xylem vessel**

Bryophyllum calcycinum **leaf powder shows (g) trichome**

Centella asiatica **leaf powder shows (h) tracheids**

FTIR- Spectroscopy **Analysis**

The functional FTIR Spectrum was used to identify group of the active components based on the peak values in region of infra-red radiation. The infra-red spectroscopic (IR) analysis of plant A,B,C,D and E in a band width ranging from 400, to 4000cm^{-1} revealed the presence of different functional groups which is shown in Tables 25-29 and Figure 6-10. The peaks showed that the extract of plant A,B,C,D and E may have compounds like Iodole compounds, aliphatic, aromatic, nitro compounds, amine, ester, pyrimidine, sulphides ,lactans, disulphide compounds pyrazola and trizola.

Ragavendra *et al.*,(2011) in *Aerva lanata* and Thangarajan Starlin *et al.*, 2012) in *Ichnocarpus frutescens* screened the functional groups of carboxylic acids, amines, amides, sulphur derivatives ,polysaccharides, organic hydrocarbons, halogens that are responsible for various medicinal properties.

Parag and Bhanu (2013) analyzed the methanolic leaf of *Ampelocissus latifolia* by FTIR and reported that the transition metal carbonyl compounds and aliphatic fluoro compounds were only present in the extract. Phytochemical analysis of *Vitex altissima* using UV-VIS, FTIR and GC-MS was carried out by Sahaya *et al.*, 2012). The FTIR spectrum confirmed the presence of alcohols, phenols, alkanes, alkynes, alkylhalides, aldehydes, carboxylic acids, aromatic, ionic compounds and amines in different extracts.

Antibacterial activity

In the present study, antibacterial activity of different solvents (acetone , methanol and water) using samples A, B, C, D and E were tested against selected strains of bacteria such as *Bacillus substilis*, *Escherichia coli*, *Proteus vulgaris*

Table: 25Spectral peak values and fu[illegible] obtained for the leaf pellets of *Abutilo[illegible]*

Peak value	Bond	Functional group
3422.42	Medium,urethanes	N-H
2923.36	Strong, alkane	C-H
2362.96	Weak, sulphinic acid	O-H
1736.16	Strong,carbonyl	C=O
1637.29	Strong,guanidine	C=N
1457.66	Strong, nitro group	N=O
1267.33	Strong,acid	C=O
1020.59	Strong,ether	C-O
880.83	Weak,sulphinic acid	O-H
669.93	Strong,primary amines	N-H
466.71	Strong,alkyl halide	C-BR

Figure:6 FTIR spectrum of leaf pellets of *Abutilon indicum.*

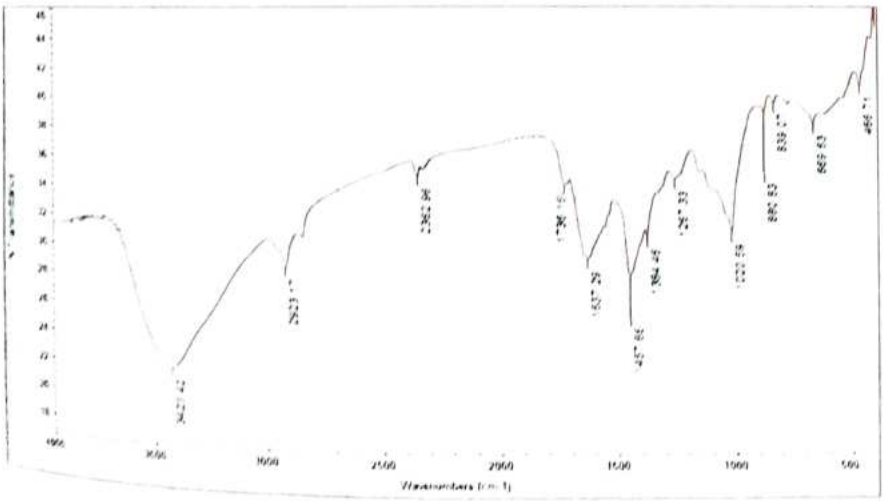


Table 26 Spectral peak values and functional group obtained for the leaf pellets of *Achyranthesaspera*

Peak value	Bond	Functional group
3423.86	Medium,urethanes	N-H
2923.30	Strong,alkane	C-H
2364.64	Weak,sulphinic acid	O-H
1637.43	Strong,guanidine	C=N
1458.77	Strong,nitro group	N=O
1384.77	Strong,nitro group	N=O
1319.18	Strong,nitro group	N=O
1261.98	Strong,acid	C-O
1020.52	Strong,ether	C-O
669.63	Strong,primary amines	N-H
466.89	Strong,alkyl halide	C-BR

Figure:7 FTIR spectrum of leaf pellets of*Achyranthesaspera*.

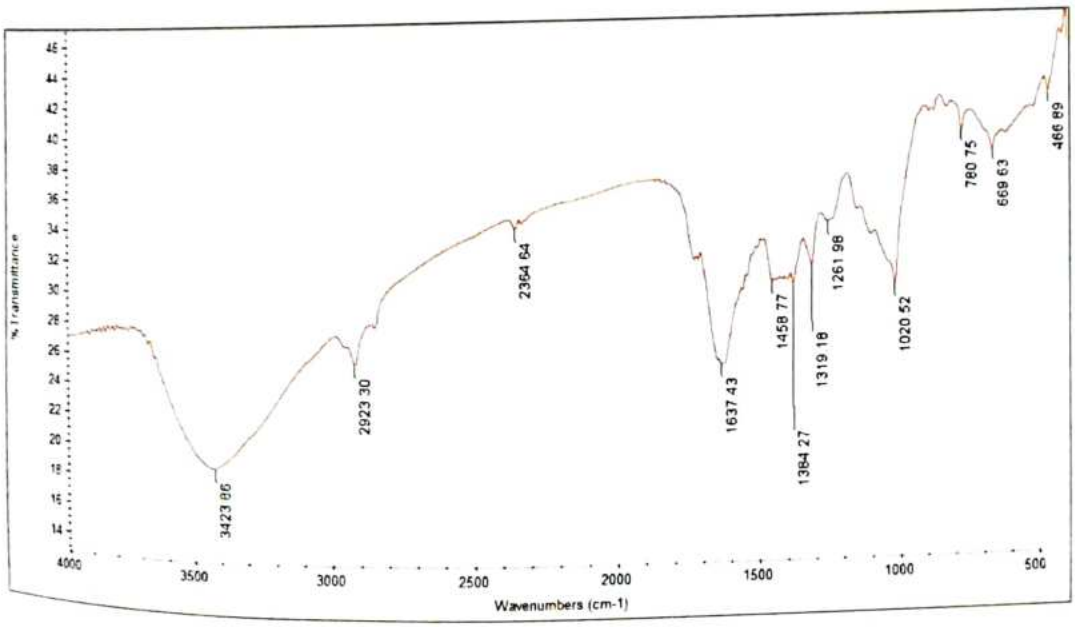


Table 27 Spectral peak values and functional group obtained for the leaf pellets of *Boerhaavia diffusa*

Peak value	Type of vibration	Funtional group
3422.42	Medium, urethanes	N-H
2923.36	Strong, alkane	C-H
2362.96	Weak, sulphinic acid	O-H
1736.16	Strong, carbonyl	C=O
1637.29	Strong, guanidine	C=N
1457.66	Strong, nitro group	N=O
1267.33	Strong, acid	C=O
1020.59	Strong, ether	C-O
880.83	Weak, sulphinic acid	O-H
669.93	Strong, primary amines	N-H
466.71	Strong, alkyl halide	C-BR

Figure:8 FTIR spectrum of leaf pellets of*Boerhaaviadiffusa*

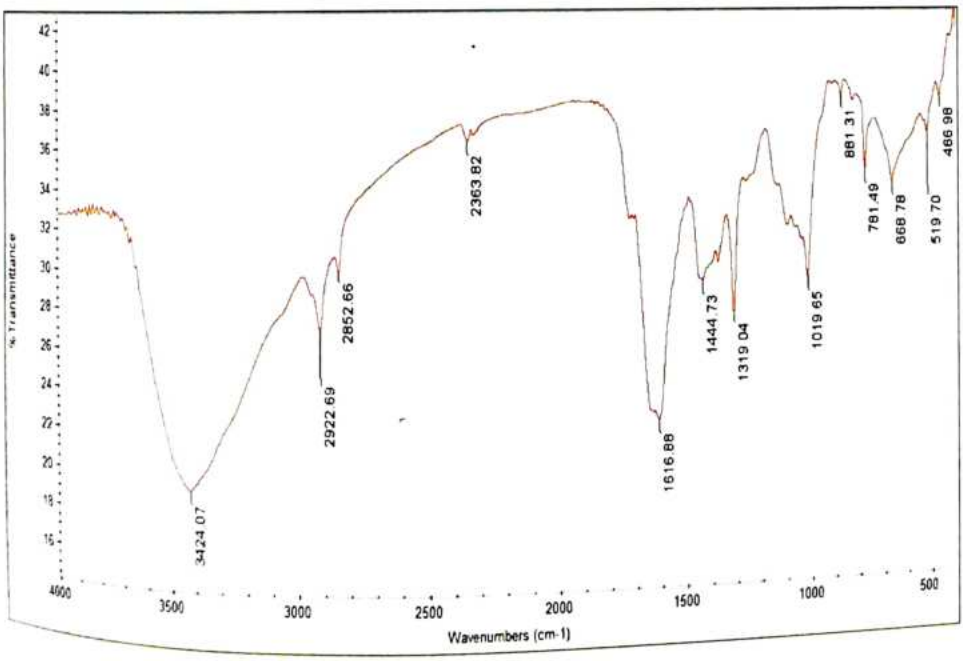
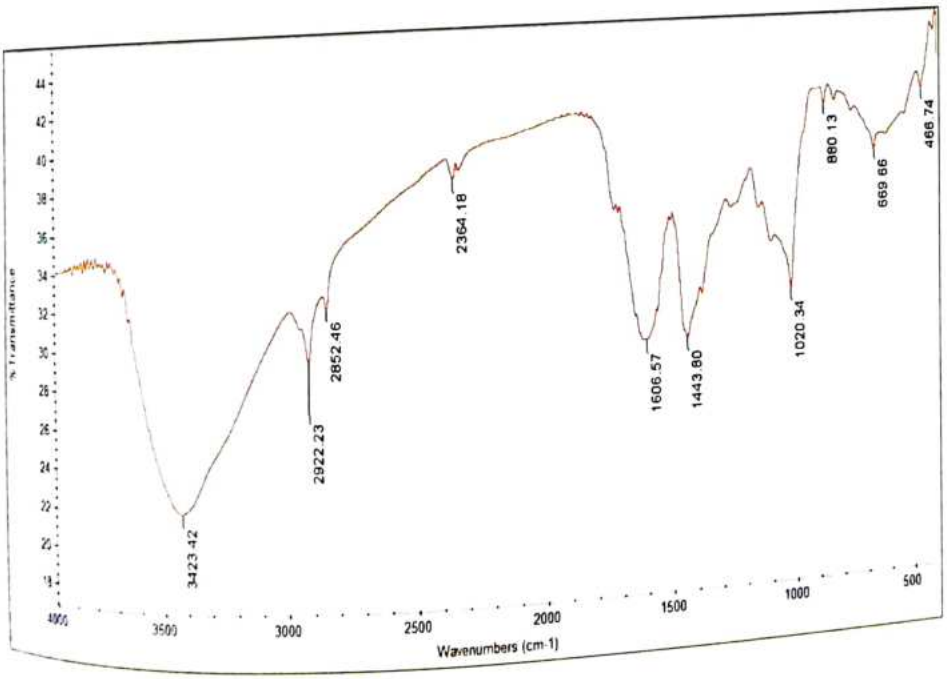


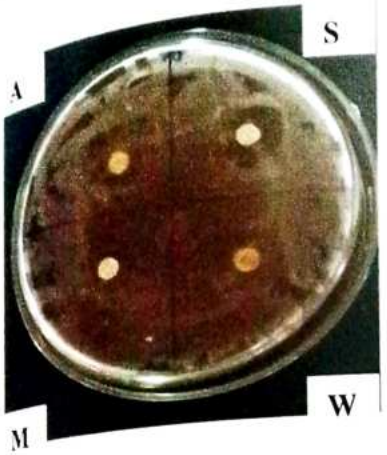
Table-28 Spectral Peak values and functional group obtained for the leaf pellets of *Bryophyllum.*

Peak value	Bond	Functional group
3423.42	Medium,urethanes	N-H
2922.23	Strong,alkane	C-H
2852.46	Medium,ester	C-H
2364.18	Weak,sulphinic acid	O-H
1606.54	Strong,guanidine	C=N
1020.34	Strong,ether	C-O
880.13	Weak,sulphinic acid	O-H
669.66	Strong,primary amines	N-H
466.74	Strong,alkyl halide	C-BR

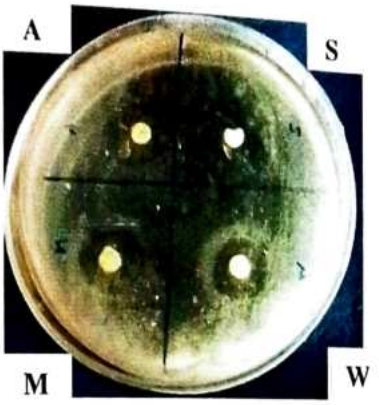
Figure:9 FTIR spectrum of leaf pellets of *Bryophyllumcalicinum.*



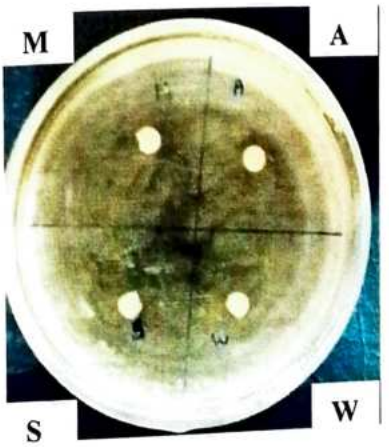
Antibacterial activity of different solvent extracts of *Abutilon indicum* leaf against bacterial strains



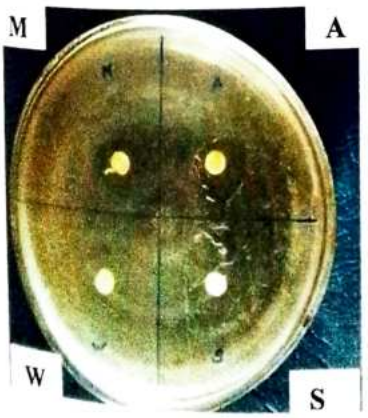
E. coli



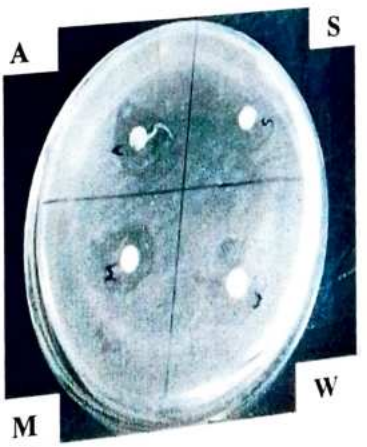
Bacillus subtills



Salmonella typhi



Proteus vulgaris

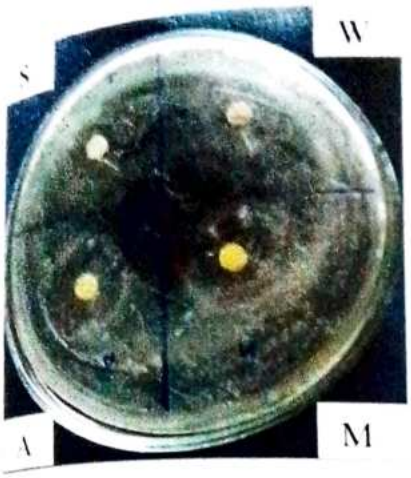


Kelbsiella pneumonia

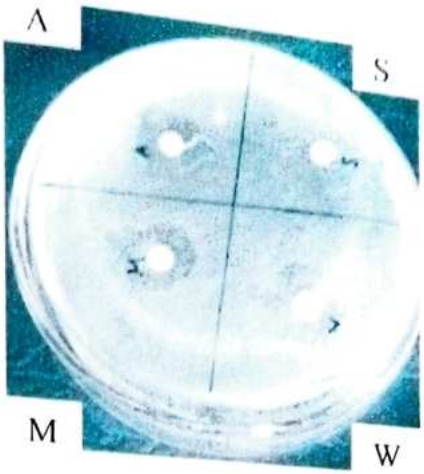
A – Acetone M – Methanol S – Streptomycin W – Water

Plate

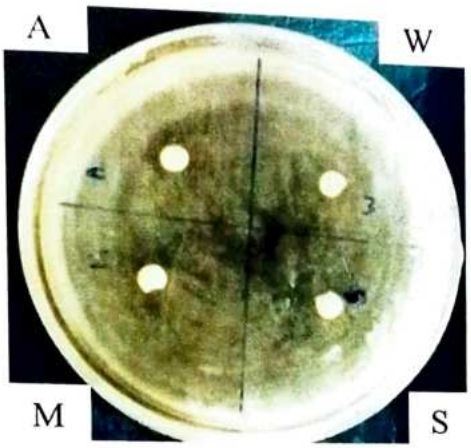
Antibacterial activity of different solvent extracts of *Achyranthus aspera* leaf against bacterial strains



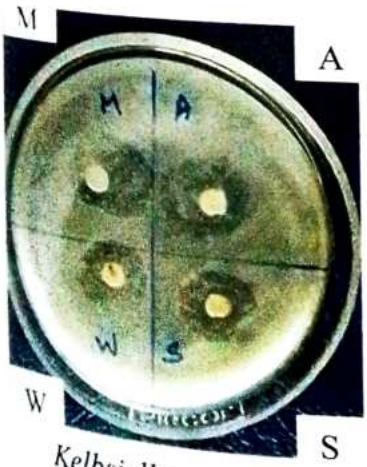
E. Coli



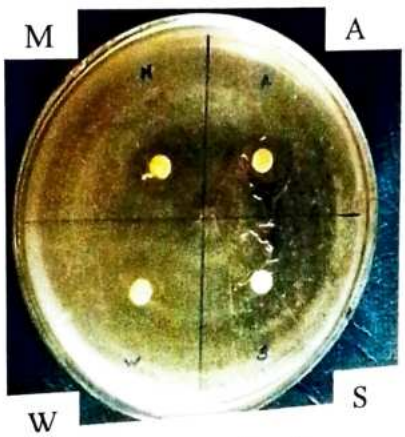
Bacillus substills



Salmonella typhi



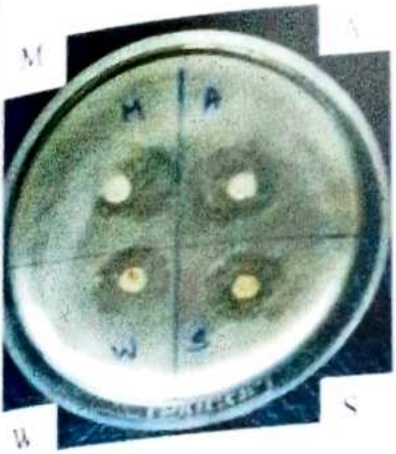
Kelbsiella pneumonia



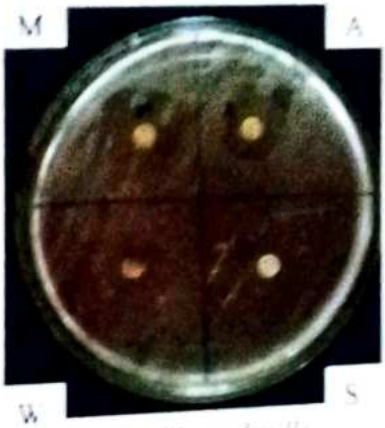
Proteus vulgaris

Plate -6

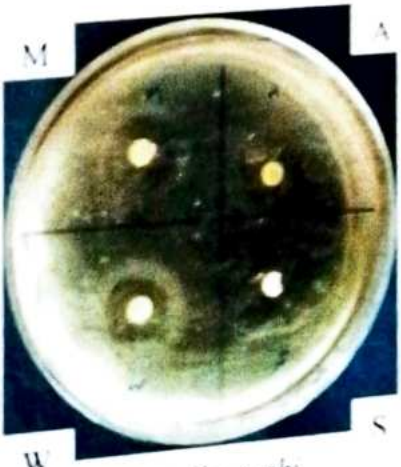
Antibacterial activity of different solvent extracts of *Boerhaavia diffusa* leaf against bacterial strain



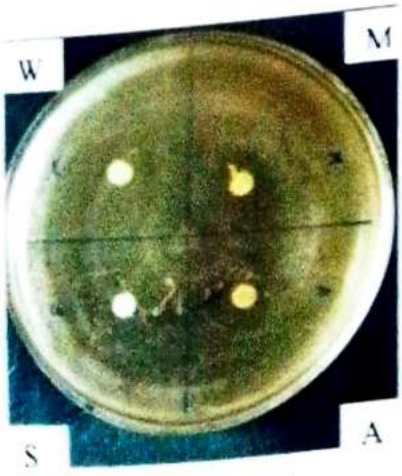
E. coli



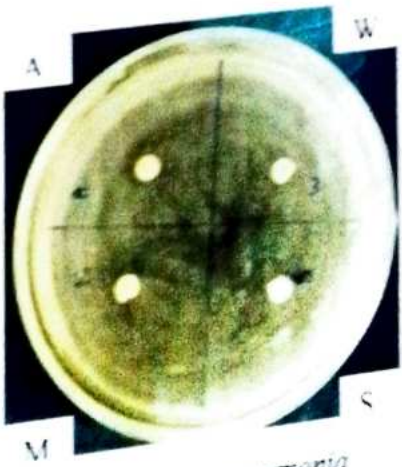
Bacillus substills



Salmonella typhi



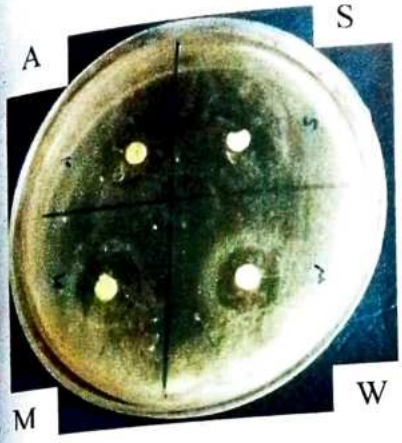
Proteus vulgaris



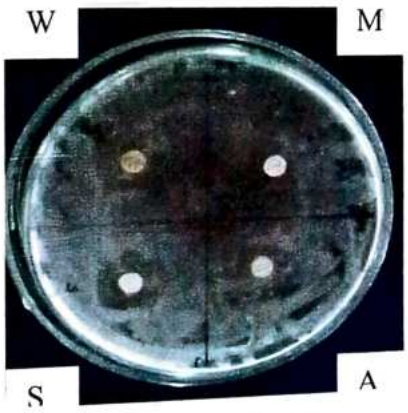
Kelbsiella pneumonia

Plate -7

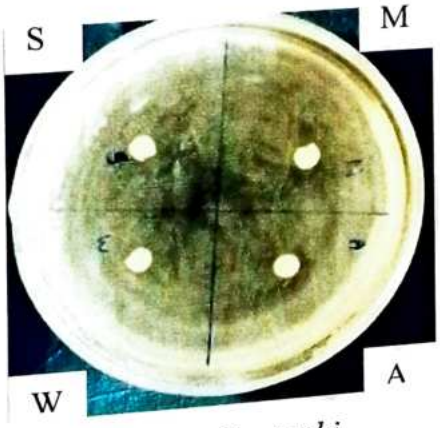
Antibacterial activity of different solvent extracts of *Bryophyllum calicycinum* leaf against bacterial strain



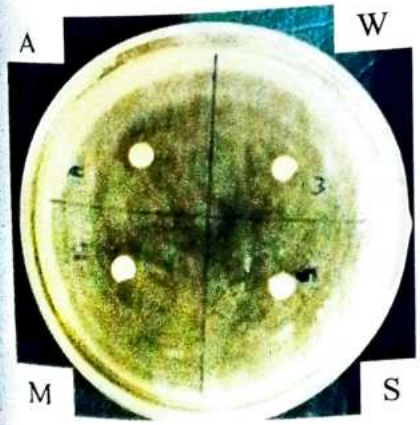
E. coli



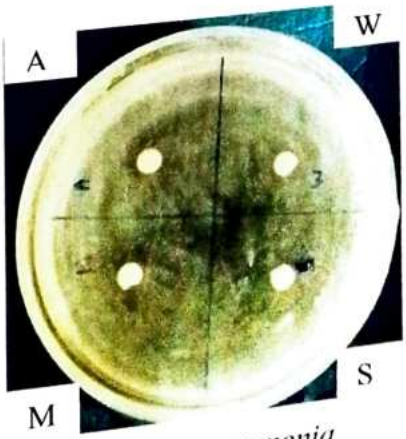
Bacillus substills



Salmonella typhi



Proteus vulgaris



Kelbsiella pneumonia

Table-30 Antibacterial activity of *Abutilon indicum and Acyranthes asperaextracted* against selected strains of bacteria

Microorganisms	*Abutilon indicum* (Inhibition zone in mm)			*Acyranthes aspera* (Inhibition zone in mm)		
	Acetone	Methanol	Water	Acetone	Methanol	Water
E.coli	8	6	2	7	5	4
Bacillus substills	7	9	5	9	4	4
Salmonella typhi	4	7	3	6	5	3
Proteus vulgaris	9	3	5	4	7	3
Klebsiella pneumonia	4	8	6	6	3	7

Table- 31 **Antibacterial activity of *Boerhaavia diffusa* and *Bryophyllum calicinum* extracted with selected solvent against selected strains of bacteria**

Sample	*Boerhaavia diffusa* (Inhibition zone in mm)			*Bryophyllum calicinum* (Inhibition zone in mm)		
Microorganisms	Acetone	Methanol	Water	Acetone	Methanol	Water
E.coli	7	5	3	8	6	3
Bacillus substills	9	6	4	5	3	2
Salmonella typhi	8	4	3	7	5	4
Proteus vulgaris	7	3	2	9	10	3
Klebsiella pneumonia	6	5	4	8	5	3

Table-32antibacterial activity of *Centella asiatica* leaf extracted with selected solvent against selected strains of bacteria

Sample	*Centella asiatica* (Inhibition zone in mm)		
Micro organisms	Acetone	Methanol	Water
E.coli	4	7	3
Bacillus substills	6	10	2
Salmonella typhi	5	7	4
Proteus vulgaris	7	3	5
Klebsiella pneumonia	8	4	3

Salmonella typhi, *Klebseiella pneumonia* and are presented in Table (34-36). The diameter of the inhibition zones against these species ranged from (2 to 10mm).

The acetone, methanol and water extracts from leaf of *Abutilon indicum* exhibited maximum activity against methanolic extract of *Bacillus substilis* and acetone extract of *Ptoteus vulgaris* which is represented in Table 30 and Plate:4.

The acetone, methanol and water extracts from leaf of *Achyranthus aspera* also shows the exhibited maximum activity against acetone extract *of Bacillus substilis*, which is represented in Table 30 and Plate:7

The acetone, methanol and water extracts from leaf of *Boerhaavia diffusa* exhibited maximum activity against acetone extract of *Bacillus substilis* minimum activity against water extract of *Ptoteus vulgaris*, which is represented in Table 31 and Plate:5.

The acetone, methanol and water extracts from leaf of *Bryophyllum calcycinum* also shows the exhibited maximum activity against methanolic extract of *Proteus vulgaris* and minimum in water extract of *Bacillis substilis* which is represented in Table 31 and Plate:8

The acetone, methanol and water extracts from leaf of *Centella asiatica* exhibited maximum activity against methanolic extract of *Bacillis substilis* and minimum in water extract of same strain, which is represented in Table 32 and Plate:6

SUMMARY AND CONCLUSION

SUMMARY AND CONCULSION

The plants are rich source of secondary metabolites with interesting biological activities. In general, these secondary metabolites are an important source with a variety of structural arrangements and properties.

Abutilon indicum G.Don. *Achyranthus aspera* L. *Boerhaavia diffusa* L., *Bryophyllum calcycinum* Kurz and *Centella asiatica* Urh are well known medicinal plants. It is used as a therapeuticagent. The shrub *A.indicum* is specially used in the treatment of anti-diabetic, anti inflammatory,cleaning wounds and ulcers. It is widely used as traditional medicine for rheumatism, conjunctivitis and diabetes (Anushia *et al.*, 2009). *Boerhaavia diffusa* is used in the treatment of anemia, nervous weakness, paralysis. Leaf of *Centella asiatica* is used to treat the wounds, cellulitis and skin ulcers. The leaf of *Achyranthus aspera* plant is used in the treatment of diarrhoea, dysentery and rheumatic pains.

The preliminary phytochemical screening was carried out in the different extract of leafs of *Abutilon indicum* G.Don, *Achyranthus aspera* L *Boerhaavia diffusa* L, *Bryophyllum calcycinum* Kurz and *Centella asiatica* Urh. After performing the analysis, the following phytochemicals such as alkaloids, terpenoids, steroids, coumarin, tannins, saponin, flavones, phenols, protein, gum, carbohydrate and quinones have been screened. The medicinal effects of plants are considered to be due to secondary metabolites, especially secondary compounds, produced by plant species. In this study, flavonoid, tannin, vitamin C, vitamin E and phenol content of *Abutilon indicum* G.Don. *Achyranthus aspera* L. *Boerhaavia diffusa* L. *Bryophyllum calcycinum* Kurz and *Centella asiatica* Urh have been quantitatively analysed using spectrophotometric methods.

The powder analysis of the selected plant specimens showed the certain properties like, xylery fibres, cortical cells, trichomes, stellate hairs, xylem vessels and tracheids. These characters plays a vital role in determining their medicinal values. This analysis provided an insight in notiying the varying possibilities in them.

The FTIR analysis showed the major compounds present in these selected plants. It helped to narrow down the research work in the next level of studying their selective compounds.

The different solvent extracts of leaf *Abutilon indicum* G.Don. *Achyranthus aspera* L. *Boerhaavia diffusa* L. and *Bryophyllum calcycinum* Kurz and *Centella asiatica* Urh. were used for antibacterial studies against human pathogenic bacteria, *Bacillus substilis, Escherichia coli, Proteus vulgaris, Salmonella typhi and Klebseiella pneumonia.* These extracts showed ranging degree of antibacterial activity. The maximum activity was found to be 10mm zone of inhibition obtained by methanol extract of *Achyranthus aspera* leaf against *Proteus vulgaris.* The maximum activity was found to be 10mm zone of inhibition obtained by methanol extract of *Bryophyllum calcycinum* 10mm leaf against *Bacillus substilis.*

From the current study it is concluded that the selected medicinal plants have immense medicinal potential based on their phytochemical screening. The quantitative analysis also indicated the presence of good amount of selected phytochemicals. The antibacterial activity showed their defence against selected microorganisms. Further study needs to be carried out in finding and isolation of certain compounds using other advanced techniques. These plants are freely available in nature so estimating their higher medicinal values will help in the utilization of these plants in treating certain important diseases known to man.

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NUTRACEUTICAL, PHYTOCHEMICAL EVALUTION AND ANTIBACTERIAL ACTIVITIES OF SELECTED GREENS

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By

LJENOBIA

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DEPARTMENT OF BOTANY

ST. MARY"S COLLEGE (Autonomous)

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

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2019-2020

CERTIFICATE

This is to certify that this dissertation entitled, **"Nutraceutical,Phytochemical Evalution And Antibacterial Activites of Selected Greens"** submitted by **I.JENOBIA Reg.No. 18APBO03** to ST.MARY'S COLLEGE (Autonomous), THOOTHUKUDI in partial fulfillment for the award of the degree of **"Master of Science in Botany"** is done by her under my supervision. It is further certified that this dissertation or any part of this has not been submitted elsewhere for any other degree.

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DECLARATION

I do here by declare that this dissertation entitled **"Nutraceutical, Phytochemical Evalution And Antibacterial Activities of Selected Greens"** Submitted by me in partial fulfilment for the award of the degree of **„Master of Science in Botany"**, in the result of my original and independent work carried out under the guidance of **Dr. G. Flora, M.Sc., M.Phil., Ph.D.,** Assistant Professor, Department of Botany, St. Mary's College (Autonomous), Thoothukudi and it has not been submitted elsewhere for the award of any other degree.

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INTRODUCTION

Introduction

Indian subcontinent is a vast repository of medicinal plant that is used in traditional medical treatment (Chopra *et al.*, 1956). Out of 17,000 species of higher plants reported to occur within India, 7500 are known to have medicinal uses (Shiva, 1996). The various indigenous systems such as siddha, ayurveda, unani and allelopathy use several plant species to treat different ailment (Rabe and Staden, 1997). The world health organization (WHO) also estimated that 80% of the population of developing countries rely on traditional medicine mostly plant drugs, for their primary health care need (Schminche, 2003).

Vegetables are the cheapest and most readily available sources of important proteins, vitamins, minerals and essential amino acid (Okafor, 1983). Many of the local vegetable materials are under-exploited because of inadequate scientific knowledge of their nutritional potentials.

S. oleraceae, A.aritis, A.gangeticus, in an annual herb belongs to the family Amaranthaceae and it is widely distributed, cultivated in India. Several parts of this plant are used in traditional Indian medicine for numerous therapeutic effects like laxative, diuretic, carminative, cooling, and flatulence. It is a rich source of vit-A ,vit-C, vit-E, vit-K, vit-B6,vit-B2,magnesium,manganese,folate,betaine,iron, calcium, potassium,folic acid ,copper, protein , phosphorous, zinc, niacin, selenium and omega-3 fatty acids MuninAgarwal (2011)

The nutrient content of different types of vegetables varies considerably and they are not major sources of carbohydrates compared to the starchy foods which form the bulk of food eaten, but contain vitamins, essential amino acids, as well as minerals

nd antioxidants (Mnzava, 1997; Fasuyi, 2006). According to Okafor (1983), egetables are the cheapest and most available sources of important proteins, Vita-mins, minerals and essential amino acids. Vegetables are included in meals nainly for their nutritional value is present.

Medicinal plants have a long-standing history in many indigenous communities nd continue to provide useful tools for treating various diseases. The practices of traditional medicine are based on hundreds of years of belief and observations, which predate the development and spread of modern medicine. Today, there is widespread nterest in herbal drugs. This interest primarily stems from the belief that herbal nedicines are safe, inexpensive and have no adverse effects. Medicinal plants are noving from fringe to main stream use with a greater number of people seeking remedies and health approach. It is no wonder that the world's one-fourth population i.e. 7.7 billion people, are dependent on traditional medicines for the treatment of various ailments. However a key obstacle, which has hindered the acceptance of the alternative medicines in the developed countries, is the lack of documentation and stringent quality control. There is a need for documentation of research work carried out on traditional medicines. With this backdrop, it becomes extremely important to make an effort towards standardization of the plant material to be used as a medicine. The process of standardization can be achieved by stepwise nutritional value and phytochemical studies. These studies help in identification and standardization of the plant material. Correct identification and quality assurance of the starting materials is an essential prerequisite to ensure reproducible quality of medicine which will contribute to its safety and efficacy.

Plants are one of the most important source of medicine and are said to be biosynthetic laboratory for most of the active principles like alkaliodes, glycosides, resins, tanins , flavonoids , volatile oil, gums, deo resins etc. which exhibit different dose dependant pharmacology and therapeutic effects. Today a large number of drugs prepared from plants are regularly used. The advantage of green drug therapy is safety economy and easy availability (prakash and guptha, 2005). Because of these advantages medicinal plants are widely used by the traditional practioners.

Amaranthus aritis (Amarantheceae), it is distributed throughout the hotter parts of India. The leaves extract was reported to contain alkaloids, flavonoids, sterols, triterpenoids, and glycosides. It has been reputed in the Siddha system of medicine as a remedy for jaundice, piles, ulcer and leprosy. The plant is also reported to possess analgesic activity and to have an effect on fertilization (Okafor , 1983). In some places, juice from the leaves of the plant is used in combination with the liquid extract of *A. Cepa* to treat jaundice, and hepatoprotective studies on experimental animals confirmed the above activity. The present study was carried out to determine the antibacterial activity of different extracts of the leaves on gram positive and gram negative organisms. A survey of literature revealed that the FTIR analysis of functional groups was not done so far with the greens such as *Amaranthus aritis, Amaranthus gangeticus,* and *Spinacia oleraceae.* Hence, an attempt is made in the present study to analyse the functional groups of phytoactive compounds present in the leaf extracts (in different solvents such as petroleum ether, chloroform, ethanol and methanol) of the four Indian medicinal plants, *Amaranthus aritis, Amaranthus gangeticus* and *Spinacia oleraceae* by FTIR spectroscopic analysis.

SCOPE AND OBJECTIVES

Scope and objective

The main objective of this study is to supplement constructive information with regard to nutrition and medicinal values of selected three greens (*A.aritis, S.oleraceae , A. gangeticus)*

Keeping these in mind the present work is concentrated on

- To detect the presence of phytochemical compounds from *A.aritis, S.oleraceae , A. gangeticus*
- To determine the proximate analysis (protein, Ash contents, moisture contents and carbohydrate) of selected three greens. To Quantify the phytochemical compounds of *A.aritis, S.oleraceae , A. gangeticus* by petroleum ether, ethanol, methanol, chloroform, and aqueous solution.
- To measure the vessels and tracheids of green leaves using maceration technique.
- To study the functional group of greens by FTIR analysis.
- To study the antibacterial activity of methanol, chloroform and aqueous extract of three greens against the bacterial strains (*Bacillus substilis, Escherichia coli, Ptoteus vulgaris, Salmonella typhi* and *Klebseiella pneumonia).*

LITERATURE REVIEW

Literature review

Phytochemical studies :

Akubugwo, I. E *et al.,* (2007) Studied the nutritional and chemical value of *Amaranthus hybridus* were investigated using standard analytical methods in order to assess the numerous potential of the plant leaves. the results revealed that the leaves contain an appreciable amount of nutrients, aminoacids and phytochemicals and low levels of toxicants.

Igbinosa *et al.*,(2009) crude ethanolic, methanolic and water extracts of the stem bark of *Jatropha curcas* exhibited antimicrobial activities. Phytochemical screening revealed the presence of saponin, steroid, tannine, glycosides, alkaloids and flavanoid in the extracts.

Huang,WY , Cai YZ (2010) Natural phenolic compounds from medicinal herbs and dietary plants: potential use of cancer prevention.

RNS Yadav and MuninAgarwal (2011) suggested that the traditional medicine practice is recommended strongly for some medicinal plants due to the presence of bioactive phytochemicals.

Bishnu *et al* (2011) performed a qualitative phytochemical analysis for the detection of alkaloids, glycosides, terpenoids, steroids, flavonoids, tannins and reducing sugars in *Ocimum sanctum, Eugenia caryophyllata, Achyranthes bidentata* and *Azadirachta indica.*

Arya.V *et al., (2012)* conducted Photochemical screening of petroleum ether,chloroform, ethanol, aqueous and hydroalcoholic extracts of leaves of *Psidim guajava* which revealed the presence of flavonoids, tannins triterpenoids, saponins,

sterols, alkaloids and carbohydrates by positive reaction with the respective test reagent. Phytochemical screening showed that maximum presence of phytoconstituents in ethanolic and hydroalcoholic extracts.

Hemalatha,K. (2012) studied two phenolic compounds (*P*- Coumaric acid and α–Tocopherol), two flavonoidal compounds (luetoline and 7-methoxy luetoline) and two iridoidal glycosides (barlerin and schanshiside methyl ester) have been isolated from leaves of *Barleria cristata* their structure were established by spectral analysis. This is the first report of occurrence of these compounds from *Barleria cristata Linn* plant.

Tariq and Reyaz (2013) conducted quantitative phytochemical analysis of traditionally used medicinal plant *Terminilia chebula.*

The phytochemical analysis showed that the ten plants (*P. amarus, S. auriculata, P. maderaspatensis, S. torvum, A.* hypochondriacus, *A. cruentus, A. caudatus, A. aritis, A. gangeticus)* are rich in at least one of alkaloids, flavonoids, terpenoids, reducing sugars and phlobatannins. Plant *Psidium gujauva* having all these phytochemicals. The phytochemical screening and qualitative estimation of 10 medicinal plants studied showed that the leaves were rich in phlobatannins, terpenoid, flavonoids, alkaloids and reducing sugar (Wadood *et al.*, 2013)

Different solvents of varying polarities were used by Malahubban M. *et al.*, (2013) for the extraction of bio-active compounds, including *Andrographolide* in *Andrographis paniculata*, and rosmarinic acid in *Orthosiphon stamineus* leaves. The methanolic extracts of *A.paniculata* and *O.stamineus* exhibited antimicrobial and antioxidant properties. Methanolic and ethanolic extracts of *A. paniculata* and *O.*

stamineus leaves also produced the strongest antioxidant activities as compared with extracts using other solvent.

Nag,S.(2013) analysed the preliminary phytochemical of the medicinal plant *Barleria lupulina Lindl.* phytochemical analysis indicated the presence of alkaloids, starch, tannin, redusing sugar, protein, Flavanoids, amino acids and lignin present in the methanolic extracts of *Barleria lupulina Lindl.*

Abdul *et al* (2013) analysed phytochemicals of medicinal plants occurring in local area of Marden which showed that the plant (*H.integrifolia, H.heteroclita, C.marginata, A.viridis, A.aritis, A.gangeticus, S.oleraceae, A.paniculata, A.indica, C.auriculata)* tested were rich in atleast one of alkaloids, flavonoids, terpenoids, reducing sugars and phlobactannin.

Venkateswarlu, G. (2014) studied the indigenous drug *Barleria montana* nees in wall used by different ethnic groups of the world for the treatment of diseases have special significance from long time like diabetes, wounds, cuts, hepatoprotective, etc. The preliminary phytochemical studies revealed presence of the presence of Alkaloids, Carbohydrates, Glycosides, Gums, Phenols, Proteins, Saponins, Steroids, Fixed oils and fats.

The photochemical screening of *Albizzia lebbeck* by Vasanthi. P *et al.*, (2014) revealed the presence of phenols, steroids, tannins, saponins and flavonoids in the hydroalcohol leave and bark extracts.

Karthik , K *et al*., (2014) studied the quantitative phytochemical analysis of *Hypochaeris radicata* species exhibited the presence of alkaloids, total phenolics, total flavonoids, tannins, saponins and ascorbic acid in considerable quantity.

Garima et al (2014) evaluated phytochemical, antibacterial and free radical scavenging properties of *Azadirachta indica* leaves.

Zayed,M.Z. *et al*.,(2014), studied of phytochemical constituents in leaf extracts of *Neolamarckia cadamba* (rubiaceae) from Malaysia. The results thus concluded that *N. cadamba* leaves possess various potent bioactive compounds and is recommended as a plant of phytopharmaceutical importance.

Martins, D and Nunez,C.V (2015) reported phytochemical studies addressing all species of Rubiaceae. Iridoids, anthraquinones, triterpenes, indole alkaloids as well as other varying alkaloid subclasses, have shown to be the most common. These compounds have been mostly isolated from the genera *Uncaria*, *Psychotria*, *Hedyotis*, *Ophiorrhiza*and *Morinda*.

Twinkle,S.,Bansode,D.R.Salalkar,P.K.(2015) recorded phytochemicals as well as the mineral content in selected medicinal plant extracts of *Trigonella foenum-graecum, Syzygium cumini, Terminalia Chebula and Salvadora persica*. It was found that flavonoid is present abundantly in all species. Saponins and tannins are also present in almost all species studied.

Kumar, S.Venkateshwar, R.C *et al*.,(2015) studied the phytochemical analysis revealed the presence of alkaloids, saponins, tannins, flavonoids, terpenoids, coumarins, quinines, cardiac glycosides, Xanthoproteins, glycosides, steroids,

phenols, resins, carboxylic acid group in varying concentrations. This research supports the local use of the leaf extract of the plant *Holoptelea integrifolia* for pediculoses and *Celestrus emarginata* for increasing male sex vigour.

Tabassum,B *et al.*,(2015) recorded the extracts (spinacia oleraceae) were found to be rich in bio active compounds like flavonoids, carbohydrates-reducing sugars, monosaccharides, glycosides of cardiac, coumarins, anthraquinones, and steroids.

Chigozie, and Ezeonu,C.S (2016) studied the qualitative and quantitative analysis reported that *Moringa oleifera, Barteria nigritiana, Anogeissus leiocarpus, Allanblackia floribunda, Albizia adianthifolia, Afzelia bipindensis, Pentaclethra macrophylla*, and *Cassipourea barteri* were contain heavy content of steroids. Glycoside was heavily present in *Moringa oleifera, Afzelia bipindensis, Tetrapleura tetraptera, Combretodendron macrocarpum*, and *Cordia millenii*.

Qualitative Phytochemical screening resulted in the presence of alkaloids, saponins, flavonoids, tannins, total phenolics and absence of terpinoids and resins in A. viridis leaf extract. Results showed the high antioxidant activity of A. viridis which is due to presence of high phenolic contents revealed by phytochemical analysis Sadia, S . *et al.*,(2016).

phytochemical analysis done by Gul,R. *et al* (2017) and result to showed that he *Ephedra intermedia* plant extract contains a mixture of phytochemicals as reducing sugars, cardiac glycoside, phenolic compounds, flavonoids, and alkaloids.

Phytochemical screening showed that maximum presence of phytoconstituents in ethanolic and hydroalcoholic extract of *Hertia cheirifolia L.* (Majoul,K. et al., 2017)

Qualitative screening using ethanolic extract indicated the presence of all the phytochemical constituents in *P. amarus and E. heterophylla*, and the absence of anthraquinone in *S.occidentalis, P.nigrum, A.conyzoides* and *G. latifolium*. Coumarin was also absent in *S.occidentalis* and *G.latifolium* Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties which are considered to be beneficial to human health. The presence of bioactive ingredients and the quantitative determination of the percentage crude yield of chemical constituents of the different plant species studied showed that the leaves are rich in alkaloids, tannins, flavonoids, saponins and phenols (Ajuru, G. 2017).

Rukshana, M.S, Doss A and Rani K.P (2017) studied the qualitative phytochemical screening of the ethanolic extracts of the leaves revealed the presence of many compounds such as flavonoids, tannins, alkaloids, terpenoids, steroids and phenols. This study result will make a way for the production of herbal medicines for various ailments by using *Pergularia daemia* leaves.

Qualitative phytochemical analysis showed the presence of flavanoids, tannins, terpenoid, saponin and steroids in the *Centella asiatica* highest amount of phenolic, flavanoid and tannin content was present in the shoot culture (Roy, A. *et al.*, 2018).

Olasupo, A.D. (2018) Preliminary phytochemical screening studied on *Spinach oleraceae* cure abundantly present.The study revealed that Cardenolides,

glycosides, flavonoids, phenol and *Spinach oleracea.* This credit to maximum activity of ethyl acetate.

Kumari, S.(2018) phytochemical analysis of the extracts of *A. viridis* revealed the presence of major phytochemical compounds, including flavonoids, alkaloids, phenolics, steroids, terpenoids, saponins, cardiac glycosides, and tannins.

Proximate analysis:

Ismail, M. (2012) to study the proximate analysis of *Peperomia pellucid* L, an underexploited weed plant in Malaysia. The results indicated (*P. pellucid)* to be rich in cured protein,carbohydrate and total ash contents.

The Proximate analysis showed that the (*Lannea schweinfurthii, Rhus natalensis Euclea divinorum)* moisture, ash, crude protein, crude lipid, crude fibre and carbohydrates in all the plants investigated. The plants showed that the leaves used in treating with headache, dermatological, gastrointestinal, gynaecological, abdominal pains(Oyugi, J.2013)

Iqbal,S.(2016) studied the proximate composition of foods includes moisture, ash, lipid, protein and carbohydrate contents. Sample(*A.aspera)* collection and preparation must be considered carefully to ensure analysis of a homogeneous and representative sample, and to obtain accurate results. Estimation methods of moisture content, ash value, crude lipid, total carbohydrates, starch.

Erhunse, N.(2018) recorded showes the proximate composition of the dried leaf of *M. cercropioides* moisture (60.58 ± 0.43 %), crude protein (6.58 ± 0.4%),

crude fibre (8.85 ± 0.05%), crude fat (7.0 ±1.0%), ash (4.75 ± 0.25%).The results of the proximate analyses revealed the plants samples have high moisture content.

Maceration studies:

Ishii,S.,and Yokotsuka,T (1971) studied the tissues of higher plant the cells are cuddled up each other with intracellular cementing materials. The degradation of cementing materials causes the loss of tissue coherence and leads to tissue-maceration. Subsequently, the cells are liberated separately from tissues. Although the nature of cementing materials has not been made clear, pectic substances have been considered main component in parenchymatous tissues.

Mahesh, S.*et al.*, (2015) studied the *B. serrata* growing in Institutes campus and their fibers were macerated using different concentrations of macerating agent (nitric acid). 50% nitric acid was found very efficient to separate out all the fibers. This maceration protocol resolves clearly all the compact fibers and made their measurement convenient.

Experimental conditions of anthocyanin sextraction from dried calyces of *H. sabdariffa*, followed by maceration technique. Ethanol – water proportion in the case of maceration, were the relevant independent variables, while for ultrasound assisted extraction, the ultrasonic power was used instead of temperature which was kept constant is recorded by Jahorina*et al.*, (2018).

Epifano, F. (2018) assessed maceration at room temperature and by heating, ultrasound-assisted, and microwave-assisted extractions were also carried out for comparison. Results obtained with electromagnetic induction heating showed that this

methodology performed largely better both in terms of time process and extraction yields.

FTIR studies:

Muruganantham *et al* (2009) carried out the FTIR analysis of plant parts like leaf,stem,of the medicinal plants, *A.indicum* and *T.populnea* are reported the functional group of carboxylic acids, amines, sulphur derivatives, nitrates, chlorates and carbohydrate that are responsible for various medicinal properties of both herbal plants.

The FTIR analysis of methanolic and aqueous leaf extracts of *Bauhinia racemosa* revealed the presence of protein, oil, fats, phenolic compounds, flavonoids, saponins, tannins and carbohydrate as major functional groups (kumar,G. *et al.*, 2010).

Ragavendran *et al.,* (2011) screened the functional groups of carboxylic acids, amines, amides, sulphur derivatives, polysaccharides, organic hydrocarbons, halogens that are responsible for various medicinal properties of *Aerva lanata.*

Starlin, T *et al.*, (2012), analysed the ethanolic extracts of *Ichnocarpus frutescens*, by FTIR, which revealed the functional group components of amino acids, amides, amines, carboxylic acid, carbonyl compounds, organic hydrocarbons and halogens.

Ashokkumar and Ramaswamy, (2013), studied the functional groups observed in the extracts, OH group was found to be present uniformly only in the methanol extracts of all plants. As OH group has got the ability of forming hydrogen bonding

capacity, presence of OH group particularly in methanol extract of leaf of all the 4 plants (*A.aritis, P.guajava, C.auriculata, S.oleraceae)* probably indicates the higher potential of methanol extract towards inhibitory activity against microorganisms.

Pednekar, P.A. and Raman, B.(2013) carried out the FTIR spectroscopic analysis of methanolic leaf extract of *Ampelocissus lantifolia* for antibacterial compound.

Kumar, S.S and Manoj, P (2015) analysed FTIR for five green leafy vegetables (GLVs) viz., *Hibiscus cannabinus* L., (kenaf), *H.sabdariffa* L., (roselle), *Basella alba* L., (vine spinach), *B.rubra* L. (malabar spinach) and *Rumex vesicarius* L., (sorrel) confirmed the presence of free alcohol, intermolecular bonded alcohol, intramolecular bonded alcohol, alkane, aromatic compounds, imine or oxime or ketone or alkene, phenol and amine stretching.

The functional group identification is made by FTIR analysis and the active components based on the peak value in the region of infrared radiation. The ethanolic flower extract of *Erythrina variegata* L. is passed into the FTIR spectroscopy and the functional groups of the components are separated based on the peak ratio. The results of FTIR analysis confirmed the presence of functional groups such as non bonded, O-H stretch, carboxylic group, acidic, H bonded, C-H stretch, asymmetric stretching of –CH (CH2) vibration, C=N (stretch), carbon-carbon triple bond, multiple bonding, carbonyl compound frequency, C=O stretch, C=C stretch, O-H bend, alcoholic group, C-N stretch, C-O stretch, PO3 stretch, =C-H bending and C-Cl (Hemmalakshmi, S *et al.*, (2017).

Antibacterial activity:

Manjusha *et al.*, (2010) methanol extracts of the dried leaves of *Amaranthus spinosus* were used for phytochemicals, physiochemical and antibacterial analysis. The minimum inhibition concentration of extracts showed that 178 µg/mL

Deshpande R. *et al.*, (2011) evaluated antibacterial activity of different extract of *J. regia* againstoral micro flora and found that acetone extract was found to be more effective against oral microflora. Similarly results were observed chloroform extract of *Barleria prionitis* L. leaves.

Diwan, P.D. and Gadhikar, Y.A. (2012) studied the antibacterial activity of aqueous, petroleum ether, chloroform and acetone extract of leaves of *Barleria prionitis* (Family-Acanthaceae) against seven bacterial species (*Lactobacillus rhamnosus*, *Staphylococcus aureus*, *Bacillus subtilis*, *Actinomyces viscoscus*, *Staphylococcus epidermidis*, *Escherichia coli*, *Streptococcus mutans*) which are known to cause oral diseases in human being. Result showed that almost all the test organism from the study were found to be effected by all the four type of extract of *Barleria prionitis* leaves but pronounced inhibition of the four extract was observed for bacterial species, *Lactobacillus rhamnosus.* Chloroform extract was found to be more effective against the entire test microorganism.

Kumar,N.U and Kumar,S (2013) evaluated that the antibacterial activity of rhizome of *Barleria prionitis* in methanol extract. The methanol extract showed antibacterial activity against two Gram's positive (S. aureus and B. cereus) and two Gram's negative (E. coli and S. typhi) bacteria. All the results fromthat study stated

that the extract can be used to prevention of bacterial infection and may have role in pharmaceutical medicine evolution.

Bindhu and Umadevi (2013) carried out synthesis of leaf extract of *Hibiscus cannabinus* showed good antibacterial activity against *Escherichia coli, Proteus mirabilis* and *Shigella flexneri.* The minimum inhibition concentration of extracts showed that 50 µg/mL.

Prema (2013) studied the antibacterial activity in four medicinal plants (*Acalypha indica, Eucalyptus globules, Spinacia oleraceae, Amaranthus ariris).* The acetone extract of *Acalypha indica* was more effective against *Staphylococcus aureus*. Ethanol extract of *A.indica* and *Eucalyptus globules* were highly sensitive to *S.aureus* and *P.Aeruginosa.*

Nayan,R.Bhalodia and Shukla,V.J (2014). The Hydroalcohol extracts of *Cassia fistula* showed significant antibacterial activities against tested gram positive and gram negative bacteria.

Ashraf *et al.,* (2018) studied the antibacterial activites of cold water, hot water and methanolic extracts studies against *Staphylococcus aureus* was more than *Escherichia coli* but *Candida albicans* was completely resistant to the extracts. The result showed the cold water extracts is high activity of *Escherichia coli.*

MATERIALS AND METHODS

Plant material

Amaranthus aritis L.

Botanical name : *Amaranthus aritis L.*

Family : Amaranthaceae

Vernacular name : Arai keerai

Distribution :

Amaranthus aritis L.(Amaranthaceae), found throughout south india. *Amaranthus aritis* L. is an Northeastern Indian state of Manipur species, a medium-sized evergreen herb. Now found in Kerala, India.

Description:

The '*Amaranthus aritis'* is known as one of the richest and medicinal herb plant of India. It is commonly known as *cheng-kruk* in India. *Amaranthus aritis'* is light green stem that grows to about 60–80 cm in height. Numerous branches emerge from the base, and the leaves are ovate, 3–6 cm long, 2–cm wide, with long petioles of about 5 cm. The plant has terminal panicles with few branches, and small green flowers with 3 stamens

Amaranthus gangeticus L.

Botanical name : *Amaranthus gangeticus L.*

Family : Amaranthaceae

Vernacular name : Thandukeerai

Distribution:

Amaranthus gangeticus L.(Amaranthaceae), found throughout India and distributed eastwards . *Amaranthus gangeticus L.* is an Africa species, a medium-sized evergreen perennial herb. Now found in China , Japan ,Korea, India.

Description:

The '*Amaranthus gangeticus'* is known as one of the richest and diets plant of Bangladesh. *A. gangeticus* is also known as elephant-head amaranth. It is an annual flowering plant with deep purple flowers. It can grow to 2–3 feet (0.61–0.91 m) tall. Ovules 2, rarely 3; style filiform, stigma small, terminal. 2-Seeded;

Spinacia oleraceae L.

Botanical name : *Spinacia oleraceae L.*

Family : Amaranthaceae

Vernacular name : Bala keerai

Distribution:

Spinacia oleraceae L.(Amaranthaceae), found throughout temperate Asia, and has been introduced to the mediterranean area early. *Spinacia oleraceae* grows cultivated or naturalized in all temperate and subtropical regions of Europe, Asia, and North America.

Description:

The '*Spinacia oleraceae*' is known as one of the richest and nutrient alue herb of Asia. *S.oleraceae* growing as tall as 30 cm(1 ft). *S.oleraceae* ay overwinter in temperate regions. The leaves are alternate, simple, ovate to iangular, and very variable in size: 2–30 cm (1–12 in) long and 1–15 cm (0.4–5.9 in) oad, with larger leaves at the base of the plant and small leaves higher on the owering stem. The flowers are inconspicuous, yellow-green, 3–4 mm (0.1–0.2 in) in ameter, and mature into a small, hard, dry, lumpy fruit cluster 5–10 mm (0.2–0.4 in) ross containing several seeds.

Collection and identification of plant material

The fresh plant materials of selected greens collected from **Ulavar santhai, Thoothukudi** in the month of june to August 2019. The plants were identified with the help of local floras. 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.

Preparation of the plant extracts

Five grams of *Amaranthus aritis, Amaranthus gangeticus* and *Spinacia oleraceae* were extracted separately with methanol, ethanol, petroleum ether, chloroform and aqueous solutions by maceration (24 hrs for each solvent) with constant shaking, the homogenates were then filtered through whatman No 2 filter paper and the extracts were stored at 4°c, the extracts thus obtained were used for various analyses.

Preliminary phytochemical screening of different extracts (Harbrone, 1998)

The qualitative phytochemical test for alkaloids, flavonoids, tannins, phenols, terpenoids, sterols, quinines, coumarins, betacyanins, saponin, anthraquinone, carbohydrates, glycosides, proteins are carried out in the concentrated extracts using the standard procedures to identify the constituents in the leaf extracts of *Amaranthus aritis, Amaranthus gangeticus* and *Spinacia oleraceae*. The chemical test for various phytoconstituents in the extracts were carried out as described below.

Test for alkaloids (Evans, 1997)

Wagner's reagent

Iodine (1.27 g) and potassium iodide (2 g) were dissolved in 5 ml of water and made up to 100 ml with distilled water.

Test for carbohydrates (Ramakrishnan *et al.*, 1994)

Fehling's test

One ml of plant extract is filtrate, was boiled on water bath with 1 ml each of Fehling solutions I and II. A red precipitate indicated the presence of sugar.

Fehling's solution

Fehling's solution I: Copper sulphate (34.66 g) was dissolved in distilled water and made up to 500 ml with distilled water.

Fehling's solution II: Potassium sodium tartarate (173 g) and sodium hydroxide (50 g) were dissolved in water and made up to 500 ml.

Test for glycosides (Evans, 1997)

Borntrager's test

To 2 ml of filtrate hydrolysate, 3 ml of chloroform was added and shaken. Chloroform layer was separated and 10% ammonia solution was added to it. Pink colour indicated the presence of glycosides.

Test for saponins (Kokate, 1999)

The extract (50 mg) was diluted with distilled water and made up to 20 ml. The suspension was shaken in a graduated cylinder for 15 min. A two cm layer of foam indicated the presence of saponins.

Test for phenolic

Ferric chloride test (Mace, 1963)

The extract (50 mg) was dissolved in 5 ml of distilled water. To this, a few drops of neutral 5% ferric chloride solution was added. A dark green colour indicated the presence of phenolic compounds.

Test for steroids: (Bishnu *et al.*, 2011)

2 ml of acetic anhydride was added to 2 ml extract of each sample followed by careful addition of 2 ml H_2SO_4. The colour changed from violet to blue or green indicate the presence of steroids.

Test for protein (Abdul *et al* ., 2013)

Ninhydrin test

About 0.5 ml of extract is taken and 2 drops of freshly prepared 0.2% ninhydrin reagent is added and heated. The appearance of pink or purple colour indicates the presence of proteins, peptides or amino acids.

Test for coumarins (Padmanabhan *et al.*, 2014)

To 1 ml of plant extract, 1 ml of 10% NaOH is added, formation of yellow colour indicates the presence of coumarins.

Test for terpenoids (Abdul *et al* ., 2013)

To 0.5 ml of the plant extract, 2 ml of chloroform along with concentrated sulphuric acid is added. Formation of red brown colour at the interface indicates the presence of terpenoids.

Test for betacyanin (selvaraj et al., 2014)

To 2 ml of the plant extract, 1 ml of 2N sodium hydroxide is added and heated for 5 min at 100°c formation of yellow colour indicates the presence of betacyanin.

Test for quinines (Egwaikhide and gimba, 2007)

A small amount of extract is treated with concentrated Hcl and formation of yellow colour precipitate indicates the presence of quinines.

Test for anthraquinone (kokate et al., 1995)

About 0.5 ml of the extract is taken into a dry test tube and 5 ml of chloroform is added and shaken for 5 minutes. The extract is filtered and the filtrate was shaken with equal volume of 10% ammonia solution a pink violet or red colour in the lower layer indicates the presence of anthraquinone.

Test for flavonoids (Harbone, 1998)

Alkaline reagent test

An aqueous solution of the extract was treated with 10% ammonium hydroxide solution. Yellow fluorescence indicated the presence of flavonoids.

Test for tannins (ciulei, 1994)

1 ml of extract, 2 ml of 5% $Fecl_3$ is added. A dark green or a blue-black indicates the presence of taninns.

Quantitative Test

The chemical tests for various phyto constituents in the extracts were carried out as described below;

Estimation of Flavonoid (Chang *et al.*, 2002)

100mg of samples were homogenized with 10ml of distilled water and filtered through a muslin cloth.0.5 ml of the extract was added with 2.5 ml distilled water and mixed. After 6 minutes 0.15 ml $NaNo_2$, was added and again after 6min 0.3 ml of 10% $AlCl_3$ is added. After 5 minutes 1 ml of 1M NaOH and 0.5 ml of water were added following through mixing of the solution the absorbance against blank were recorded at 510nm. Quercetin was used as standared and the results were expressed as my qucertin equivalents (QE) 1 g fresh weight.

Estimation of Tannin (Julkunen-Tritto, 1985)

100 mg of the samples were homogenized with 10 ml of distilled water and filtered through a muslin cloth. 0.1 ml aliquots of aqueous extract were mixed with 3 ml of 4%vanillin (prepared with methanol) and 1.5 ml of concentrated HCl. The solution was shaken vigorously and left to stand at room temperature for 20 minutes in darkness. Absorbance against blank was measured at 500 nm using UV visible spectrophotometer. Results were expressed as mg catechin equivalent (CE)/g dry weight.

Estimation of phenol

Total phenol of methanol extracts were estimated using Folin phenol reagent based on the reaction between phenol and oxidizing agent phosphomolybdate according to Slinkard and Singleton, (1977). Hundred milligrams of methanol extracts of different parts of *A.aritis, A.gangeticus, S.oleraceae* were dissolved 24 in 10 ml of methanol. One ml of methanol extract was added into test tube and 1 ml of folin

phenol reagent was added followed by 3 ml of (2%; w/v) sodium carbonate solution. The mixture was allowed for 1 h with intermittent shaking. The reaction mixture was measured at 760 nm. Blanks were prepared without sample. Simultaneously gallic acid standard was also prepared. The total phenol contents are expressed mg GAE/g DW of methanol extracts.

Proximate analysis of Green's Leaf :

Nutritional analysis

To assess nutritive values of *A. aritis, A. gangeticus, S. oleracea* were dried in a specially designed electrical drier at 40^0c for 24 h, powdered using a mixer grinder and stored for further analysis. Ash contents, moisture contents, carbohydrate, protein, were determined by the different method.

Preparation of the Samples

Green leafy vegetables were cleaned and washed with distilled water followed by washing with de-ionized distilled water, and allowed to air dry. Two gram portion of the dry sample were weighed and made into paste using pestle and motor. The juice was extracted and made upto 5ml with de-ionized distilled water and poured into a centrifuge tube and after the process of centrifuging the aqueous extract collected was packed in polythene pouches and stored in the refrigerator. Fresh leaves were also used for certain biochemical analysis namely crude fibre and moisture content whereas aqueous extract were used for carbohydrate and protein, All glass ware used for the analysis were washed with detergent and rinsed with water and soaked overnight in 1N HCL and rinsed again with distilled water and acetone before drying. Care was taken to prevent contamination at every stage.

Carbohydrate content (Dubois *et al.*, 1956)

Two grams of leaves were ground with 10 ml of 80% hot ethanol. The homogenate was centrifuged at 5000 g for 20 min. The clear supernatant was evaporated to dryness over boiling in water bath and the residue was dissolved in 20 ml of distilled water. Each of this (20 ml) was used as carbohydrate source. One ml was mixed with 1 ml of 5% of phenol (aqueous w/v), and 5 ml of conc. H_2SO_4 was added rapidly and mixed thoroughly and the tubes were incubated for 10 min and then placed in water bath for 20 min at 30^0c. The colour development was read at 490 nm in spectrophotometer.

Proteins content (Lowry *et al.*, 1951)

Fresh leaf sample (1.0 ml) was added to 1 ml of NaOH and kept at 100°C for 4-5 min. Then, 5 ml of alkaline copper reagent was added and it was allowed to stand at room temperature for 10 min. Folin-phenol reagent was added rapidly and mixed immediately. Optical density was read after 30 min at 750 nm using spectrophotometer.

Fibre content (IS, 1990)

Two gram green sample leaves were taken and boiled for 30 minutes with sulphuric acid for acid treatment and washed with boiled deionized distilled water. Then the greens were treated for alkali treatment, in which the sample were heated for 30 minutes along with sodium hydroxide and again washed with deionized distilled water. The sample was then kept in hot air oven and made into dry ash under 600^0c and kept for cooling. After 24 hours, the dry ash weights were noted.

Moisture content (IS, 1990)

Moisture content was determined by weight loss an drying method in terms of present weight as per procedure mentioned in WHO library.

Technique for Maceration (Shashank Mahesh *et al.*,2015)

Preparation of Solvent

Cut the material (either fresh or dry) into small slices about 0.5mm thick. Macerate in a solution of equal parts of 10% aqueous nitric acid and 10% aqueous chromic acid. The solution may be heated in the flame for woody tissues (not for soft herbaceous tissues). Using a glass rod, tab the material gently in order to loosen the cells. Wash thoroughly in water to remove the acid stain the material with water soluble safranin. Mount in 50% glycerine and observe.

FTIR spectrum analysis:

The powder of *Amaranthus aritis* , *Amaranthus gangeticus* and *Spinacia oleraceae* (leaves) were mixed with KBr salt, using a mortar and pestle and compressed into a thin pellet, Infared spectra were recorded on a model-perkin spectrophotometer FTIR spectrum MRX-1 in the range 4000-400cm^{-1}

Antibacterial activity

Extraction of plant materials

5 mg of plant powder was extracted with different solution like methanol, chloroform and aqueous.

Bacterial strains used

The test organisms were obtained from the Department of Microbiology, St. Mary's College (Autonomous), Thoothukudi. *Proteus vulgaris, Salmonella typhi, Escherichia coli , Bacillus substilis, Kelbsilla pneumonia* 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 of bacterial colony was transferred to the broth medium. This microbe culture was incubated at room temperature for 24 hours.

Agar medium

- Nutrient ager 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

Antibacterial activity was evaluated by agar disc diffusion method (Kirby Bauer *et al.*,1986). The solutions were prepared with known weight of extracts dissolved in 5% dimethyl sulphoxide (DMSO). What man No.1 filter paper

discs (5mm) were impregnated with these extracts and allowed to dry at room temperature. The spread plates were prepared by proper concentration of inoculation. Each sample loaded discs was placed in the seeded agar plate. After 24- 48 hours of $\pm 37^{0}c$ incubation, the diameter of the inhibition zone was measured for positive control. streptomycin discs (100 g/ml) was used , whereas for negative control; repective solvents loaded on the sterile disc.

RESULT AND DISCUSSION

Result and Discussion

Qualitative analysis

Phytochemical analysis of plant is predicted by the need for drug alternative of plant origins. These secondary plant metabolite extractable by various solvents exhibit valued biochemical and phermacutical activities in animals when investigated. Preliminary phytochemical analysis of the various solvent extracts in leaf of *Amaranthus aritis, Spinacia oleraceae* and *Amaranthus gangeticus,* showed different results **(Table:1,2 &3).** Many herbaceous and medicinal plant contain important phytochemical such as alkaloids, phenols, tannin, saponins, glycosides, quinones, flavonoids, terpenoids and coumarins, which are utilized by humans.

Among the five different extracts of leaves (*A. aritis*), petroleum ether extract showed the presence of maximum number of compounds than the other solvent. Quinines is absent in petroleum ether extract.

Methanol extract of *S.oleraceae* showed the presence of maximum number of compounds than the other solvents, where Terpenoid is absent.

Chloroform extract of *A.gangeticus* showed the presence of maximum number of compounds than the other solvent. coumarins is absent in chloroform extract. So it is found that all the treated plant have considerable proportion of important secondary phytichemicals.

Table 1: Phytochemical screening of different extracts of *Amaranthus aritis L.* Leaf

Phytochemical Tests	Methanol	Ethanol	Chloroform	Petroleum ether	Water
Alkaloids	-	-	++	+++	+
Flavanoids	++	-	-	-	-
Tannins	-	+	++	+++	+
Phenol	-	+	++	-	-
Terpenoids	-	+	-	-	-
Sterol	-	-	-	+++	++
Quinines	-	-	-	-	-
Betacyanin	-	-	+	+++	-
Coumarins	+	-	++	+++	-
Saponin	-	-	++	+++	-
Anthraquinone	-	+	+	+	+
Carbohydrate	-	-	+	+	+
Protein	+	-	-	+	+
Glycoside	+	-	+	+	+

+++ .... High, ++.... Moderate, + ... Low, -- ....Absent

Table 2: Phytochemical screening of different extracts of *Spinacia oleraceae L.* Leaf

Phytochemical Tests	Methanol	Ethanol	Chloroform	Petroleum ether	Water
Alkaloids	+++	++	-	-	-
Flavanoids	++	+	-	-	+
Tannins	+++	+	++	-	-
Phenol	+++	++	-	-	-
Terpenoids	-	-	-	-	+
Sterol	++	-	++	-	-
Quinines	-	-	-	-	+
Betacyanin	++	-	+	-	-
Coumarins	++	-	++	-	-
Saponin	++	+	+	-	-
Anthraquinone	++	-	-	-	+
Carbohydrate	++	+	+	+	+
Protein	++	+	-	-	+
Glycoside	+++	-	-	-	+

+++ .... High, ++.... Moderate, + ... Low, -- ....Absent

Table 3 : phytochemical screening of different extracts of *Amaranthus gangeticus L.* Leaf:

Phytochemical Tests	Methanol	Ethanol	Chloroform	Petroleum ether	Water
Alkaloids	+	-	++	-	-
Flavanoids	-	+	++	-	+
Tannins	+	-	+++	++	-
Phenol	+	-	+++	+	+
Terpenoids	+	-	++	-	+
Sterol	+	+	++	-	-
Quinines	+	-	-	+	+
Betacyanin	++	-	+	-	+
Coumarins	+	-	-	-	-
Saponin	+	+	++	+	-
Anthraquinone	++	-	+	-	+
Carbohydrate	++	+	+	+	+
Protein	-	+	++	-	+
Glycoside	-	-	+	+	+

+++ .... High, ++.... Moderate, + ... Low, -- ....Absent

Quantitative analysis

The total phenol, flavonoid and tannin, were analysed in extract of *A. aritis*, *A. gangeticus*, and *S. oleraceae* belonging to the family Amaranthaceae.

The phenol content of the aqueous extract of *A.gengeticus* was found tobe 1.42 (mg QE/g) which is higher than *S. oleraceae* and *A. aritis*. Natural phenolic compounds play an important role in cancer prevention and treatment. Various bioactivities of phenolic compounds are responsible for their chemopreventive properties (antioxidant, anticacinegetic, antimutagenic and anti inflammatory effects).

The flavonoind contents of selected greens as shown in **Table 4** is 0.27 (mg QE/g in *A.gangeticus* which is cure than that of *A.aritis(*0.15 (mg QE/g) and *S.oleraceae* 0.14 (mg QE/g). The presence of flavanoid in the leaf of *A.gangeticus* could accurent for its use as an antiinflammatry agent (Ekwueme *et al* .2011) and for treatment of diarrhoea (Scheie *et al*,2005) fever reducing, pain relieving and anticancer activities.

The tannin content of the aqueous extract of *A.aritis* to be 6.61(mg QE/ g) which is more than the tannin content of *A.gangeticus* 3.91 (mg QE/g) and *S.oleraceae* 3.41 (mg QE/g). Tannins are known to possess immunostinulating activities (Kumar and Subramanyan 2013).

Table 4: Quantitative analysis of phytochemicals in greens leaves

Sample	Amount of phenol mg/(QE)/g	Amount of flavonoid mg/ (QE)/g	Amount of tannin mg/ (QE)/g
A.aritis	0.91 ± 0.061	0.15 ± 0.029	6.611 ± 0.320
S. oleraceae	0.79 ± 0.097	0.14 ± 0.021	3.41 ± 0.284
A.gangeticus	1.42 ± 0.099	0.27 ± 0.027	3.91 ± 0.416

Each value represents the mean ± SD of three determination of dry weight (DW)basis

Proximate analysis of Green's Leaf :

Nutritional analysis

The findings for proximate composition of selected three greens were presented in **Fig(1-4)** The moisture content of dry leaf was found to be 94% for *S.oleraceae,* 87% for *A.aritis* and 70.3% for *A.gangeticus*. The high water content in *S.oleraceae* leaves were ideal for vegetable juicing, natural product stabilities. High moisture content tends to promote microbial contamination and chemical degradation (Hussain *et al* 2009)

The ash content is generally recognized as a measure of quality for the assessment of the functional properties of food (Hofman *et al* 2002). *A.aritis* contained high levels of total ash (3g) in the dried plant.

The carbohydrate content of the selected green leaves were 4.5%, 2.5%,3% in *A.aritis, S.oleraceae,* and *A.gangeticus* respectively.

The protein content of the selected green leaves were 1.9%, 2.4%,3.8% in *A.aritis, S.oleraceae,* and *A.gangeticus* respectively.

Maceration:

Evaluation of the crude drug is an integral part of establishing correct identity of the drug. Pharmacognostical parameters are necessary for the confirmation of the identity of the crude drug. This maceration evaluation of leaf of *Amaranthus aritis, Spinacia oleraceae, Amaranthus gandeticus* sample were useful for setting standards for identification and authentication of the drug not only in crude form but also in finished products.

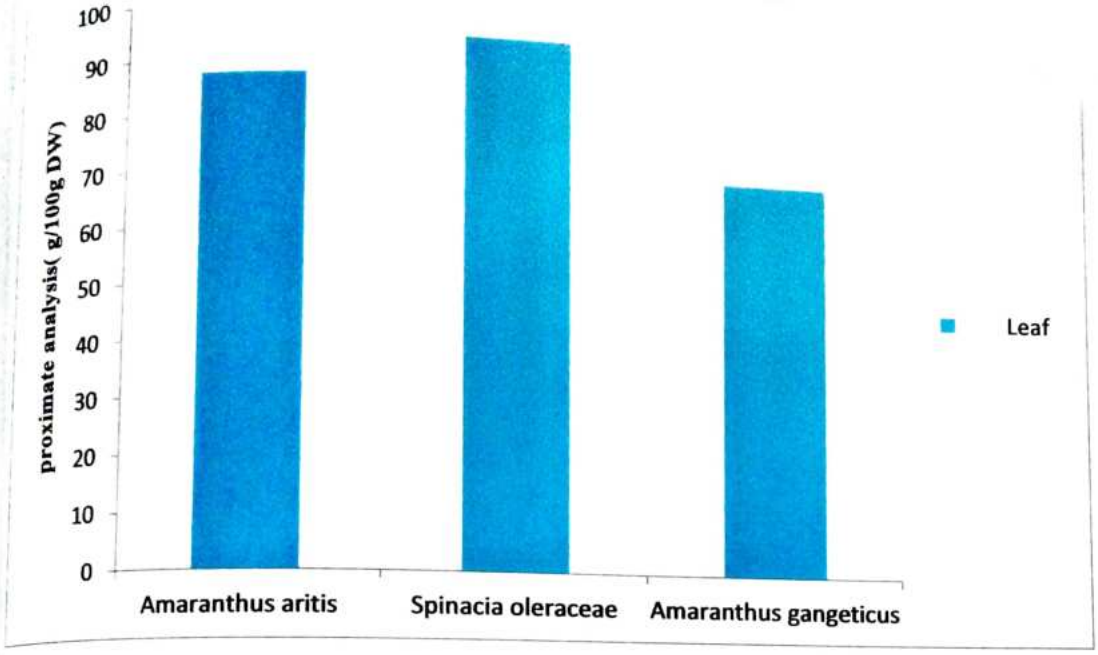


Fig.1 Ash content in Green's leaf powder

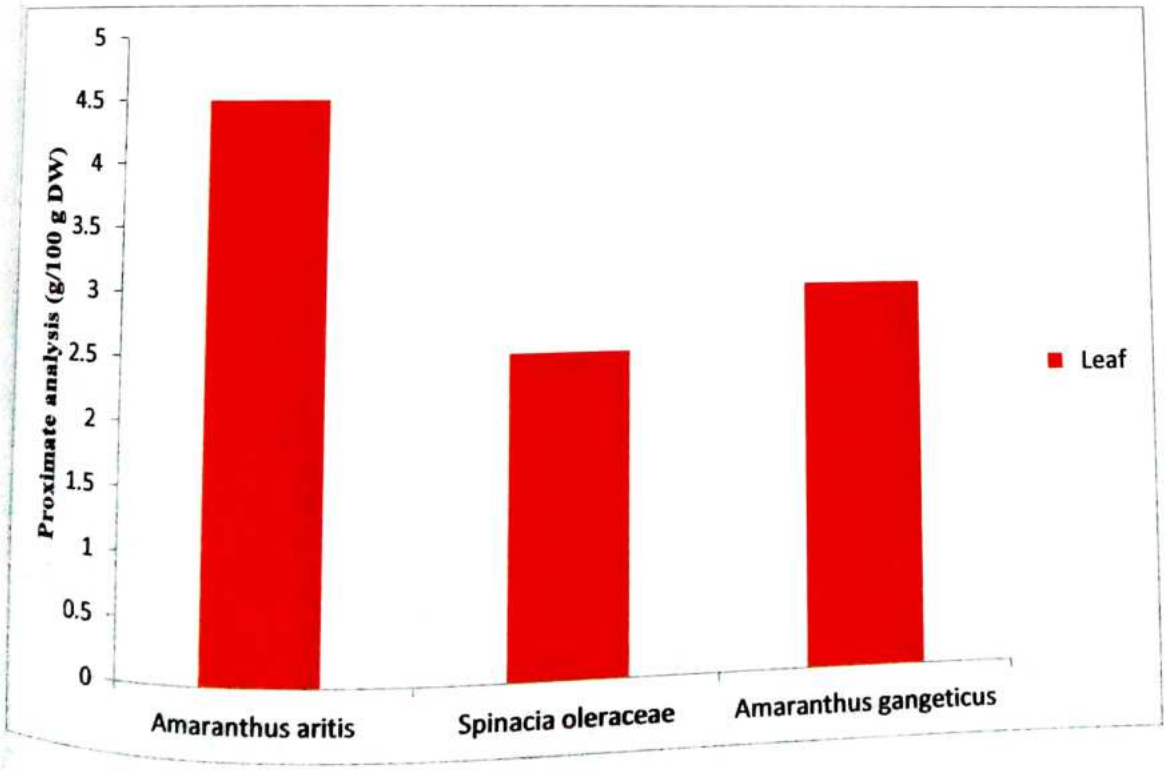


Fig. 2 Carbohydrate content in Green's leaf powder

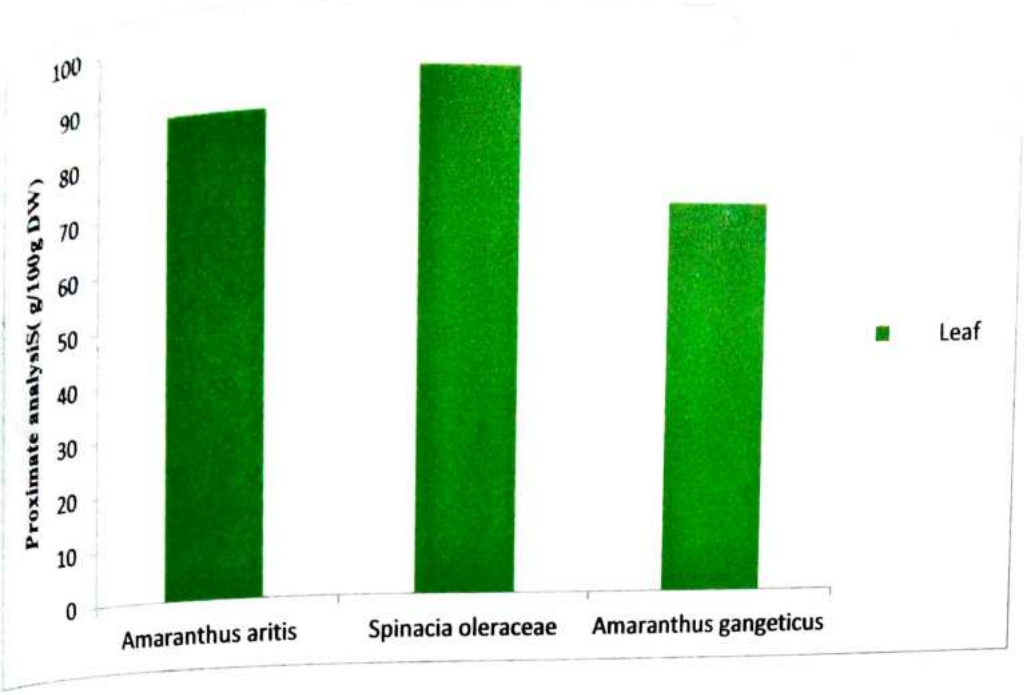


Fig. 3 protein content in Green's leaf powder

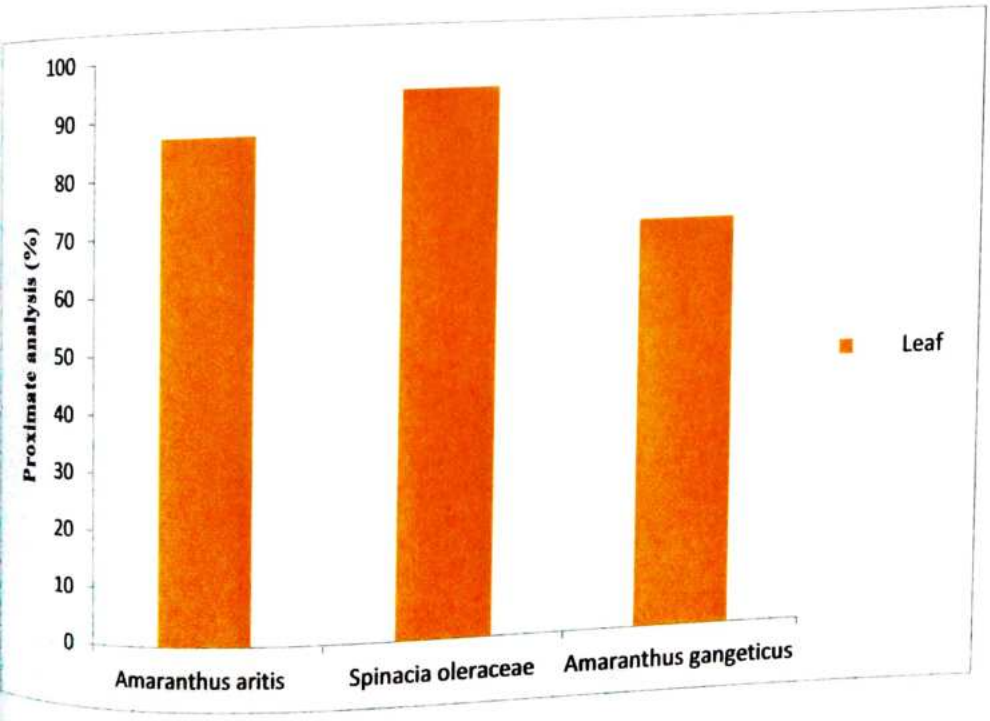


Fig. 4 Moisture content in Green's leaf powder

Table: 5 Length of vessels and tracheids in dry leaf powder of greens using maceration techniques

S.No.	Sample	vessels	Trachied
1.	*A.aritis*	86µm	84µm
2.	*S.oleraceae*	169µm	118.4µm
3.	*A.gangeticus*	344µm	140.3µm

Plate 4

Maceration in *A.aritis* leaf indicated the presence of vess

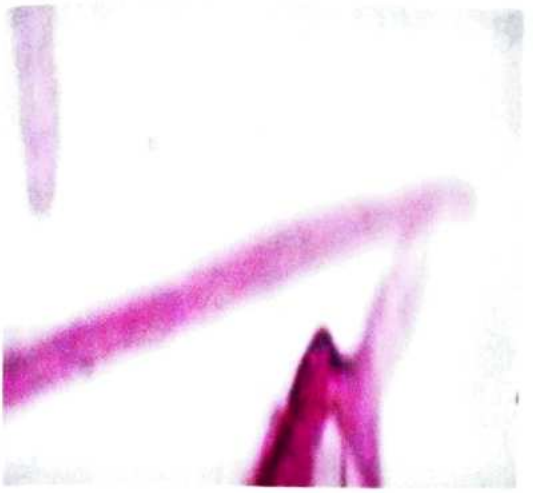


Plate a: Microscopy observation of vessel cell

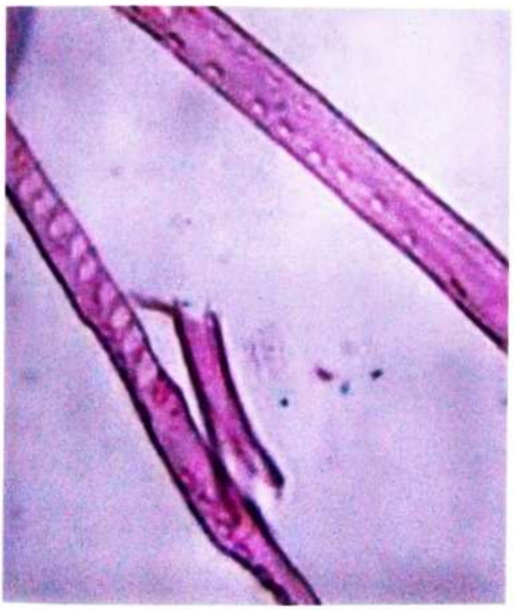


Plate b: Microscopy observation of tracheid cell

Plate 5

Maceration in *S.oleraceae* leaf indicated the prese vessel and Tracheid cells

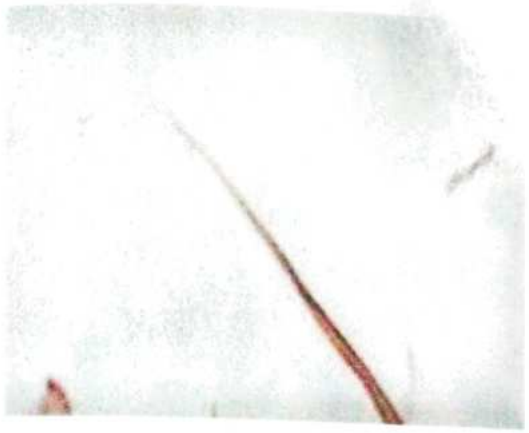


Plate a: Microscopy observation of vessel cell

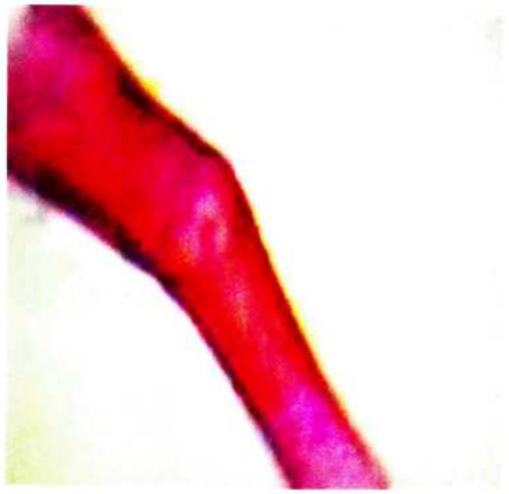


Plate b: Microscopy observation of Tracheid cell

Plate 6

Maceration in *A.gangeticus* leaf indicated the presence of cheid cells

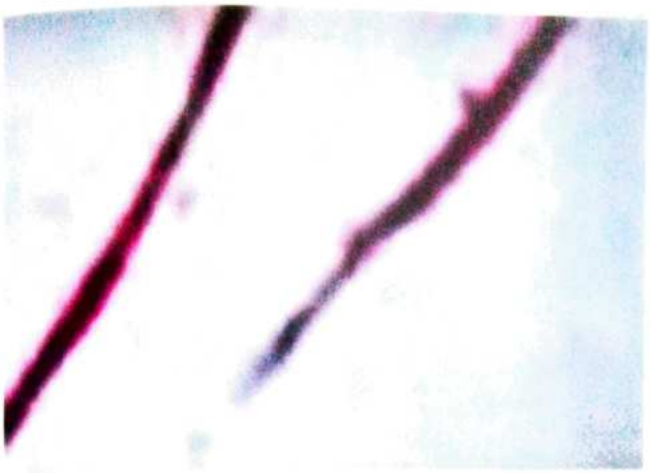


Plate a: Microscopy observation of vessel cell

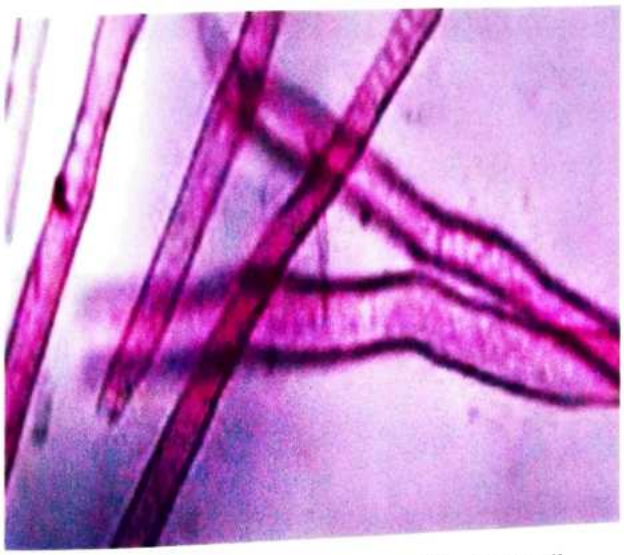


Plate b: Microscopy observation of Tracheid cell

Under microscopical examination of *A.aritis, S. oleraceae, A.gandeticus* leaf powder shows the vessels, and trachied cells. The photographs were taken and shown **(Plate-4-6).**

Jeffrey (1917) proposed maceration by using mixture of equal portions of freshly combined 8 to 10% nitric acid and chromic acid. Slightly heating hastens the reaction and macerates wood samples. Jeffrey mixture of *A.gangeticus* exhibited maximum maceration activity against *A.gangeticus* and less with *A.aritis* and *S.oeraceae.*

The maceration of vessel length in dry leaf powder was found to be 344µm in *A.gangeticus,* 169µm in *S.oleraceae* and 86µm in *A.aritis.* Measurement of vessel length is high in *A.gangeticus,* compare able to *A.aritis,* and *S.oleraceae.*

The maceration of tracheid length in dry leaf powder was found to be 140µm in *A.gangeticus,* 118µm in *S.oleraceae* and 85µm in *A.aritis.* Measurement of vessel length is high in *A.gangeticus,* compared to *A.aritis,* and *S.oleraceae* **(Table (5)).**

FTIR

Fourier Transform Infrared Spectroscopy was used to analysed the functional group present in the leaf of *A.aritis, S.oleraceae,* and *A.gangeticus.*

The FTIR spectroscopy analysis of *A. aritis* leaf obtained peaks at 3902.69 cm^{-1}, 3786.97 cm^{-1}, 3230.74 cm^{-1}, 2927.74 cm^{-1}, 1181.32 cm^{-1}, 780.15 cm^{-1}, 621.04 cm^{-1}, 515.92 cm^{-1}. These absorption peaks are known to be associated with the

Table: 6 FTIR spectral peak values and functional group obtained for the leaf extract of *A.aritis.*

Peak Value	Bond	Functional Group
3302.69	Medium , Aromatic amines	N-H
	Strong, Alcohol group	O-H
3225.25	Medium, Urethanes	N-H
3230.74	Strong, Alkane	C-H
2927.74	Medium, Ester	C-H
2856.38	Weak, Sulphinic Acid	O-H
2380.00	Medium	O-H
2307.67	Strong, Carbonyl	C=O
1846.71	Strong, Guanidine	C=N
1627.81	Medium, AromatiC	C=C
1511.12	Strong, Nitro Group	N=O
1395.40	Strong, Acid	C-O
1239.18	Strong, Acetals	C-H
1114.78	Strong, Ether	C-O
1034.74	Weak, Sulphinic Acid	O-H
901.66	Strong, Aliphatic Chloroformate	C-O-H
780.15	Strong, Primary Amines	N-H
621.64	Strong, Alkyl Halide	C-Br
515.92		

Table: 7 FTIR spectral peak values and functional group obtained for the leaf extract of *S.oleraceae*

Peak Value	Bond	Functional Group
3399.80	Medium , Aromatic amines	N-H
3259.86	Strong, Alcohol group	O-H
3187.35	Medium, Urethanes	N-H
3070.46	Strong, Alkane	C-H
2933.53	Medium, Ester	C-H
2309.60	Weak, Sulphinic Acid	O-H
2300.45	Medium	O-H
1749.32	Strong, Carbonyl	C=O
1628.77	Strong, Guanidine	C=N
1511.12	Medium, AromatiC	C=C
1317.29	Strong, Nitro Group	N=O
1241.11	Strong, Acid	C-O
1173.60	Strong, Acetals	C-H
1011.59	Strong, Ether	C-O
930.59	Weak, Sulphinic Acid	O-H
780.15	Strong, Aliphatic Chloroformate	C-O-H
645.14	Strong, Primary Amines	N-H
516.89	Strong, Alkyl Halide	C-Br

Table: 8 FTIR spectral peak values and functional group obtained for the leaf extract of *A.gangeticus*

Peak Value	Bond	Functional Group
[illegible]	Medium , Aromatic amines	N-H
	Strong, Alcohol group	O-H
[illegible]	Medium, Urethanes	N-H
[illegible]	Strong, Alkane	C-H
[illegible]	Medium, Ester	C-H
[illegible]	Weak, Sulphinic Acid	O-H
[illegible]	Medium	O-H
[illegible]	Strong, Carbonyl	C=O
[illegible]	Strong, Guanidine	C=N
[illegible]	Medium, AromatiC	C=C
[illegible]	Strong, Nitro Group	N=O
[illegible]	Strong, Acid	C-O
[illegible]	Strong, Acetals	C-H
[illegible]	Strong, Ether	C-O
[illegible]	Weak, Sulphinic Acid	O-H
[illegible]	Strong, Aliphatic Chloroformate	C-O-H
[illegible]	Strong, Primary Amines	N-H
[illegible]	Strong, Alkyl Halide	C-Br

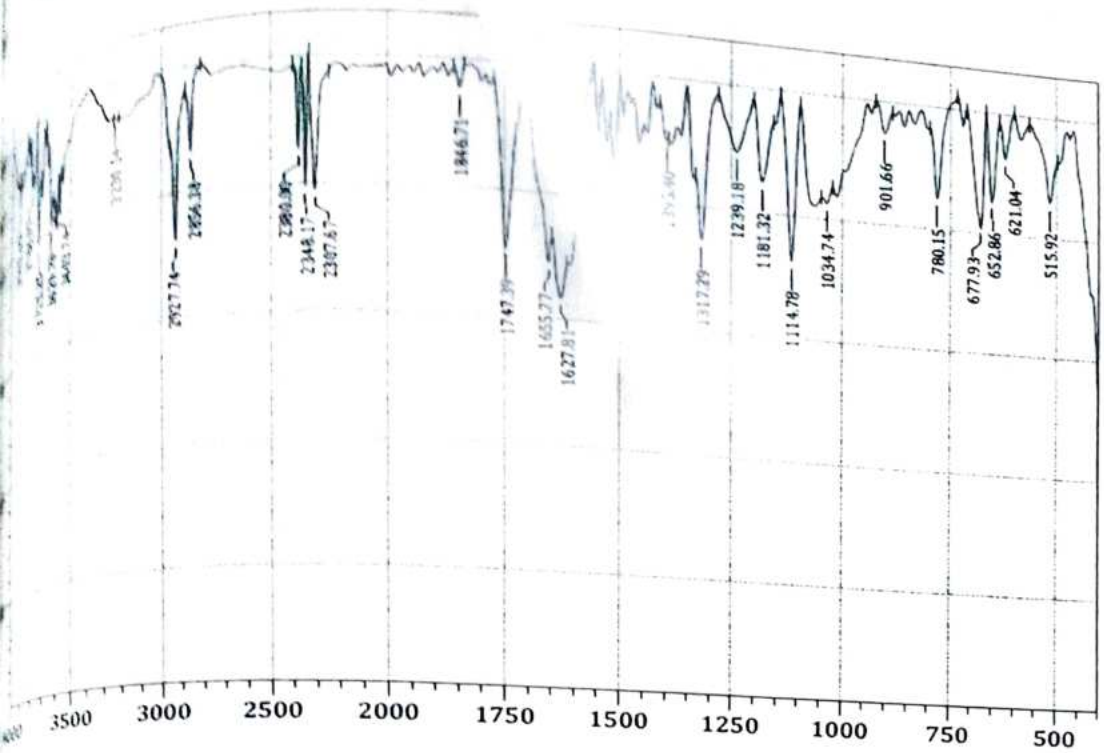


Fig.5 FTIR spectrum of chloroform extract of *A.aritis*

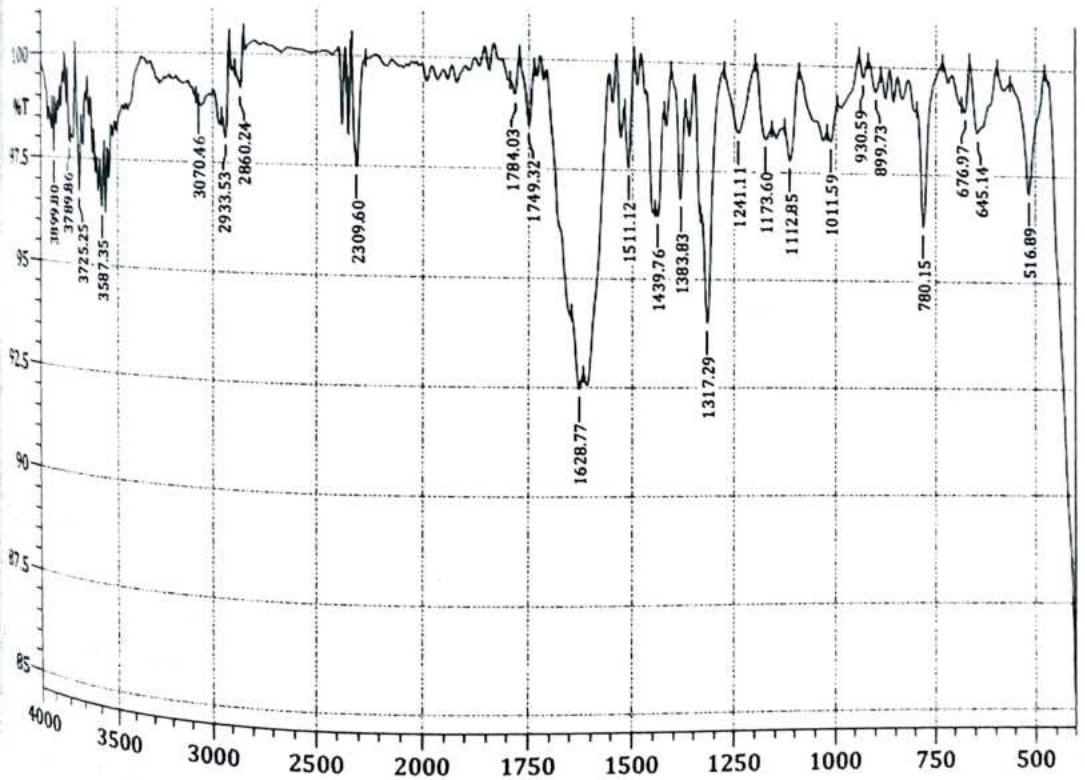


Fig.6 FTIR spectrum of chloroform extract of *S.oleraceae*

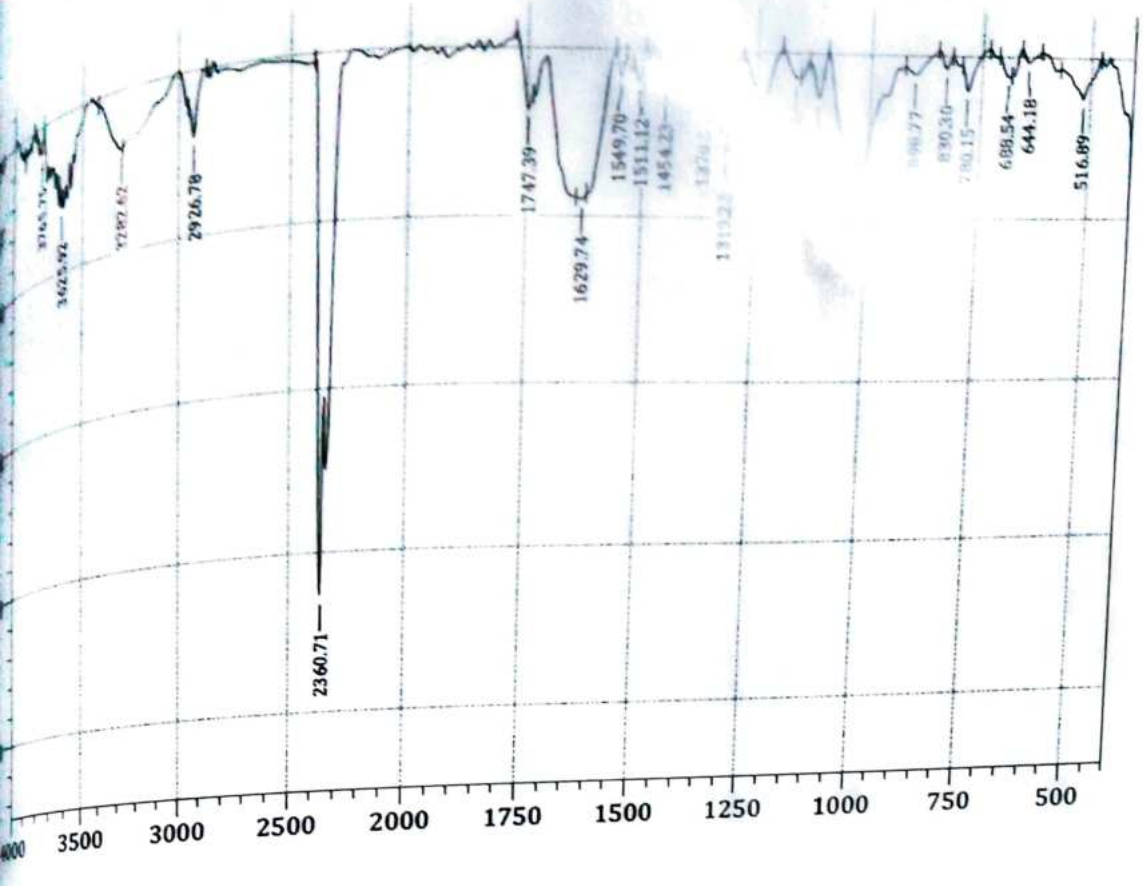


Fig.7 FTIR spectrum of chloroform extract of *A.gangeticus*

stretching vibration for N-H in aromatic amines, O-H in alcohol group, N-H in urethanes, C-H in alkane, C=O in carbonyl, C=N in guanidine, N=O in nitro group, C-O-H in aliphatic chloroformate, C-BR in alkylhalide. Fig: (5), Table :(6)

The FTIR spectroscopy analysis of *S.oleraceae* leaf obtained peaks at 3899.80 cm^{-1}, 3789.86 cm^{-1}, 3070.46 cm^{-1}, 2933.53 cm^{-1}, 2309.60 cm^{-1}, 1784.03 cm^{-1}, 1628.77 cm^{-1}, 1241.11 cm^{-1}, 930.59 cm^{-1}. These absorption peaks are known to be associated with the stretching vibration for N-H in aromatic amines, O-H in alcohol group, C-H in alkane, C=N in guanidine, N=O in nitro group, C-O-H in aliphatic chloroformate, C-BR in alkylhalide. Fig: (6), Table :(7)

The FTIR spectroscopy analysis of *A.gangeticus* leaf obtained peaks at 3765.75 cm^{-1}, 3282.62 cm^{-1}, 1747.39 cm^{-1}, 1163.96 cm^{-1}, 1027.02 cm^{-1}, 730.59 cm^{-1}, 780.15 cm^{-1}, 645.14 cm^{-1}, 516.89 cm^{-1}. These absorption peaks are known to be associated with the stretching vibration for N-H in aromatic amines, O-H in alcohol group, O-H in sulphinic acid, N=O in nitro group, C-O-H in aliphatic chloroformate, C-BR in alkylhalide. Fig: (7), Table :(8)

From the spectral data presence of C=O , C-H, N=O, O-H, C-O-H, C-BR, C-N were identified. These bonding are responsible for the presence of alkyl group, methyl group, alcohol, ether, carboxylic group, aliphatic nitro group, and indo group. Carboxylic acid present in the medicinal plant serves as main pharmaceutical product in curing ulcer, head ache, pain in lever , treatment of endama and rheumatic joint pain. Amides, amine, and amino acid are the main groups which are involved in protein synthesis(Devakia,K 2017). The study revealed that the whole plant of *A*

aritis, S.oleraceae, and *A.gangeticus* contain a considerable amount of secondary metabolites and in future to be used human disease management.

Antibacterial activity

In the present study, antibacterial activity of *A.aritis, S .oleraceae* and *A.gangeticus* using different solvents (chloroform, methanol and aqueous) were tested against five human pathogenic bacteria (*Bacillus substilis, Escherichia coli, Ptoteus vulgaris, Salmonella typhi , Klebseiella pneumonia*) presented. The diameter of the inhibition zones against these species ranged from 2 to 13mm.

The different solvents of chloroform and methanol and aqueous extracts of *A.aritis* exhibited maximum activity against tested bacterial species. The inhibition zone observed against the tested bacteria *E.coli* (4mm), *B. substilis* (7mm), *P. vulgaris* (11mm), *S.typhi* (12mm) and *K. pneumonia* (3mm) respectively.

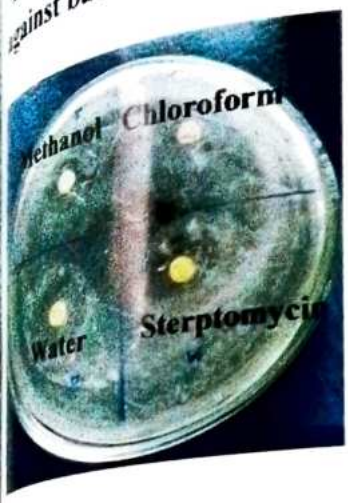
Methanol extract of *S.oleraceae* showed maximum antibacterial activity against *S.* typhi (10mm), (chloroform and aqueous extract of *S.oleraceae* exhibited minimum antibacterial activity against *K.pneumonia* (9mm).

Methanol extract of *A.gangeticus* exhibited maximum antibacterial activity against *P.vulgeris* (8mm) and less with *E.coli* (4mm) and *S. typhi* (3mm) **(Plate:7-9)**.

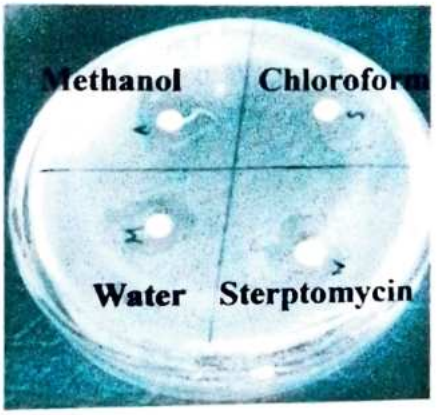
The maximum activity was found as 12mm zone of inhibition obtained by methanol extract of *A. aritis* against *B. substilis*. The methanol extract of *A. aritis* leaf exhibited more or less same zone of inhibition compared to standard antibiotics

Plat

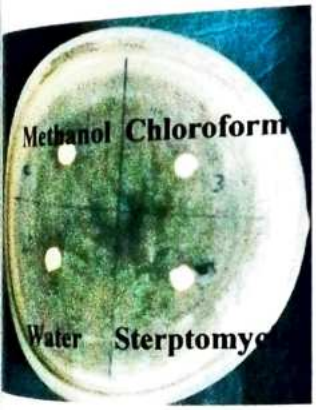
Antibacterial activity of different solvent ... ts of Amaranthus aritis leaf against bacterial strains



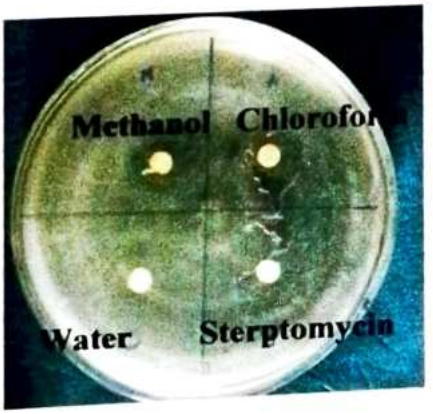
a: *E.coli*



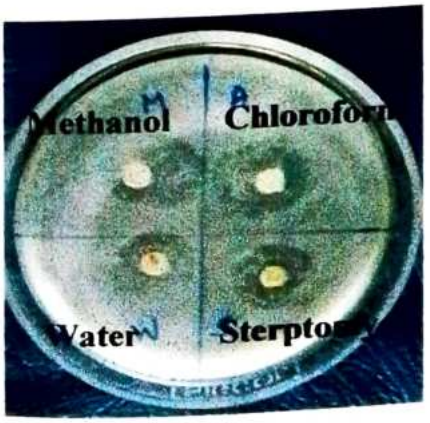
b: *Bacillus substilis*



c: *Salmonella typhi*



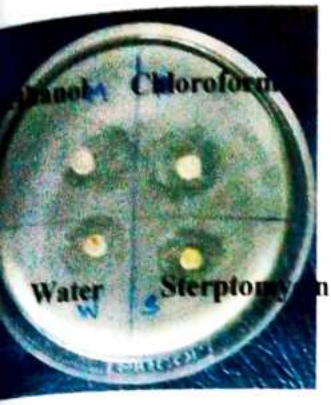
d: *Proteus vulgaris*



e: *Kelbsiella pneumonia*

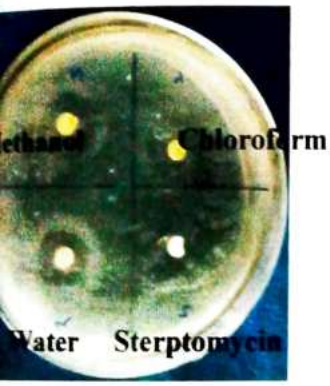
Plate 8

ntibacterial activity of different solvent extracts of *Spinacia oleraceae* leaf
ainst bacterial strains

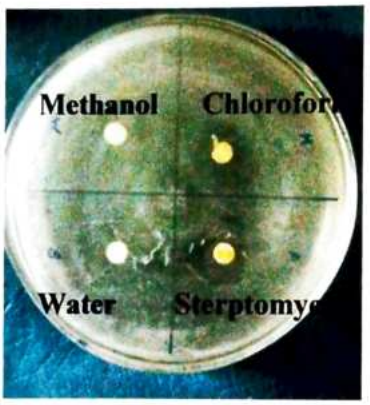


a: *E.coli*

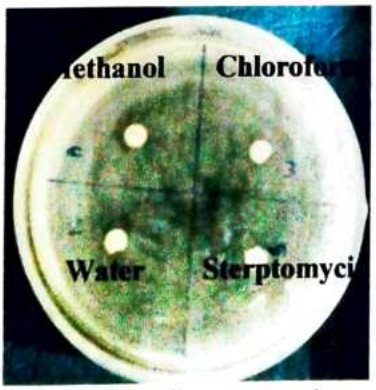
b: *Bacillus substilis*



c: *Salmonella typhi*

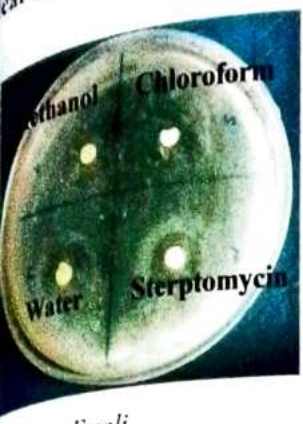


d: *Proteus vulgari*



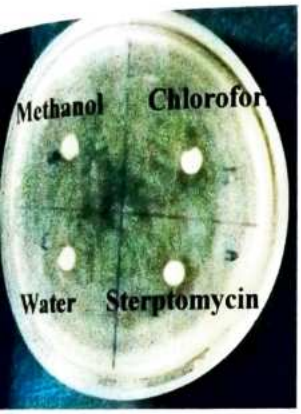
e: *Kelbsiella pneumonia*

Antibacterial activity of different solve[illegible] of *Amaranthus gangeticus* leaf against bacterial strains

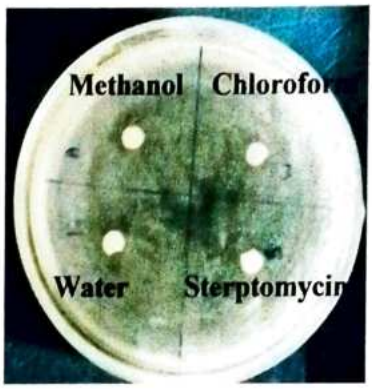


a: *E.coli*

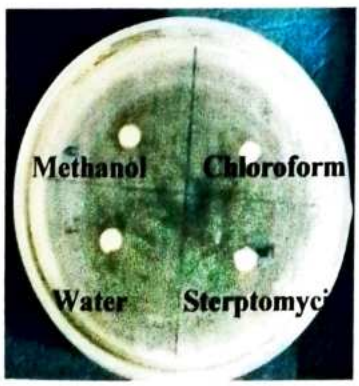
b: *Bacillus substilis*



c: *Salmonella typhi*



d: *Proteus vulgaris*



e: *Kelbsiella pneumonia*

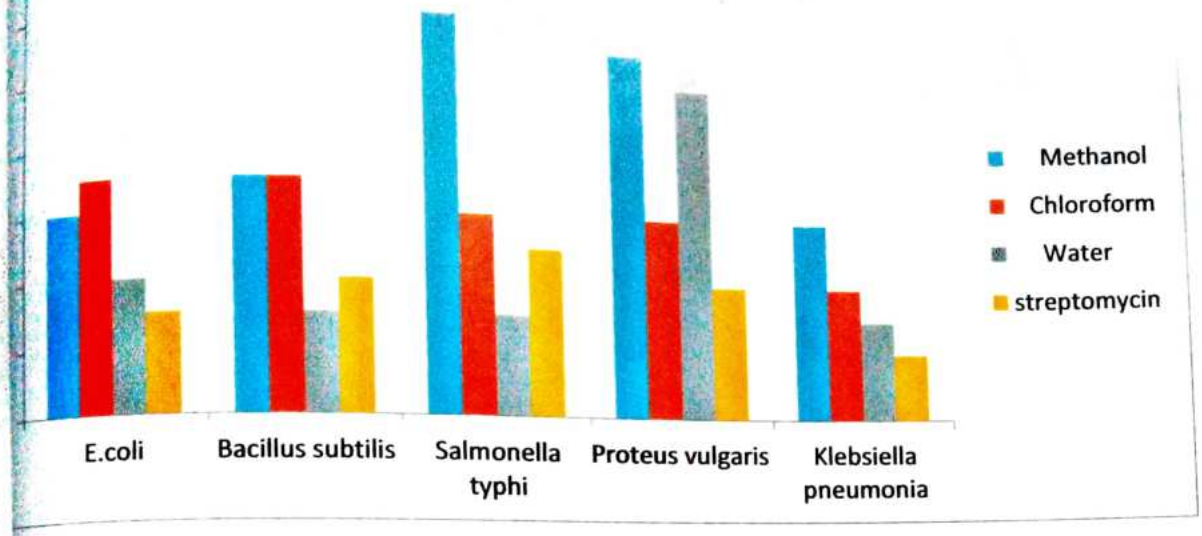


Fig. 8 Antibacterial activity of *A. aritis* leaf extracted with different solvent against bacterial strains

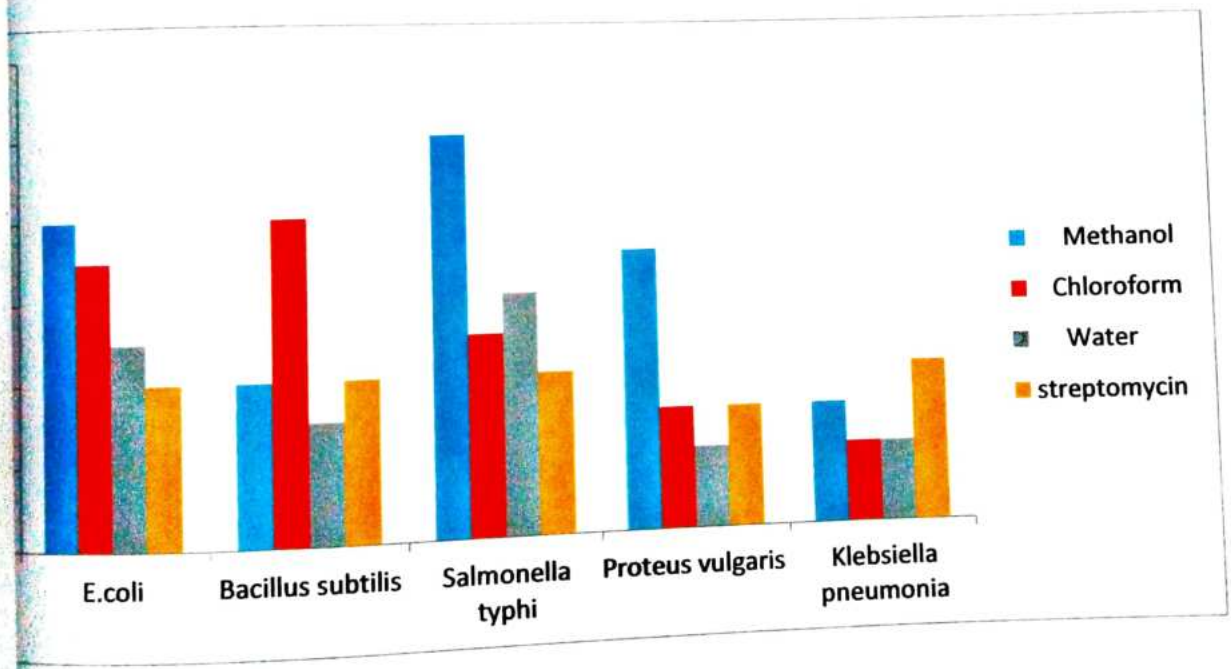


Fig.9 Antibacterial activity of *S. oleraceae* leaf extracted with different solvent against bacterial strains

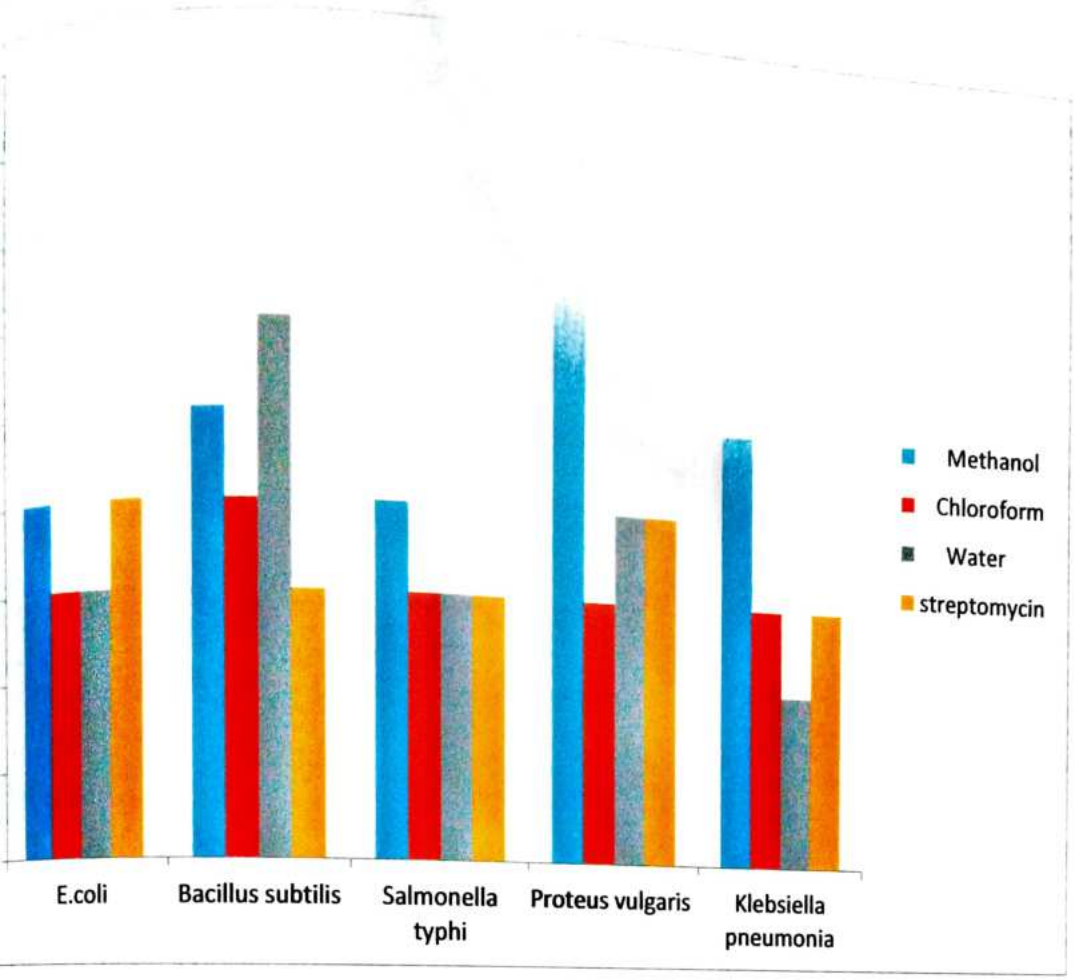


Fig.10 Antibacterial activity of *A.gangeticus* leaf extracted with different solvent against bacterial strains

streptomycin. Maximum antibacterial effect was found in *B.substilis* for methanol extracts of *A. aritis* leaf.

The antibacterial activity of leaf extract of *A. aritis*, *A.gangeticus* and *Spinacia oleraceae* were nearly similar to streptomycin. The antibacterial activities of *A. aritis*, *A. gangeticus* and *S. oleraceae* 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 *A. aritis*, *A.gangeticus* and *S. oleraceae* showed the presence of saponins on quantitative test. The antimicrobial activity of these plants may be due to the presence of saponions. The presence of tannins were also reported in *A. aritis*, *A. gangeticus*, and *S. oleraceae* . The antibacterial activity of tannins may due to their intercalation with enzymes, cell envelope transport proteins and also complex with cell wall polysaccharides (*Ya et al.*, 1998). Hence these plants stand as a potential candidate as a source of ingredients in drug formulation for the treatment of bacterial infection.

SUMMARY AND CONCLUSION

ummary and conclusion

Green leafy vegetables have been used as medicine since ancient times and ve been playing a very important role in our diet and nutrition. They are the most adily available sources of carbohydrates, fats, proteins,vitamins, mineral essential mino acids and fibers. Green vegetables as medicinal plants contain none or less xic effects, and have the ability to synthesize several secondary metabolites of latively complex structures possessing antimicrobial activities. The present work as therefore designed to investigate the antibacterial effects of three green egetables namely *Amaranthus aritis, Spinacia oleraceae* and *Amaranthus angeticus* against some bacterial strains (*Bacillus substilis, Escherichia coli, Ptoteus ulgaris, Salmonella typhi , Klebseiella pneumonia*) and their qualitative and uantitative screening of *A. aritis, S. oleraceae* . Various leaf extracts (chloroform, ethanol and aqueous) showed varied antimicrobial activity to the test organism hich was species dependent. The presence of phytochemicals including alkaloid, henols, tannin, saponins, glycosides, quinines, flavonoids and coumarins were etermined in all the three greens leaves. But the quinines is absent in the *A. aritis*. he presence of these bioactive constituents is associated with the antibacterial ctivity of the plant. The proximate composition of these three green leaves were arried out using standard methods. The mean leaves moisture content is 94%, 87% nd 70.3% respectively, for *A. aritis, S. oleraceae, A. gangeticus*. The tested greens have high ash content, carbohydrate, protein respectively. Maceration evaluation of leaves of selected three greens was useful for setting standards for identification of the drug not only in crude form but also in finished products. *A.gangeticus* dry leaf

wder showed large vessel length (344μ) and large tracheid length (140μm) mpared to other two greens. The FTIR spectroscopy analysis of selected three eens showed the functional group of alkyl, methyl, alcohol, ether, carboxylic, phatic nitro and indo.

In conclusion present study illustrated that *A.aritis, S.oleraceae* and *gangeticus* leaves can be used in energy drinks or formulations as have high tritive value. It can be a good source of antioxidant agent and can replace harmful thetic antioxidants. It may become a part of natural antibiotics, cosmetics, and dicine as it provides potent antimicrobial activity towards wide range of microbes. sence of various active secondary metabolites is the main cause of its different logical efficacy. However these active principles need to be isolated, identified and racterized, and the structure need to be elucidated.

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PHYTOCHEMICAL SCREENING, NUTRITIVE VALUE, ANTIBACTERIAL ACTIVITY, FTIR AND GC-MS ANALYSIS OF SELECTED CAESALPINIACEAE MEMBERS

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October-2019

CERTIFICATE

This is to certify that this dissertation entitled, **"PHYTOCHEMICAL SCREENING, NUTRITIVE VALUE, ANT IBACTERIAL ACTIVITY, FTIR AND GC-MS ANALYSIS OF SELECTED CAESALPINIACEAE MEMBERS"** submitted by **S.JESI HEBZIBA, Reg. No. 18APBO04** to ST.MARY'S COLLEGE (Autonomous), THOOTHUKUDI in partial fulfillment for the award of the degree of **"Master of Science in Botany"** is done by her under my supervision. It is further certified that this dissertation or any part of this has not been submitted elsewhere for any other degree.

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DECLARATION

I do here by declare that this dissertation entitled **"PHYTOCHEMICAL SCREENING, NUTRITIVE VALUE, ANTIBACTERIAL ACTIVITY, FTIR AND GC-MS ANALYSIS OF SELECTED CAESALPINIACEAE MEMBERS"** Submitted by me in partial fulfilment for the award of the degree of **'Master of Science in Botany'**, in the result of my original and independent work carried out under the guidance of **Dr. Mrs. F. Dayana Lobo, M.Sc., M.Phil., Ph.D.**, Assistant Professor, Department of Botany, St. Mary's College (Autonomous), Thoothukudi and it has not been submitted elsewhere for the award of any other degree.

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INTRODUCTION

Indian has an ancient heritage of traditional medicine. Materia Medica of India provides lots of information on the folklore practices and traditional aspects of therapeutically important natural products (Umesh *et al*., 2010). Out of 17,000 species of higher plants reported to occur within India, 7500 are known to have medicinal use. The various indigenous systems such as siddha, ayurveda, unani and allelopathy use several plant species to treat different ailments (Perumal, 1997).

Medicinal plants possess therapeutic properties or exert beneficial pharmacological effects on the human and animal body (Ghani, 2003). Plants are the source of about 25% of prescribed drugs in the world (Rate *et al*., 2001). In developing countries about 80% people rely on traditional plant based medicines for their primary health care needs. There is abundant number of medicinal plants and only small amounts of them were investigated for its biological and pharmacological activities. The wide range of medicinal plant parts like flowers, leaves, barks, stems, fruits, roots extracts are used as powerful raw drug, possessing a variety of pharmacological activities. Discovery of new pharmaceutical agents from medicinal plants can combat the drastic increase in infectious diseases in many countries especially in rural areas and it has been used as an economic reason as well. Nowadays, there is widespread interest of drugs derived from plants which reflect its recognition of the validity of many traditional claims regarding the value of natural products in health care (Nair *et al*., 2005). Thus, in order to determine the potential use of medicinal plants, it is essential to intensify the study of medicinal plants that finds place in folklore. The application of herbs and medicinal plants in traditional medicine to diagnose, prevent or treat diseases dates back to many centuries among rural communities throughout the world (Conco *et al*., 1999).

The active Phytochemical produced by plants include, alkaloids, phenolic, anthraquinones, flavonoids, phenols, saponins, steroid, tannins, terpenes and etc., (Gahukar, 2010). In recent years, advances have been made in the development of antimicrobial compounds in an effort to check the harmful effects of microorganisms (Rao, 1995). Bacterial disease results when the harmful bacteria enter the organism then multiply and invade the body's defence mechanism. These pathogenic bacteria enter the body through inhalation, ingestion or damaged skin tissue. The inability of the immune system to stop the bacteria from reproducing and spreading consequently results in the symptoms of bacterial disease (Namukobea *et al.*, 2011). The antimicrobial resistance is the foremost problem all over the world with present antibiotic therapy in treating infectious diseases (Manikandan *et al.*, 2011). The development of dug resistance by microorganisms reduces the effectiveness of modern drugs (WHO, 2000). Thus, resistance to antibacterial agents poses threat in many areas of the world especially in the developing countries (Shears, 2000). The integration of traditional and modern medicine is gaining increase recognition globally (Abebe, 1996; WHO, 2000).

Caesalpinaceae is commonly called cassia family. The sub-family contains 135 genera which are cosmopolitan in distribution. *Cassia fistula* Linn. is a very common plant and is widely known for its medicinal properties. In the Indian literature, this plant has been described to be useful against skin diseases, liver troubles, tuberculosis glands and its use in the treatment of rheumatism, hematemesis, pruritus, leucoderma and diabetes. *Cassia siamea* Lam leaf of this plant has been used as vegetables in Thailand (Otimenyi *et al.*, 2007). Furthermore, Aliyu, (2006) revealed that *Cassia siamea* is ethno medicinally used as laxative, blood cleaning agent, cure for digestive system, urinogenitory disorders, herpes and rhinitis. A

traditional claim have cited *Cassia Siamea* Lam to be used for the treatment of typhoid fever, jaundice, abdominal pain, menstrual pain, in addition, it is claimed to be used for reducing sugar level in the blood. It has become necessary to further evaluate the pharmacological potential use of *C. siamea* leaves for the treatment of many other diseases. *Peltophorum petrocarpum*. Linn (belonging to Caesalpiniaceae family) regarded as one of the most significant plant species in traditional system of medicine. The plant is used in different parts of the world for the treatment of several ailments like stomatitis, insomnia, skin troubles, constipation, ringworm, insomnia, dysentery, muscular pains, sores, and skin disorders and is the source of a diverse kind of chemical constituents such as aliphatic alcohols, fatty acids, amino acids, terpenoids, phenolics, flavonoids, alkaloids, steroids etc. In India it is represented Phyto-chemical investigations of crude plant extracts shows the presence of active principles in the plant part of leaves. Plant derived substances has obtained greater attention in the recent years to prevent and cure human diseases as they are considered to be more bio-friendly. It is generally estimated that over 6000 plants in India are in use in traditional, folk, and herbal medicine, representing about 75% medicinal needs of the third world countries (Rajashekaran, 2002). Chemicals are non-nutritive plant chemicals that have protective or disease preventive properties. Plant produces these chemicals to protect itself but research works demonstrates that many phytochemicals can protect humans against diseases. Knowledge of the chemical constituents of plants is desirable because such information will be of value for the synthesis of complex chemical substances. So it is essential to work on medicinal plants to bring neutraceutical and pharmaceutical values.

SCOPE AND OBJECTIVES

Plants derived natural products such alkaloids, flavonoids, terpenoids have received considerable attention in recent years due to their diverse pharmacological properties including antimicrobial, antioxidant and anticancer activities. Plant crude extracts were containing large amount of natural antioxidants, which are used as folkloric medicines. According to World Health Organisation, medicinal plants would be greatest source to obtain an array of drugs. Thus, such plants should be investigated to better understanding for their properties, safety practices in addition to usefulness. In India, the ayurvedic system has features a numerous of such medicinal remedies on plants or plant products and the determination of their morphological, pharmacological characters can provide a better understanding of their active principle and mode of action. However it is essential to work on locally available resources to bring out their neutraceutical and pharmaceutical values. So the present work was carried out in three genus of cassia family. Selected plants such as *Cassia fistula, Cassia siamea* and *Peltophorum petrocarpum* are rich in medicinal properties and many people are not aware of the therapeutic activities of such medicinal plants. So, in this regard we focused to study the phytochemical analysis, nutritive value and antibacterial activities of selected Caesalpiniaceae members. Hence the present investigation was undertaken with the following objectives:

- To qualitatively screen the presence of different phytochemicals of ethanol, methanol, petroleum ether, chloroform and aqueous extracts of leaves (*Cassia fistula, Cassia siamea* and *Peltophorum petrocarpum*).

- To evaluate the nutritional components of selected plants (*Cassia fistula*, *Cassia siamea* and *Peltophorum petrocarpum*) with special reference to protein, carbohydrate, lipid and vitamin
- To quantify the major antioxidant chemicals (Phenol, flovonoids and tannin)
- To elucidate the effectiveness of medicinal plants in controlling human pathogenic bacteria such as *Escherichia coli*, *Bacillus subtilis* and *Serratia marcescens*
- To identify and compare the functional group of leaf extracts of (*Cassia fistula*, *Cassia siamea* and *Peltophorum petrocarpum*) by Fouriour transform infrared spectroscopy (FTIR) analysis.
- To analyze the secondary metabolites present in the methanolic leaf extracts of *Cassia fistula*, *Cassia siamea* and *Peltophorum petrocarpum* by Gas chromatography – Mass Spectrum (GC-MS).

REVIEW OF LITERATURE

Medicinal value

The medicinal plants are not only used as medicines to maintain their health care, also consumed as food by several Tribes of Indian subcontinent. Phytochemicals are responsible for medicinal activities of the plants. Based on this fundamental knowledge several pharmaceutical industries are established. The phytochemical constituents that are playing a significant role in medicines can be identified using crude extracts drugs of the plants (Savithramma *et al*., 2011). These are non-nutritive chemicals that protect human beings from various diseases. Phytochemicals are basically divided into two major groups, there are primary and secondary metabolites that are categorized based on the function in plant metabolism. Primary metabolites comprise common carbohydrates, amino acids, proteins and chlorophylls, while secondary metabolites consist of alkaloids, saponins, steroids, flavonoids and tannins. (Kumar *et al*., 2009)

Aliyu *et al*. (2006) revealed that *S. siamea* is ethno medicinally used as laxative, blood cleaning agent, cure for digestive system, urinogenitory disorders, herpes and rhinitis. A traditional claim have cited *Senna Siamea* Lam to be used for the treatment of typhoid fever, jaundice, abdominal pain, menstrual pain, in addition, it is claimed to be used for reducing sugar level in the blood.

Kumar *et al*., 2010 *Bauhinia acuminata* of the family Caesalpiniaceae is reported as medicinally important in traditional system of medicine and are used extensively for the treatment of inflammation, headache, fever, tumors and skin infections etc.,

Agarwal *et al.*, 2005). The plant contains rhein glucoside, rhein, fistulic acid, sennoside A & B (Nair *et al.*, 2005).

Aurantiamide acetate (0.011), β sitosterol (0.006) and its β D glucoside (0.02%) has been isolated from flowers. The roots contain 7-methylphyscion, betulinic acid and βsitosterol. The stem bark contains two flavonol glycosides, 5,7,3',4'-tetrahydroxy-6,8-dimethoxyflavone-3-O-α-arabinopyranoside ($C_{22}H_{22}O_{13}$, m.p.285°), 5,7,4'-trihydroxy 6,8,3'trimethoxyflavone-3-O- α-L -rhamnosyl (1→2)-O-β-D glucopyranoside ($C_{35}H_{36}O_{18}$m.p. 210°) a xanthone glycoside, 1,8-dihydroxy-3, 7-dimethoxyxanthone-4-O- α-L rhamnosyl(1→2)-O- β-D-glucopyranoside ($C_{27}H_{32}O_{16}$, m.p. 217°). The cuticular wax of leaves contain hentriacontanoic, triacontanoic, nonacosanoic and heptacosanoic acids. The seed oil contains cyclopropenoid fatty acids, viz, vernolic, malvalic and steteulic acids. (Nair *et al.*, 2005).

Mahajan *et al.*,(2016) analysed of the pulp seed and shell (dry basis) gave: moisture, 60.4, 70.1, 34.2; protein, 5.8, 15.9, 3.8; total N, 0.93, 2.5, 0.6; ash, 5.6, 4.5, 1.8% respectively; and energy (fruit) 4.25 kcal/g. The pulp contains sucrose, 31.3; fructose, 26.2, and glucose, 42.5% and high concentration of potassium (1809mg/100g dry basis). The pods contain 5 nonatetracontanone, 2-hentriacontanone

Fruit pulp contained proteins (19.94) and carbohydrates (26.30%); arginine, leucine, methionine, phenylalanine, tryptophan, aspartic and glutamic acids isolated from fruit pulp; a new dimeric proanthocyanidin CF1 isolated along with (-) epiafzelechin, (+) catechin, kaempferol, dihydrokaempferol and 1,8-dihydroxy-3-methylanthraquinone and its structure was determined (Nair *et al.*, 2005).

Mahajan *et al.* (2016) investigated that, studies on the plant revealed the presence of chrysophanol, rhein, physicion, and kaempferol. The identities of the compounds were confirmed by spectrometry (NMR, MS, IR) and direct comparison (Co-TLC, MMP) with authentic samples.

Kalimuthu *et al.* (2010) reported the presence of proanthocyanidins containing flavon-3-ol (epiafzelechin and epicatechin) units with 2S- configuration, *viz.* catechin, epiafzelechin, epicatechin, procyanidin B-2 and its enantiomer, epiafzelechin-(4β→8)-epicatechin and its enantiomers, epicatechin-(4β→8)-ent- epiafzelechin and its enantiomer. An anthraquinone derivative: 3-formyl-1-hydroxy-8-methoxy anthraquinone, 3β-hydroxy-17 norpimar -8(9)-en-15- one and 26-methylheptacosanoic acid.

Bauer *et al.* (1996) reported that the fruit was a good source of Fe and Mn, and their concentrations were considerably higher than those in apple, apricot, peach, pear and orange. Aspartic acid, glutamic acid and lysine constituted 15.3, 13.0 and 7.8%, respectively, of the total amino acids in the pulp. In the seeds the same amino acids constituted, respectively, 16.6, 19.5 and 6.6%.

Vaishnav *et al.* (1996) confirmed that Rhamnetin 3-*O*-gentiobioside was isolated from the roots of Cassia fistula.

Misra *et al.* (1996) reported that the hexane fraction of fruits (collected from India) exhibited antibacterial activity against *Klebsiella* sp. 5-Nonatetracontanone, 2-hentriacontanone, triacontane, 16-hentriacontanone and beta-sitosterol was isolated from the hexane fraction.

Misra *et al.* (1997) isolated a new diterpene, 3 beta -hydroxy-17-norpimar-8(9)-en-15-one from the pods of *Cassia fistula*.

Misra *et al.* (1997) reported that from the pods of *Cassia fistula*, an anthraquinone derivative, characterised as 3-formyl-1-hydroxy-8-methoxy-anthraquinone *1*, was isolated. This is the first report on the isolation and characterisation of this compound.

Kulkarni *et al.* (2015) analysed phytochemical of *Cassia fistula* using different solvents (petroleum ether, mthanol and aqueous extracts) for presence of various phytoconstituents. They also performed *in vitro* studies of these extracts for antimicrobial, antioxidant and anti-inflammatory activities. From studies they found that methanolic extracts showed maximum activities due to presence of more total phenolic content followed by aqueous and petroleum ether extracts.

Joseph Joseline *et al.* (2014) explored the phytochemical constituents in the aqueous, petroleum ether, chloroform, ethanol and acetone extracts of petals of *P. pterocarpum* and founded the phenolic compounds, phytosterols, coumarins in all the extracts. Flavanoids in aqueous, ethanol and acetone extracts. Terpenoids in all the extracts of *P. petrocarpum* except the ethanolic extract. Steroids in petroleum ether and chloroform extracts. Quinones in petroleum ether extract. Proteins were detected in aqueous, petroleum ether, chloroform and ethanol extracts and carbohydrates in aqueous and chloroform extracts. The presence of diverse secondary metabolites like saponins, terpenoids, steroids, tannins, phenols, alkaloids and coumarins in the leaf extract of *P. pterocarpum* were confirmed by Amala *etal.* (2015).

Pooja Moteriya *et al.* (2015) performed the preliminary qualitative phytochemical screening and found to possess the phytoconstituents like alkaloids,

flavanoids, triterpenes and tannins in different amounts in the *P. pterocarpum* flower extracts.

Duke *et al.* (1981) isolated three new anthraquinone glycosides from seeds of *Cassia tora*. Their structures were elucidated by spectral analysis. Two compounds showed weak protective activity on primary cultured hepatocyte against carbon tetrachloride induced toxicity.

Chanda *et al.* (2009) revealed the presence of phenolic compounds, flavanoids, saponins, steroids, tannins, xanthoproteins, carboxylic acids, coumarins and carbohydrates in the methanol extract of *P. pterocarpum* flowers.

Das *et al.* (2011) evaluated *Cassia tora* stem bark for pharmacognostic and phytochemical studies. In pharmacognostic studies, they evaluated the plant for macroscopy, microscopy, various physico-chemical parameters and behaviour of bark powder with different chemical reagents. The phytochemical studies of different extracts showed the presence of carbohydrate, glycosides, alkaloids, steroids, flavonoid, tannins and phenols.

Pandey *et al.*(2011)carried out phytochemical analysis in leaves of *Cassia fistula* Linn. which belongs to family Leguminosae is a medium-sized tree and its different parts are used in ayurvedic medicine as well as home remedies for common ailments. Sequential extraction was carried out using solvents viz. petroleum ether, chloroform, ethanol, methanol and water from leaf of the plant were investigated for preliminary phytochemical and antibacterial property. The minimum inhibitory concentration ranged in between 94 to 500 Mg/ml. Evaluation of phytochemicals such as alkaloids, flavonoids, carbohydrates, glycosides, protein and amino acids, saponins, and triterpenoids revealed the presence of most of constituents in polar extracts.

Usha Veerachari *et al* (2011) investigated the phytochemical screening in *Cassia spectabilis*, *Cassia siamea*, *Cassia fistula*, *Cassia biflora and Cassia hirsuta*. Qualitative analysis was done for various phytoconstituents like alkaloids, tannins, saponins, anthraquinones, anthocyanosides, phenolic, flavonoids, carbohydrates, proteins, steroids, terpenoids, cardiac glycosides and phlobatannins. The leaf extracts were prepared using various solvents like ethanol, methanol and ethyl acetate to detect the presence of the active components. The phtyo chemical screening revealed the presence of all the above chemical constituents except anthraquinones, anthocyanosides and phenolic flavonoids.

Verma *et al*. (2007) observed that *Cassia fistula* seed grown under different soil and climatic conditions of Bangladesh, contained 3% golden coloured oil. The oil was fractionated into mono, di, and triglycerides by silicic acid column chromatography. The triglycerides varied from 89.16% to 91.01%, diglycerides from 2.51% to 3.32% and monoglycerides from 0.91% to 0.98% depending on the areas from which the seeds were collected. Fractionation of lipids into three major lipid groups neutral lipids, glycolipids and phospholipids was carried out by silicic acid column chromatography. The neutral lipids were accounted for over 89.80% of the total weight of the lipid employed. Saturated and unsaturated fatty acids present in the oil were separated and varied from 23.79% to 28.20% and 63.28% to 66.71% respectively depending on the areas. The fatty acid composition of the oil was analysed by gas liquid chromatography (GLC). The major fatty acids found in the oil were linoleic acid (42.42%), oleic acid (29.62%), stearic acid (14.33%) and palmitic acid (11.41%). In addition to the above, caprylic acid (0.76%) and myristic acid (1.44%) were also present in minor amounts (Verma *et al*.,2007)

Lee *et al.* (2001) reported that twenty-seven compounds including eight long-chain hydrocarbons. 1-hexacosanol. 1-octacosanol. palmitic acid. stearic acid. oleic acid. linoleic acid.heptacosyl eicosanate. glyceryl-1-tetraeicosanoate: three sterols. beta -sitosterol. stigmasterol. beta -sitosteryl-3-*O*-D-glucopyranoside: one triterpene. lupeol: eight anthraquinones. chrysophanol. emodin. physcion. citreorosein. rhein . rhein methyl ester. ziganein. 1.4.5- trihydroxyanthraquinone: two coumarins. isoscopoletin. scopoletin: two chromones. 2.5- dimethyl-7-hydroxychromone. 2.5-dimethyl-7-methoxychromone: three aromatic compounds. isovanillic acid. vanillic acid and 2.4-dihydroxybenzaldehyde were isolated and identified from the aril of *Cassia fistula*. Their structures were determined on the basis of spectral data (Lee *et al.*, 2001)

Yueh-Hsiung Kuo *et al.* (2002) revealed that four new compounds. 5-(2-hydroxyphenoxymethyl) furfural. (2′*S*)-7-hydroxy-5-hydroxymethyl-2-(2′-hydroxypropyl) chromone. benzyl 2-hydroxy-3.6-dimethoxybenzoate and benzyl 2β-*O*-D-gluco-pyranosyl-3.6- dimethoxybenzoate. together with four known compounds. 5-hydroxymethylfurfural. (2′*S*)-7- hydroxy-2-(2′-hydroxypropyl)-5-methylchromone. and two oxyanthraquinones. chrysophanol and chrysophanein. were isolated and identified from the seeds of *Cassia fistula*. The structures were determined on the basis of spectral data explanation. and the synthesis of a compound was carried out.

Yadav *et al.* (2003) isolated a new bioactive flavone glycoside 1 (mp 252-2540C. $C_{28}H_{32}O_{16}$. [M]− 624 (EIMS)) was isolated from the acetone soluble fraction of the defatted seeds of *Cassia fistula*. It was characterized as a new bioactive flavone glycoside 5.3′.4′-trihydroxy- 6-methoxy-7-*O*- alpha -L-rhamnopyrano syl-(1 -> 2)-*O*-

beta –D galactopyranoside by several colour reactions, spectral analysis and chemical degradations.

Ali *et al.* (2003) isolated three lectins, i.e. CSL-1, CSL-2 and CSL-3, purified from the *Cassia fistula* seeds and were tested for their antibacterial activities against different pathogenic bacteria. The neutral sugar contents of CSL-1, CSL-2 and CSL-3 were estimated to be 3.5, 3.1 and 2.0%, respectively. The sugar composition of the lectins was found to be galactose in CSL-1, galactose and glucose in CSL-2, and galactose and mannose in CSL-3.

Sartorelli *et al.* (2007) examined the bioguided fractionation which resulted in the isolation of a sterol, cholesterol, which was further analysed in different models [30].

Tzakou *et al.* (2007) examined the chemical compositions of the flower and leaf essential oil of *Cassia fistula* by GC and GC/MS. Forty-four compounds were identified representing 92.6% and 90.7% of the flower and leaf oil, respectively. The main components of the flower oil were (E)-nerolidol (38.0%), and 2-hexadecanone (17.0%), while the leaf oil consisted mainly of phytol (16.1%).

Sartorelli *et al.* (2009) discovered that the fractionation through bioguided antileishmanial activity of the dichloromethane extract of *Cassia fistula* fruits (Leguminosae) led to the isolation of the active isoflavone biochanin A, identified by spectroscopic methods.

Antioxidant

Amitabye *et al.* (2002) investigated the total phenolic, proanthocyanidin, and flavonoid contents and the antioxidant activities, of fresh vegetative and reproductive organs of Cassia fistula harvested at different stages of growth were determined using the Trolox equivalent antioxidant capacity (TEAC) and ferric-reducing antioxidant power (FRAP) assays. The antioxidant activities were strongly correlated with total phenols (TEAC r) 0.989; FRAP r) 0.951) in all organs studied, and with proanthocyanidins (TEAC r) 0.980; (FRAP r) 0.899; in reproductive organs including fruits. The antioxidant activities of reproductive parts were higher than those of the vegetative organs, with the pods having highest total phenolic, proanthocyanidin, and flavonoid contents and antioxidant potentials (TEAC) 992 (0.4 *i*mol/g dry weight; FRAP) 811 (23 *i*mol/g dry weight).

Manonmani *et al.* (2005) reported that aqueous extract of *Cassia fistula* (Linn.) flowers (ACF) was screened for its antioxidant effect in alloxan induced diabetic rats. An appreciable decrease in peroxidation products viz thiobarbituric acid reactive substances, conjugated dienes, hydroperoxides was observed in heart tissues of ACF treated diabetic rats. The decreased activities of key antioxidant enzymes such as superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase and glutathione in diabetic rats were brought back to near normal range upon ACF treatment. These results suggest that ACF has got promising antioxidative activity in alloxan diabetic rats.

Luximon *et al.* (2002) estimated antioxidant activity and total phenolic content, proanthocynidine and flavonoid content in vegetative and reproductive organs of *Cassia fistula.* They found that antioxidant activity was higher in reproductive organs including fruits than vegetative parts. They also correlate the antioxidant activity with total phenols.

Siddhuraju *et al.* (2002) evaluated antioxidant activity of 90% ethanol extract of leaves and 90% methanol extract of stem bark, pulp and flowers from Indian laburnum (*C. Fistula* L.). The antioxidant activity was maximum in stem bark minimum in flowers and pulp.

Pawanjit Kaur and Saroj Arora, (2009) investigated to explore the antioxidant potential of hexane, chloroform, ethyl acetate, methanol, and water extract of bark and leaves of *C. seamia* and *C. javanica* by superoxide anion radical scavenging assay. The different extracts showed significant inhibition of superoxide radicals in a dose-dependant manner. Among all the bark extracts of the methanol extract showed the maximum inhibition of

60.5% at 800 µg/ml concentration and water extract also showed strong antioxidant potential of 51.3% at 1000 µg/ml concentration. The water extract of bark of showed strong antioxidant potential of 55% at 1000 µg/ml concentration. The various leaf extracts of *C. siamea* showed moderate antioxidant potential of 25–50% at 1000 µg/ml, whereas methanol leaf extract of *Cassia javanica* showed strong antioxidant potential of 50.4% at 300 µg/ml concentration. This preliminary study indicates the antioxidant activity of the bark and leaves of *C. siamea* and *C. javanica.*

Ali *et al.* (2012) studied phytochemical and pharmacological aspects of *Cassia fistula*. In traditional medicine, it has been used in the treatment of diabetes, hematemesis, leucoderma, pruritis, intestinal disorder and as antipyretics, analgesic and laxative. The fruits, stem bark, and leaves of this plant contain a variety of biologically active compounds. Such as anthraquinones, flavonoids, flavon-3-ol derivatives, alkaloid, glycosides, tannin, saponin, terpenoids, reducing sugar and

steroids those have various medicinal properties. The fruit and stem bark extract shows various activities like antipyretic, anti-inflammatory, antioxidant, antidiabetic, hypolipidemic, hepato-protective, antimicrobial, antitumor, antiulcer etc. The article reviews the various activities of the plant.

Antimicrobial activity

Yadav *et al.* (2003) isolated compound from *C. fistula* which showed antimicrobial activity against *Staphylococcus aureus, Bacillus subtilis, Klebsiella pneumoniae, Escherichia coli, Aspergillus niger* and *Fusarium oxysporum.*

Ali *et al.* (2004) reported that the antibacterial and antifungal activities of *C. fistula* and *M. ferrea* extracts were tested on 14 bacteria and 6 fungi. *C. fistula* extracts showed stronger antibacterial activity than *M. ferrea.*

Sundararaju *et al.* (2006) reported that 100% mortality was recorded from the *C. fistula* extract at 48 h at 50 and 100% concentrations. At 72 h, 100% mortality was observed in all extracts at all three concentrations. The mortality rate was minimum at 24 h in all three extracts. All plant extracts exhibited a high degree of nematicidal action against the adults and juveniles of *P. coffeae.*

Ali *et al.* (2006) reported that three lectins, i.e. CSL-1, CSL-2 and CSL-3, purified from the *Cassia fistula* seeds, were tested for their antibacterial activities against different pathogenic bacteria, i.e. *Bacillus subtilis, B. megaterium, Streptococcus haemolyticus, Streptococcus aureus, Sarcina lutea, Shigella sonnei, Escherichia coli, Klebsiella sp., Shigella shiga, Shigella boydii, Shigella flexneri, Shigella dysenteriae, Salmonella typhi* and *Pseudomonas aeruginosa*, using 30 micro g/disc. CSL-3 was active against all bacterial strains and showed strong activity

against *B. megaterium*, *Streptococcus haemolyticus* and *Shigella boydii* CSI-2 showed poor activity.

Chukwujekwu *et al.* (2006) isolated and identified emodin from the ethanolic root extract of *Cassia occidentalis* and reported antibacterial activity of isolated emodin against *Bacillus subtilis* and *Staphylococcus aureus*.

Aliyu *et al.* (2008) evaluated the phytochchemicals and antibacterial potentials of ethanol and Acetone extracts of *Sianna siamea* leaves on *Escherichia coli* and *Staphylococcus aureus*. The phytochemical test was carried out using standard methods and investigation the presence of saponin, glycoside, steroid phenol and reducing sugar, while alkaloid and flavonoid were absent. The antibacterial activity of the leaf extract was bio-assayed using the agar well diffusion method and broth (tube) dilution method.

Sharma *et al.* (2010) analyzed the antibacterial activity of ethanolic and aqueous extracts from *Cassia tora* leaves and found that both extracts exhibited significant antibacterial activity.

Gaurav Kumar *et al.* (2010) evaluate the antimicrobial activity of the leaves of the *Bauhinia racemosa L.* In the current study, the aqueous and methanol extract of leaves of *B. racemosa L.* was tested against standard bacterial and fungal cultures. *In vitro* antimicrobial test was performed by agar well diffusion method on Mueller hinton agar and Sabouraud dextrose agar for bacterial and fungal cultures respectively. The methanol extract showed a broad spectrum of antimicrobial activity as it inhibited Gram negative bacteria (*Escherichia coli, Micrococcus luteus* and *Pseudomonas aeruginosa*), Gram positive bacteria (*Bacillus subtilis*) and fungi

(*Candida albicans* and *Aspergillus niger*). Both extracts showed maximum relative percentage inhibition against *A. niger*.

Kumar *et al.* (2006) reported that antimicrobial activity of fruit of *C. fistula* by agar dilution streak method at a concentration of 500 µg/ml. Only *E. coli* was moderately inhibited, whereas no inhibition was found in case of *B. subtilis* and *S. epidermidis*. However, in our study, *B. subtilis* was completely inhibited at concentration of 375 µg/ml by ethanol, methanol, and aqueous extract, while *S. epidermidis* was inhibited at concentration of 187.5 µg/ml by the same extract.

Perumasl Samy *et al.* (1998) reported moderate antibacterial activity of *C. fistula* against a wide spectrum of bacteria such as *E. coli, Bacillus mycoides, B. subtilis, Mycobacterium smegmatis, Klebsiella aerogenes, Pseudomonas aerogenes*, and *Proteus vulgaris*.

Jadav *et al.* (2010) reviewed on *C. occidentalis* which is used to cure various diseases in traditional medicine. It possess antibacterial, antifungal, antidiabetic, anticancer, antimutagenic and hepatoprotective activity. Wide range of chemical constituents including achrosin, aloeemodine,emodine, anthraquinone, chrysophanol etc., have been isolated.

Choudhary and Nagori (2014) evaluated the *in vitro* antimalarial activity of alcoholic extract of leaves of *C. occidentalis* on *Plasmodium falciparum* strain. They also fractionated the compound quinine and found that the antimalarial activity may be due to this compound.

Verma *et al.* (2010) analysed the antidiabetic activity of ethanolic extract of *C. occidentalis* in normal and alloxan-induced diabetic rats. The extract exhibited

significant antidiabetic activity and also resulted in improvement in parameters like body weight and lipid profile as well as regeneration of cells of pancreas and so it might be of value in the treatment of diabetes.

Usha veerachari *et al.* (2012) isolated Ononitol monohydrate from leaves of *Cassia tora* L. and evaluated for hepatoprotective activity against CCl4 induced hepatotoxicity in rats. They found that at concentration of 20mg/kg it decreased the levels of serum transaminase, lipid peroxidation and TNF-α but increased the levels of antioxidant and hepatic glutathione enzyme activities when compared with standard reference compound Silymarin and showed hepatoprotective activity.

Hugh *et al.* (2011) studied composition and antifungal activity of *Cassia fistula* pulp and seed oil in *Candida albicans*. GC-MS analysis of *Cassia fistula* oil showed presence of antimicrobial compounds like beta-sitosterol, stigmasterol, ergosterol, lupeol, fucosterol, alpha-amyrin and friedelin. They revealed that mechanism of antifungal activity was due to decrease in biosynthesis of ergosterol in *Candida* cell wall.

Kulkarni *et al.* (2015) studied methanol extract of whole plant of *C. fistula* for GC-MS analysis and anticancer activity on human prostate cancer line and identified 10 different phyto-constituents from GC-MS analysis. The methanolic extract also showed significant anticancer activity in MTT assay.

Yunfeng zhang *et al.* (2007) isolated volatile oil from seeds of *Cassia tora* by supercritical fluid extraction method using CO2 and analyzed by gas chromatography and GC-Mass spectroscopy. They found that volatile oil was rich in aliphatic acids and anthraquinones and showed significant antioxidant activity with IC50 value of 137 µg/ml.

FTIR

The FTIR analysis of methanol and aqueous leaf extracts of *Bauhinia racemosa* revealed the presence of protein, oil, fats, phenolic compounds, flavonoids, saponins, tannins and carbohydrate as major functional groups. (Gaurav Kumar *et al.*, 2010)

Starlin *et al.* (2013) analyzed the ethanol extracts of *Ichnocarpus frutescens* using FTIR analysis that revealed functional group components of amino acids, amides, amines, carboxylic acid, carbonyl compounds, organic hydrocarbons and halogens.

Nithyadevi and Sivakumar (2015) worked on the methanol leaf extracts of *Solanum torvum* to confirm the presence of alcohol, alkanes, aromatic carboxylic acid, halogen compound, alkyl halide through the FTIR analysis.

Kalimuthu and Prabakaran (2013) reported 28 compounds with different chemical structures in the methanol extract of *Ceropegia pusilla*.

Palawat and Payal (2014) carried out the Gas chromatography mass spectroscopic investigation of methanol extract of *Ceropegia bulbosa*, an annual land plant using GCMS technique. They compared the mass spectra of the compounds with the standard library of NIST. Maximum peak area % found in leaf extract are 2H-Azepin-2-one, 3-(dimethylamino) hexahydro, 9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z) and followed by 2H-Azepin-2-one, 3-(dimethylamino) hexahydro, 2-Amino-9-(3,4-Dihydroxy-5- Hydroxymethyl-(16.08%) in the methanol tuber extract of *C. bulbosa*. Though 29 compounds were identified using GC-MS analysis, the maximum amount of bioactive compound found in the methanol extract

of *C. juncea* is p-(Dimethylamino) benzaldehydeoxime. The compound is recognized by its peak area percentage (39.89%) on comparing with the data library.

Collection of plants

Selected plants such as *Cassia fistula*, *Cassia siamea* and *Peltophorum petrocarpum* were collected from St. Mary's College (Autonomous) Thoothukudi, Tamilnadu, India.

Extraction of plant materials:

Plant materials such as *Cassia fistula*, *Cassia siamea* and *Peltophorum petrocarpum* were thoroughly washed and shade dried at room temperature. The dried materials were ground well using pulveriser into powder and packed with No.1 Whatman filter paper. Package was placed in a Soxhlet apparatus and running with different solvents. The crude extracts were collected and dried at room temperature. 30°C after which yield was weighed and taken for further analysis.

The powdered samples (25 gms) were extracted with ethanol, chloroform, methanol, petroleum ether and water using soxhlet apparatus. The powder sample was bagged in a polythene bag and stored in air tight container for further work Solvent will be evaporated under vacuum and the concentrates were used for the study.

Cassia fistula L.,

Class	Dicotyledons
Sub Class	Polypetalae
Series	Calyciflorae
Order	Rosales
Family	caesalpinaceae
Genus	Cassia
Species	fistula

Plate: 1

Description

Cassia fistula, commonly known as golden is a flowering plant in the Family, Caesalpiniaceae of the legume family, Fabaceae. It is a popular ornamental plant and is also used in herbal medicine. The golden shower tree is a medium-sized tree, growing to 10–20 m (33–66 ft) tall with fast growth. The leaves are deciduous, 15–60 cm (6–24 in) long, and pinnate with three to eight pairs of leaflets, each leaflet 7–21 cm (3–8 in) long and 4–9 cm (1.6–3.5 in) broad. The flowers are produced in pendulous racemes 20–40 cm (8–16 in) long, each flower 4–7 cm (1.6–2.8 in) diameter with five yellow petals of equal size and shape. The fruit is a legume, 30–60 cm (12–24 in) long and 1.5–2.5 cm (0.6–1.0 in) broad, with a pungent odor and containing several seeds. The tree has strong and very durable wood.

Cassia siamea, (Lam.)

Class	Dicotyledons
Subclass	Polypetalae
Series	Calyciflorae
Cohort	Rosales
Family	Caesalpiniaceae
Genus	*Cassia*
Species	*siamea*

Plate 2

Plant description

Cassia siamea is a large tree bearing yellow flowers, generally found in the western peninsular-India, Sri Lanka, Malaysia and Thailand. The tree grows to a height of 15-20 m, with a high crown and straight bole. The alternate leaves are pinnate, with about 25-30 cm long rachis, and 8-13 pairs of shortly haired leaflets. The yellow colored senna siamea flowers, measure about 3.5 cm in length, and are placed in dense, 15-30 cm long racemes at shoot-ends. The glabrous, slightly curved and brown-colored pods grow in dense clusters, measure up to 25 cm in length, and contain about 20 seeds. The tree is commonly planted in avenues and gardens, the leaves can be used as manure and the flowers are used as a vegetable. *Cassia siamea* possesses many medicinal properties and has a sporadic effect. Propagation is performed by seeds.

Peltophorum pterocarpum (DC.).,

Class	Dicotyledons
Subclass	Polypetalae
Series	Calyciflorae
Cohort	Rosales
Family	Caesalpiniaceae
Genus	Peltophorum
Species	*P. pterocarpum*

Plate 3

Description

It is a deciduous tree growing to 15–25 m (rarely up to 50 m) tall, with a trunk diameter of up to 1 m belonging to Family Caesalpiniaceaea. The leaves are bipinnate, 30–60 cm long, with 16–20 pinnae, each pinna with 20–40 oval leaflets 8–25 mm long and 4–10 mm broad. The flowers are yellow, 2.5–4 cm diameter, produced in large compound raceme up to 20 cm long. Pollens are approximately 50 microns in size. The fruit is a pod 5–10 cm long and 2.5 cm broad, red at first, ripening black, and containing one to four seeds. It is a deciduous tree commonly used for ornamental purpose and as an avenue tree. Different parts of this tree are used to treat many diseases like stomatitis, insomnia, skin troubles, constipation, ringworm and its flower extract is known to be a good sleep inducer and used in insomnia treatment.

Preliminary phytochemical investigation

The petroleum ether, methanol, ethanol, chloroform and aqueous extracts were subjected to phytochemical screening to identify the presence of alkaloid, tannins, saponin, flavonoid, terpenoid, glycosides, quinine, anthroquinone, carbohydrates, protein and phenol.

Qualitative test

Test for tannins (Ciulei, 1994)

Ferric chloride solution test

Little quantity of extract was taken in a test tube. To this, 2 ml ethanol was added and mixed well followed by the addition of 1ml of 5 %ferric chloride reagent. Deep blue colour was observed which inferred the presence of tannins.

Test Flavonoids (Savithramma *et al.*, 2011)

Lead acetate test:

Small quantity of residue was taken in a tube to which lead acetate solution was added. Yellow colour precipitate formed which inferred the presence of flavonoids.

Test for Alkaloid (Kumar, 2011)

Mayer's test:

Little quantity of extract was taken in a test tube. To this, 2 ml dil. HCl was added. The solution was shaken well and filtered. This filtrate was used to perform the following test. 2 to 3 ml of filtrate was taken in a test tube followed by the addition of mayer's reagent. A white precipitate not formed which confirmed the absence of alkaloids.

Test for Quinines (Hugh,2008)

A small amount of extract was treated with concentrated HCl and observed for the formation of yellow precipitate (or colouration).

Test for Anthraquinone (kokate *et.al.*, 1995)

1 ml of the filtrate with 10ml benzene, filter and now add 5 ml of 10% (v/v) ammonia to the filtrate and shake well. Development of pinkish coloured solution indicated the presence of anthraquinones.

Test for Glycosides (Vaghasiya *et al.*, 2019)

Keller – Killiani test

2 ml of extract was taken in at test tube. To this, 1ml glacial acetic acid and 1 ml 5 %ferric chloride solution were added followed by the addition of 2 ml conc. sulphuric acid along the sides of the test tube. Reddish brown colour appeared at the junction of the two liquid layers. Appearance of this colour confirmed the presence of glycosides.

Test for Phenols (Padmanabhan *et al.*, 2014)

Ferric chloride test

A fraction of the extracts was treated with aqueous 5% ferric chloride and observed for formation of deep blue or black color presence of phenols.

Test for Carbohydrate (Palawat *et al.*, 2014)

Molish's test

To 2 – 3 ml extract few drops of molish's reagent (alpha naphthol solution in alcohol) was added. The test tube was shaken well and conc. sulphuric acid was added

Nutritive value of medicinal plants

Total carbohydrate (Ghani, 2003)

Reagents

5% Phenol

96% Sulphuric acid

Procedure

100 mg of leaf tissue was homogenized with 10 ml of distilled water and filtered through a muslin cloth. To 0.1 ml of the filtrate, 0.9 ml of distilled water, 1 ml of 5% phenol and 5 ml of 96% sulphuric acid were added. The contents were shaken well and after 30 minutes absorbance was read at 490 nm. Glucose was used as standard.

Total soluble protein (Lowry *et al.*, 1951)

Reagents

Alkaline copper reagent

Solution A – 2% Sodium carbonate in 0.1N sodium hydroxide

Solution B – 1% Sodium potassium tartarate

Solution C – 0.5% Copper sulphate

Procedure

To prepare 100 ml alkaline copper reagent, 98 ml of solution A, 1 ml of B and 1 ml of C were mixed together freshly. Folin-ciocalteau reagent (commercial reagent was diluted in the ratio of 1:1 distilled water at the time of use). 100 mg of sample was homogenized in 10 ml of distilled water and filtered through a muslin cloth and centrifuged at 3000 rpm for 10 minutes. To the supernatant 10% trichloro acetic acid (TCA) was added in 1:1 ratio and left in an ice bath for 30 minutes to precipitate

protein. Then, centrifuged at 3000 rpm for 5 minutes and discarded the supernatant. The precipitate was dissolved in 0.1 N sodium hydroxide and diluted to a known volume. To 0.5 ml of protein extract, 5 ml of alkaline copper reagent was added. After thorough mixing 0.5 ml of folin ciocalteau reagent was added and allowed to stand for 30 minutes; the blue color appeared and absorbance was measured at 650 nm using UV visible spectrophotometer (Model No: UV 2371). Amount of protein was calculated and expressed as mg/g DW. Bovine serum albumin (BSA) was used as standard.

Lipid (Kumar, 2010)

One gram of plant sample was ground in a pestle and mortar with about 10 ml of distilled water. The pulp was transferred to a conical flask and 30 ml of chloroform-methanol mixture (2:1 v/v) was added and mixed well. For complete extraction, it was kept for overnight at room temperature under darkness. At the end of this period, 20 ml chloroform and 20 ml water was added. The resulting solution was subjected to centrifugation, and 3 layers were formed. A clear lower layer of chloroform containing all the lipids, a coloured aqueous layer of methanol with water soluble material and a thick pasty interface were seen. The methanol layer was discarded and the lower layer was carefully collected free of interface by sucking out with fine capillary tube and transferred to a pre-weighed beaker and evaporated. When the solution is free of organic solvents, the weight was determined again. The difference in the weight was recorded as weight of lipid. The total amount of lipid was expressed in mg g^{-1}DW.

Vitamin-C (Ascorbic acid) (Ali, 2008)

Indophenol reagent (20 mg of dichlorophenol indophenol was dissolved in 10 ml of warm distilled water)

DT reagent (2 g of 2, 4 dinitrophenyl hydrazine and 1 g of thiourea were dissolved in 100 ml of 9 N sulphuric acid)

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

Antioxidant chemicals

Total phenolic content (Duan *et al.*, 2006)

Reagents

50% Folin – ciocalteaureagent

20% sodium – carbonate

Gallic acid – standard

Procedure

100 mg of plant sample was homogenized with 10 ml of distilled water and filtered through a muslin cloth 1ml of the filtrate was added to 1.5ml of deionized water and 1mlof 50% folinciocalteau reagent and the contents were mixed thoroughly. After 1 minute, 1 ml of 20% sodium carbonate solution was added mixed the control

contained all the reagents except the sample. After 30 minutes of incubation at 37°C, the absorbance was measured at 750 nm. Total phenolicswere calculated as Gallic acid equivalent (GAE) per gram fresh weight.

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

Reagents

5% sodium nitrate ($NaNo_2$)

10% Aluminium chloride ($Alcl_3$, H_2O)

1 N sodium hydroxide (NaoH)

Quercetin standard

Procedure

100 mg of plant material was homogenized with 10 ml of distilled water and filtered through a muslin cloth. 0.5 ml of the extract was added with 2.5 ml of distilled water and mixed. After 6 minutes 0.15 ml $NaNo_2$, was added and again after 6 minutes 0.3 ml of 10% $Alcl_3$was added. After 5 minutes 1 ml of 1M NaoH and 0.5 ml of water were added. Following through mixing of the solution the absorbance against blank was recorded at 510 nm. Quercetin was used as standard and the results were expressed as my quercetin equivalents (QE) 1g fresh weight.

Estimation of Tannin (Julkunen-Titto, 1985)

Procedure

100 mg of sample was homogenized with 10 ml of distilled water and filtered through a muslin cloth. 1 ml of aliquot of aqueous extract was mixed with 1.5 ml of 4% vanillin (Prepared with methanol) and 750 µl of concentrated HCl was added the solution was shaken vigorously and left to stand at room temperature for 20 minutes

in darkness the absorbance against blank was read at 500 nm using uv-visible spectrophotometer. Results were expressed as g catechin equivalent (CE) 1g tissue.

Antibacterial activity

The powdered plant samples (20 g) were extracted with Ethanol, chloroform, methanol, petroleum ether and water using soxhlet apparatus. Solvent was evaporated under vacuum and the concentrates were used for antibacterial assay.

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* and *Serrattia marcescens* obtained from the Department of Botany; St. Mary's College (Autonomous), Thoothukudi. Each bacterial pathogen was sub cultured in agar medium and maintained. What man No. 1 sterile filter paper discs (6 mm) were impregnated with different plant extract (Ethanol, Methanol, Chloroform, Petroleum ether and water). The spread plates were prepared by proper concentration of inoculation. 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 seaweed extract concentration which has effected minimum inhibition (MIC = 2.5 mg/ ml) was used for further studies.

GC-MS (Manikandan G *et al.*, 2019)

Gas chromatography-Mass spectrometry (GC-MS) analysis of the methanolic extracts was performed by using a GC-MS (Model: QP 2010 series, Shimadzu,

Tokyo, Japan) equipped with a VF-5ms fused silica capillary column of 30 m length, 0.25 mm dia. and 0.25 µm film thickness. For GC-MS detection, an electronionization system with ionization energy of 70 eV was used Helium gas (99.99%) was used as a carrier gas at a constant flow rate of 1.51 ml/min. Injector and mass transfer line temperature was set at 200 and 240°C respectively. The oven temperature was programmed from 70 to 220°C at 10°C/min, held isothermal for 1 min and finally raised to 300°C at 10°C/min. 2 µl of respective diluted samples was manually injected in the split less mode, with split ratio of 1:40 and with mass 18 scan of 50-600 amu. Total running time of GC-MS is 35 minutes. The relative percentage of the each extract constituents was expressed as percentage with peak area normalization

Identification of phytochemical components

The identity of the components in the extracts was assigned by the comparison of their retention indices and mass spectra fragmentation patterns with those stored on the computer library and also with published literatures. NIST08s.LIB and WILEY8. LIB library sources were used for matching the identified components from the plant material.

Fourier Transform Infrared Spectrophotometer (FTIR)

(Muruganantham *et al.*, 2009)

Preparation of leaf extract

The shade dried leaves of each plant (at20° C) were powdered in mechanical grinder. 20 grams of leaf powder (of each plant) was weighed, 150 ml of solvent was added and kept for 3 days. The extract was filtered using Whatman No.1 filter paper and the supernatant was collected. The residue was again extracted two times (with

3 days of interval for each extraction) and supernatants were collected. The supernatants were pooled and evaporated (at room temperature, 28 ± 1 C) until the volume was reduced to 150 ml. Extracts of the leaf powder of the three plants (*C. fistula, C. siamea, P. petrocarpum)* were prepared and stored in air tight bottles for further analysis.

Fourier Transform Infrared Spectrophotometer (FTIR) is perhaps the most powerful tool for identifying the types of chemical bonds (functional groups) present in compounds. The wavelength of light absorbed is characteristic of the chemical bond as can be seen in the annotated spectrum. By interpreting the infrared absorption spectrum, the chemical bonds in a molecule can be determined. Dried powder of different solvent extracts of each plant materials were used for FTIR analysis. 10 mg of the dried extract powder was encapsulated in 100 mg of KBr pellet, in order to prepare translucent sample discs. The powdered sample of each plant specimen was loaded in FTIR spectroscope (Shimadzu, IR Affinity 1, Japan), with a Scan range from 400 to 4000 cm^{-1} with a resolution of 4 cm^{-1}.

RESULT AND DISCUSSION

Preliminary qualitative phytochemical analysis

Phytochemicals are chemical compounds that are naturally found in plants. They are responsible for the colour and organoleptic properties of the plant. It is also referred to as those chemicals that may have biological significance but are not established as an essentials nutrient in plant. Phytochemicals could be available as dietary supplements, but the potential health benefits of phytochemicals are derived from consumption of the whole plant. Several phytochemicals have a wide range of activities, which helps to give immunity against long term disease. The phytochemicals like alkaloids, flavonoids, tannins, saponins, carbohydrates, glycosides, phytosterols, phenols, protein and amino acid, diterpens etc. are known to show medicinal activity as well as exhibit physiological activity. These chemicals are produced by plants, particularly the secondary metabolites which are synthesized as a measure for self-defense against insects, pests, pathogens, herbivores, UV exposure and environmental hazards. Phytochemistry takes into account the structural compositions of these metabolites, the biosynthetic pathways, functions, mechanisms of actions in the living systems and it's medicinal, industrial, and commercial applications. The proper understanding of phytochemical is essential for drug discovery and for the development of novel therapeutic agents against major diseases.

The results of the phytochemical analysis of the leaf extracts in various solvents has shown a remarkable variation in the presence the above studied phytochemical compounds in the studied taxa. The detailed investigations of phytochemicals in various solvents are shown in Table 1 to 3. The study revealed that the leaf extracts of *Cassia fistula* was showing maximum presence of alkaloids,

Table 1 : Phytochemical constituents present in the different solvent extracts of leaf of *Cassia fistula*

Name of the phytochemical	Qualitative test	Ethanol	Methanol	chloroform	Petroleum ether	Water
Alkaloids	Mayer's reagent	+++	+++	+++	+++	+
Anthraquinone	With Conc. Ammomia	–	–	–	–	–
Carbohytrates	Molish's reagent	+	+	+	+	+
Flavonoids	With Lead acetate	–	–	–		–
Glycosides	Keller-Kiliani test	+	–	–	–	–
Phenols	With ferric chloride	–	–	–	–	–
Protein	Xanthophylls test	+++	+++	+++	+++	+++
Quinine	With conc.HCL	+++	–	–	+	
Saponins	Distilled water	+	+	+	+	+
Steroids	Salkowski'test	+++	+++	+++	+++	+++
Tannins	With ferric chloride	+++	+++	–	+	–
Terpenoids	With $CHCL_3$, H_2SO_4	–	–	–	–	–

+++ High

++ Moderate

\+ Low

– Absent

Table 2 : Phytochemical constituents present in different solvent extracts of leaf of *Cssia siamea*

Name of the phytochemical	Qualitative test	Ethanol	Methanol	chloroform	Petroleum ether	Water
Alkaloids	Mayer's reagent	+	+	+	+	+++
Anthraquinone	With Conc. Ammomia	–	–	–	–	–
Carbohytrates	Molish's reagent	+	+	+	+	+
Flavonoids	With Lead acetate	+++	+	–	+++	–
Glycosides	Keller-Kiliani test	+	+	+	+	+
Phenols	With ferric chloride	–	–	–	–	–
Protein	Xanthophylls test	++	+++	+++	+++	+++
Quinine	With conc.HCL	+	+++	+	+	+
Saponins	Distilled water	+++	+++	+++	–	+++
Steroids	Salkowski'test	+++	+++	+++	+++	+++
Tannins	With ferric chloride	+++	+++	–	+	–
Terpenoids	With $CHCL_3$, H_2SO_4	+++	+++	+++	+++	+++

+++ High

++ Moderate

+ Low

– Absent

Table 3 : Phytochemical constituents present in different solvent extract of leaf of *Peltophorum petrocarpum*

Name of the phytochemical	Qualitative test	Ethanol	Methanol	chloroform	Petroleum ether	Water
Alkaloids	Mayer's reagent	+++	+++	+++	+++	+
Anthraquinone	With Conc. Ammomia	–	–	–	–	–
Carbohytrates	Molish's reagent	+	+	–	–	–
Flavonoids	With Lead acetate	–	+	–	–	–
Glycosides	Keller-Kiliani test	–	–	–	–	–
Phenols	With ferric chloride	–	+	–	–	–
Protein	Xanthophylls test	+++	+	+++	+	+++
Quinine	With conc.HCL	+	++	++	++	++
Saponins	Distilled water	+++	+++	+++	–	+++
Steroids	Salkowski'test	+++	+++	–	–	–
Tannins	With ferric chloride	–	–	–	–	–
Terpenoids	With $CHCL_3$, H_2SO_4	+	+	–	–	–

+++ High

++ Moderate

+ Low

– Absent

proteins and steroids in all solvents. Steroids in modern clinical studies have supported their role as anti-inflammatory and analgesic agents (Perumal,2012). Tannins reduce the risk of coronary heart diseases (Ghani *et al.*, 2003). Tannin was highly present in methanol and ethanol extracts. Quinine has diuretic property (Perumal*et al.*, 1998). Quinine was maximum in ethanolic extract, but were completely absent in methanol, chloroform and aqueous extracts. Terpenoids and anthraquinone were completely absent in all extracts. Saponin was low in all extracts.

In *Cassia siamea*, proteins, steroids and terpenoids were showing maximum presence in all extracts. Phenols and anthraquinone were absent in all extracts. Flavonoids were absent in chloroform and aqueous extracts but highly present in ethanolic and petroleum ether extracts. Tannin was adequately present in methanolic and ethanolic extracts but completely absent in chloroform and aqueous extracts. The present study regarding the qualitative analysis of the selected medicinal plants is in agreement with the previous findings of the various researchers (Bukar *et al.*, 2009; Mohammad *et al.*, 2013). Bukar *et al.*, 2009 who reported the absence of flavonoids, saponins and alkaloids in ethanolic extract of *C. siamea* leaves. Mohammad *et al.*, 2013 who reported the presence of flavonoids, tannins, polyphenols, anthraquinones, saponins and glycosides in *C. siamea* leaves extract.

The present study revealed that the various alcoholic and aqueous extracts of leaf of *Peltophorum petrocarpum* contained alkaloids, cardiac glycosides, flavonoids, glycosides, phenols, resins, saponins, steroids, tannins, terpenoids and triterpenoids (Table 3). Alkaloids are a diverse group of secondary metabolites found to have antimicrobial activity by inhibiting DNA topoisomerase (De Bruyne *et al.*, 1999). It is found in large amounts in all extracts of *Peltophorum petrocarpum* except aqueous extract. Saponins, present in plants, have been suggested as possible

anti-carcinogens. The proposed mechanisms of anticarcinogenic properties of saponins include direct cytotoxicity, immune modulatory effects, bile-acid binding and normalization of carcinogen-induced cell proliferation. However, the anticarcinogenic effects of saponins from commonly consumed plant foods have not been studied (Rao *et al.*, 1995). Soybeans are the most important sources of dietary saponins ans the main protein supplier in many vegetarian diets. Our results show that saponin was present in higher amounts in ethanol, methanol, chloroform and water extracts and absent in petroleum ether extract of *Peltophorum petrocarpum.*

However, phenols were detected in methanolic extracts and the carbohydrates were found in the solvents of ethanol and methanol. Next to methanol extract, ethanol extracts of leaf of *P. petrocarpum* showed the presence of rich variety of secondary metabolites. Petroleum ether, chloroform and water extracts showed the less variety of these secondary metabolites. Compared to all other solvent extracts, methanolic and ethanolic leaf extracts had higher number of secondary metabolites with high degree of precipitation (+++). Triterpenoids and flavonoids were determined to be present with lesser amount (+)in all extracts. Tannin, glycosides and anthraquinone were absent in all extracts. The Medicinal plants are rich in secondary metabolites which include alkaloids, flavonoids, saponins and related active metabolites which are of great medicinal value and have been active metabolites which are of great medicinal value and have been extensively used in the drug and pharmaceutical industry. These secondary metabolites are reported to have many biological and therapeutic properties. Recently number of studies had been reported on the phytochemistry of medicinal plants, particularly on the vegetative parts like leaves and stems etc (Kaur *et al.*, 2015).

Nutritive value of medicinal plant

The biochemical composition of plants is the most important parameter used for the characterization of plants. Proteins, carbohydrates, fats, vitamins, minerals and water are the nutrients that are essential for life and contribute to the caloric content of the body. Due to rapid population growth and climate change, the demand on conventional plants based food would increase in future. It is therefore, necessary to search for the alternatives in order to meet the growing demand for food. Hence, detailed information on the nutritional status and traditional uses of the documented medicinal plants is of utmost importance in upcoming era of climate change and food insecurities because it will play a significant role in the overall benefits to the health of people.

Estimation of carbohydrate

Carbohydrates are widely prevalent in the plant kingdom, comprising the mono-, di-, oligo-, and polysaccharides. Presence of Carbohydrate is generally not appreciated when the plant is considered for therapeutics. In this present study, total soluble carbohydrate was estimated quantitatively by using Lowry method. Total soluble carbohydrate was calculated with the help of a reference curve using D-glucose as standard. The absorbance was taken 630 nm by using the UV-spectroscopy instrument. This was observed that the carbohydrate level of ranged from 22.37 ± 0.06 mg/g DW to 68.75 ± 0.75 mg/g DW. Among three selected members the highest amount of carbohydrate was found in *C. siamea* (68.75 ± 0.75 mg/ g DW). Moderate amount of carbohydrate was noted in *C. fistula* (36.27± 0.08 mg/ g DW) and the lowest was observed in *P. petrocarpum* (22.37± 0.08 mg/ g DW). Carbohydrate is one of the important components for

Table 4 : Nutritive value of medicinal

S. No.	Plants	Carbohydrate (mg/ g DW)	Protein Mg/g DW	Lipids Mg/g DW	Vitamin C (mg/gDW)
1.	*C. fistula*	36.27± 0.08	10.51 ± 0.07	9.13± 0.10	14.85±0.042
2.	*C. siamea*	68.75 ± 0.75	20.83±0.02	9.81 ± 0.10	9.772±0.08
3.	*P. petrocarpum*	22.37± 0.08	18.25±0.1	5.55± 0.01	8.05±0.03

Values are the mean of 3 replicates ±SD

metabolism and it supplies the energy needed for respiration and other most important processes (Hedge and Hofreiter, 1962).

Protein

Proteins are large biomolecules, or macromolecules, consisting of one or more long chains of aminoacid residues the protein are major components of blood, muscles and connective tissues in animals. When protein is fed in excess it serves as a source of energy and fat. The protein are complex organic compounds of high molecular weight which are involved to make up different tissues and organs in the body. In our result the selected members of the plants highest amount of protein was found in *C. siamea* 20.83±0.02, Moderate amount of protein was noted in *Peltophorum petrocarpum* 18.25±0.1 and the lowest amount was observed in *cassia fistula* 10.51 ± 0.07 and proteins are composed of 21 biological amino acids. Nine of these are essential amino acids, which mean our body cannot produce them, and they must be derived from the food sourced. The presence of higher protein level in the plant parts towards their possible increase food value or that protein base bioactive compound could also be isolated in future. Similar results were results were reported by Kaur *et al.* 2015) in *cassia alata.*

Lipid

The lipids are a large and diverse group of naturally occurring organic compounds that are related by their solubility in nonpolar organic solvents, and general insolubility in water. Lipids molecules are containing hydrocarbons and make up building blocks of the structure and function of living cells. Example of lipids include fat oils, waxes, certain vitamins, hormones and most of the non-protein membrane of cells. Lipids are providing energy in oxidation processes than any other

biological compounds. It constitutes a convenient storage materials for living organisums (Sadasivam and Manickam2005).The cholesterol content range from 5.51±0.01 to 9.81±0.08 mg/Gdw). Among three selected members the highest amount of lipids was found in *C. siamea* (9.81±0.10 mg/g DW). Moderate amount of carbohydrate was noted in *C. fistula* (9.13±0.10 mg/g DW) and the lowest was observed in *P. petrocarpum* (5.55± 0.01 mg/g DW). The result was closed agreement to (Chanda *et al*., 2012) in *C. auriculkata*.

Antioxidant chemicals

Phenolic compounds are effective hydrogen donors, making them good antioxidants (Amic *et al*., 2003). Plant derived polyphenolic flavonoids are also well known to exhibit antioxidant activity. Flavonoids reduce free radicals by quenching, upregulating, or protecting antioxidant defences and chelating radical intermediate compounds (*Mahmooduzzfar et al*., 1993). It is also reported that tannins are 15–30 times more effective in quenching peroxyradicals than simple phenolics (Mahmooduzzfar *et al*., 1993).

The phenolics and polyphenols are one of the largest groups of secondary metabolites to have exhibited antimicrobial activity (Stefanvic *et al*., 2012). The site(s) and number of phenol groups are thought to be related to their relative toxicity to microorganisms, with evidence that increased hydroxylation results in increased toxicity (Agrawal 2014). Naturally occurring plant flavonoids have also been reported to possess antimicrobial activities (Kumar.2011]). The variation in the antibacterial activity of flavonoids is known to be related to their chemical structure, especially in regard to the number and positions of methoxy and phenolic groups within their structures (Otimenyin *et al*., 2010). The antimicrobial effects of tannins have also

Table 5 : Antioxidant chemicals value

S. No.	Plants	Phenol (mgGAE/g DW)	Flavonoid (mgQE/g DW)	Tannin (mgCE/g DW)
1.	*C. fistula*	0.91±0.061	0.14±0.029	6.61±0.32
2.	*C. siamea*	0.79±0.97	0.15±0.021	3.41±0.28
3.	*P. petrocarpum*	1.42±0.099	0.27±0.027	3.91±0.41

Values are the mean of 3 replicates ±SD

mg GAEs/g DW = milligram gallic acid equivalents per gram dry weight

mg QEs/g DW = milligram quercetin equivalents per gram dry weight

mg CE/g DW = milligram catechin equivalents per gram dry weight

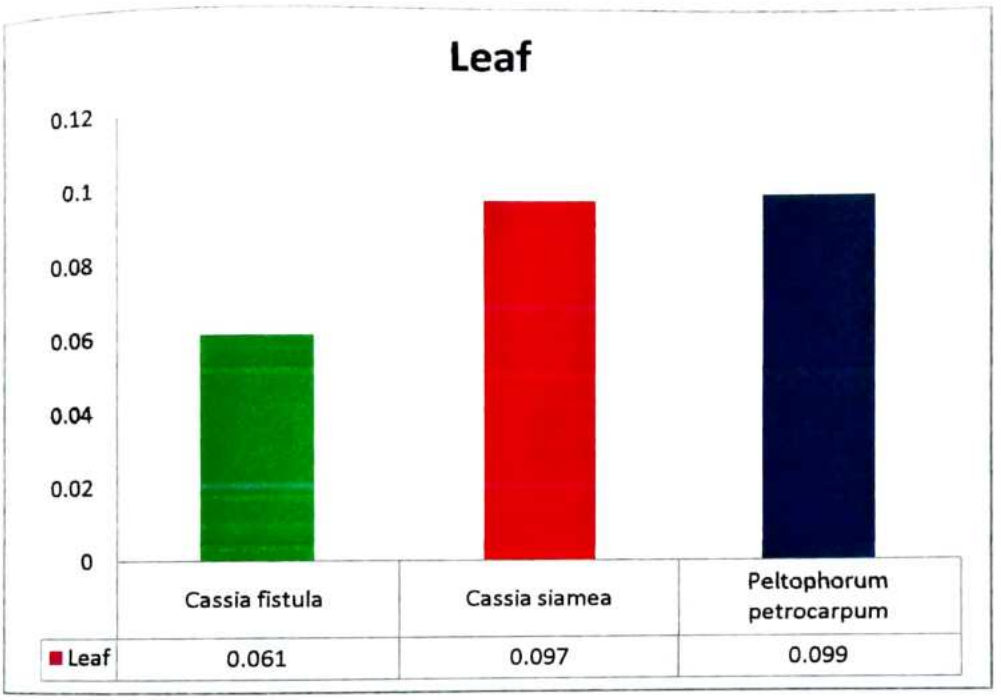


Fig. 1 : Total phenol content of selected plants

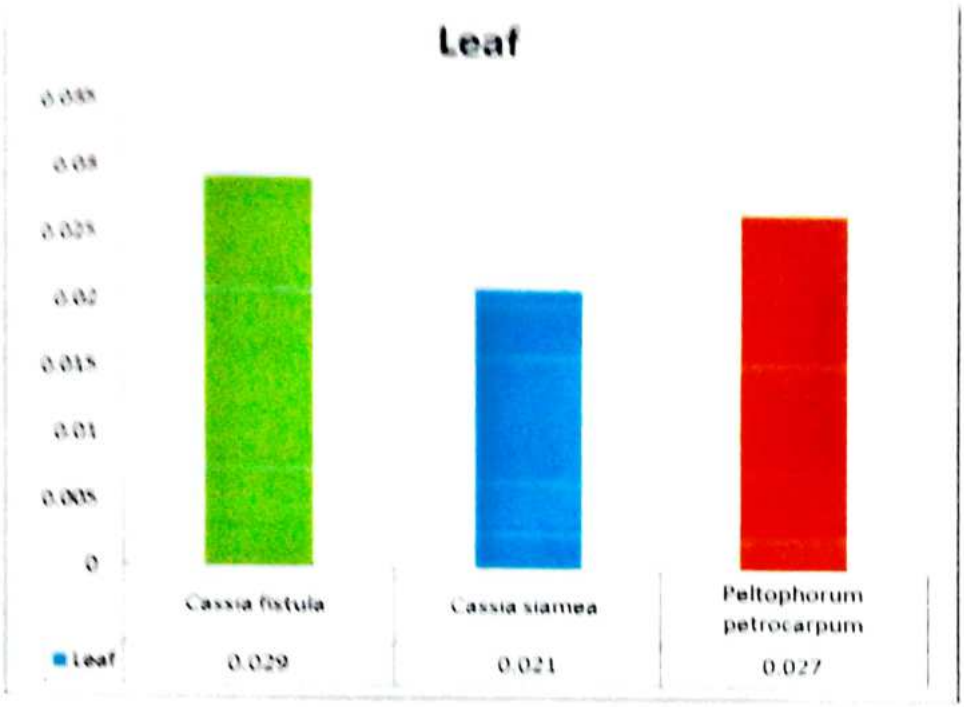


Fig. 2 : Total Flavonoid content of selected plants

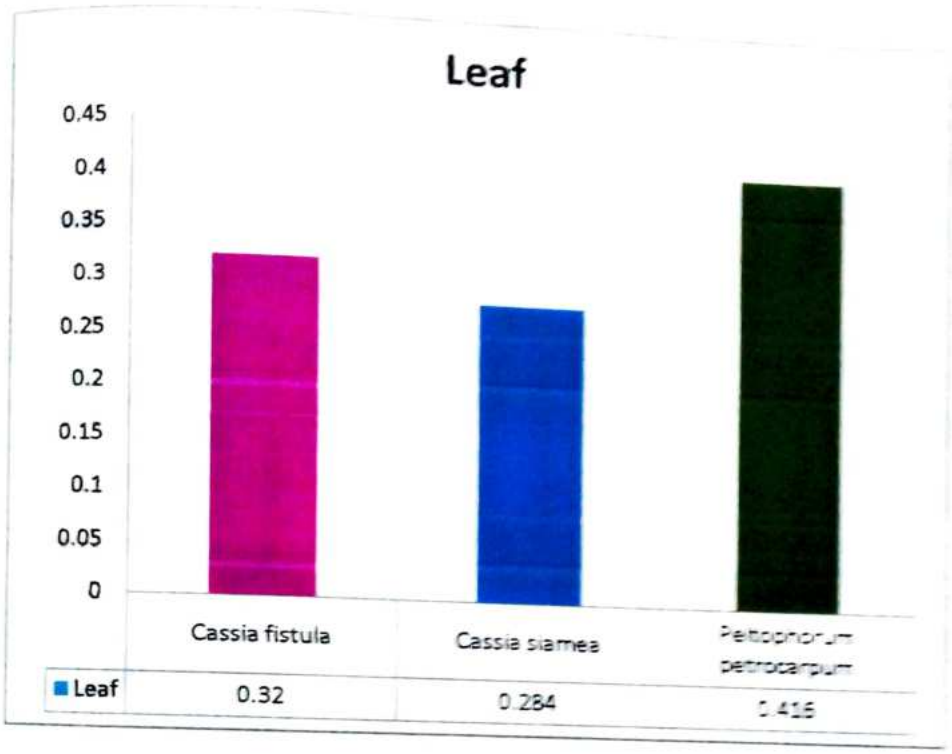


Fig. 3 : Total Tannin content of selected plants

been widely recognised (De Bruyne *et al.*, 1999). Therefore, the total phenolic, flavonoid, and tannin contents of the three plant extracts (*Cassia fistula, Cassia siamea, Peltophorum petrocarpum)* of belonging to the family caesalpiniaceae were examined for their antioxidant chemicals.

Total phenol

The total phenolic contents were determined as mg□GAE/g extract on comparison with a standard gallic acid graph. The total phenolic content of studied plants ranged from 0.79 to 1.42 mg GAE/g. Leaf extract of *Peltophorum petrocarpum* (1.42 mg QE/g) contained comparatively higher amount of total phenol whereas *Cassia fistula* (0.79 mg QE/g) and *Cassia siamea* (0.91 mg QE/g) had very low quantity (Table 4). Phenolic compound are useful in treating inflammation, stomach ulcers and diarrhoea. (Kumar *et al.*, 2010)

Total Flavonoid

The total flavonoid content was determined as mg□CE/g extract after comparison with a catechin standard graph. Total flavonoid level was ranged between 0.27 and 0.14 mg QE/g DW. The study revealed that *Cassia fistula* and *Cassia siamea* leaf extract possessed very low quantity of flavonoid. It was found that *Peltophorum petrocarpum* leaf extract was endowed with higher amount of flavonoid than other plants. Flavonoids are useful in reduce body heat and remarkable activity in cancer prevention and anticancer activities. (Veerachari, 2011)

Tannin

The total condensed tannin content was evaluated as mg□CE/g of extract after comparison with a catechin standard graph. Total condensed tannin content varied

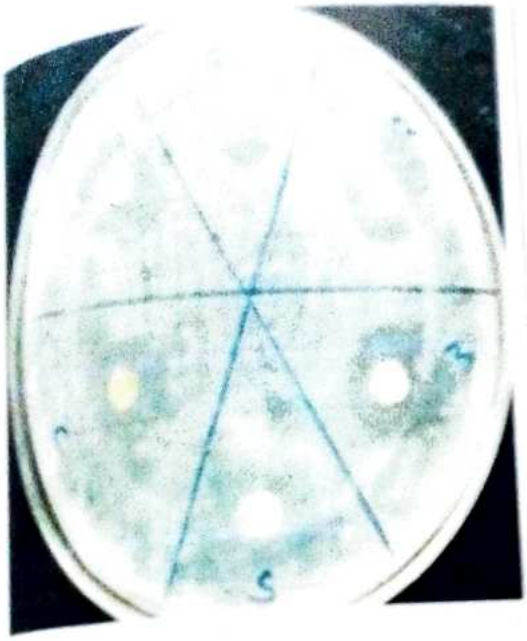
among three plants. *P. petrocarpum* (6.61 mg CE/g DW) was found to have more tannin than other plant. *C. siamea* showed lower value. Tannins are useful in stimulating growth in children, anti ulcer activities. (Ail, 2009)

Antibacterial activity

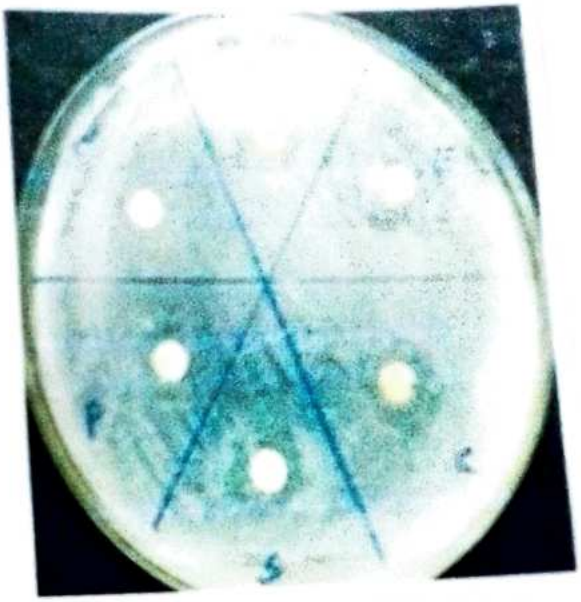
Plant extract was prepared by dissolving the dried powder in sterilized water, ethanol, methanol, petroleum ether and chloroform (1:1 w/v). Antibacterial activity of these extracts were screened against three strains viz., *Bacillus subtilis*, *Escherichia coli* and *Serratia marcescens*. *Bacillus subtilis* is a gram-positive, catalase-positive bacterium found in soil and the gastrointestinal tract of ruminantus and human. *Bacillus subtilis* is rod shaped, and form a tough, protective endospore, allowing it to tolerate extreme environmental conditions. *Escherichia coli* (also known as *E. coli*) is a gram-negative, facultative anaerobic, rod shaped bacterium of the genus *Escherichia* that is commonly found in the lower intestine of warm- blooded organisms. *Serratia marcescens* is a human pathogenic bacterium. *Serratia marcescens* is a rare opportunistic bacterium that is categorized in the enterobateriaceae family commonly found in the environment.

In the present study, antibacterial activity of three plant leaf extracts (*Cassia fistula*, *Cassia siamea* and *Peltophorum petrocarpum*) of five different solvents were tested against three human pathogenic bacteria (*Bacillus subtilis*, *Escherichia coli* and *Serratia marcescens*) were presented in Table (6 to 8). The diameter of the inhibition zones against these species ranged from 4 to 12 mm. The study revealed that except aqueous extract, all other extracts inhibited the growth of all the pathogens tested. As shown in Table 6 methanolic extracts of *Cassia fistula* and ethanolic and chloroform extract of *P. petrocarum* exhibited maximum activity against *Serratia marscens*

<antibacterial activity of plant extracts against *Bacillus subtilis*



A



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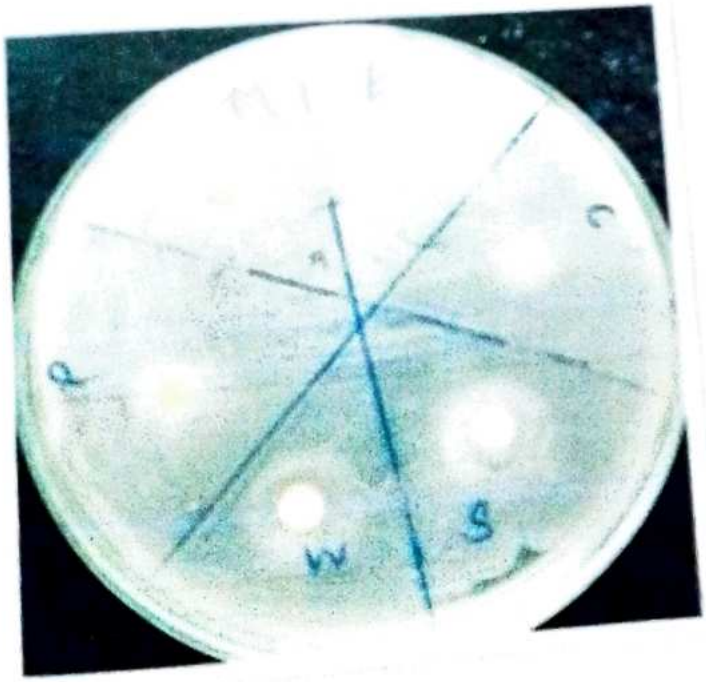
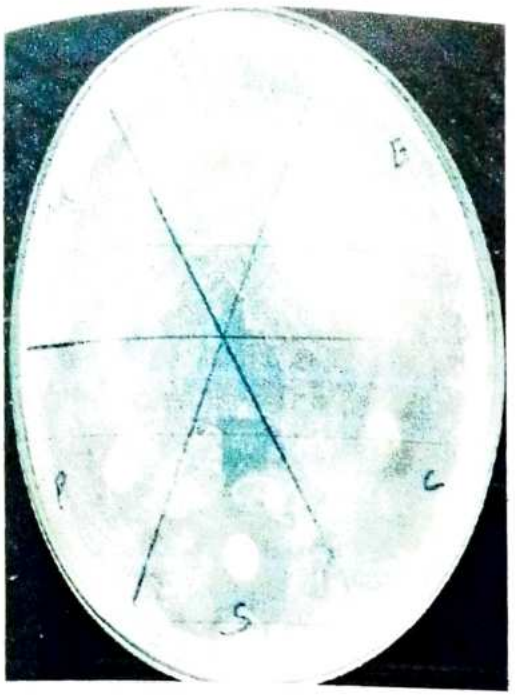


C

A. *Cassia fistula* leaf B. *Cassia siamea* leaf C. *Peltophorum petrocarpum* leaf

E-Ethanol, M-Methanol, C-Chloroform, P-Petroleum ether, W-Water, S-Streptomycin



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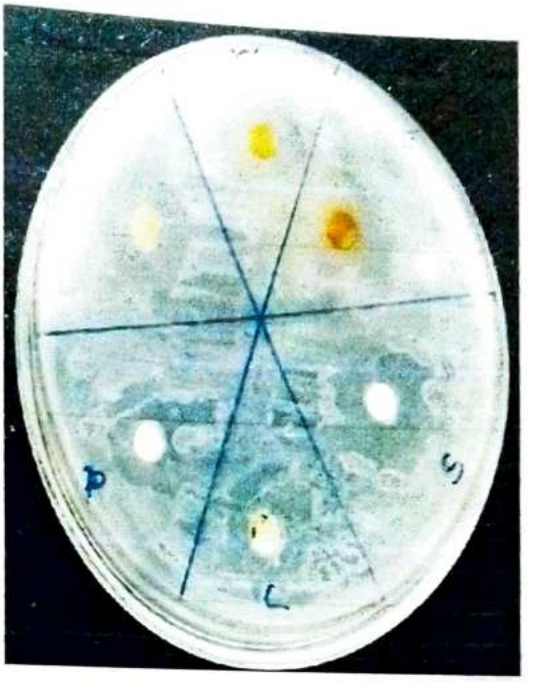
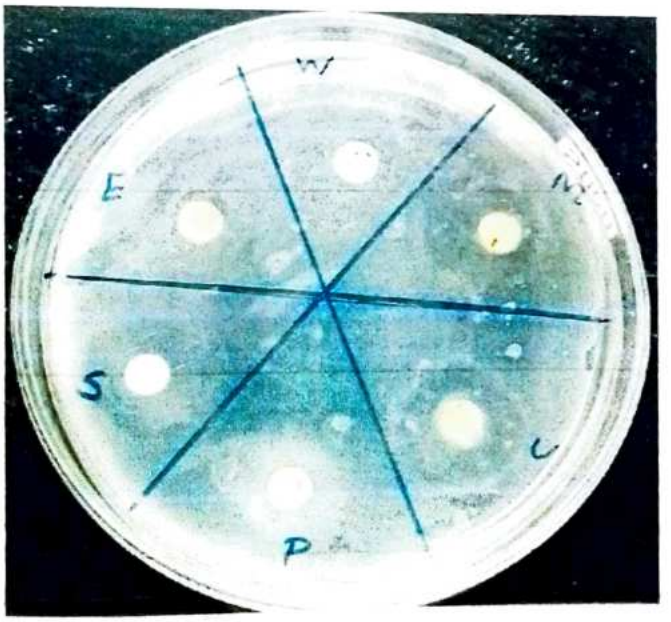


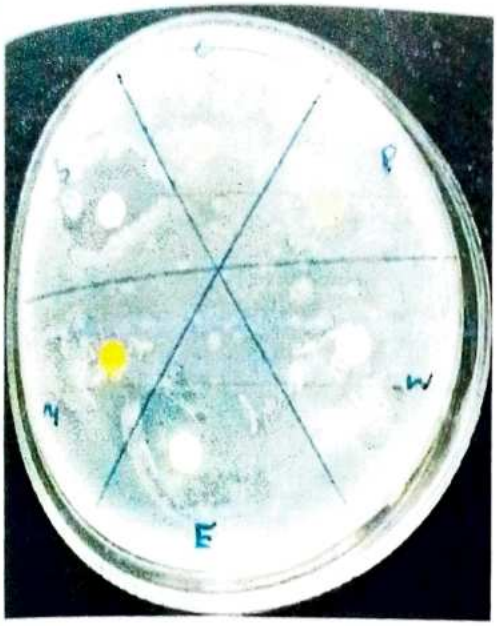
B



C

A *Cassia fistula* leaf, B. *Cassia siamea* C.*Peltophorum petrocarpum*

E- Ethanol, M-Methanol C- Chloroform, P-Petroleum ether, W- Water S-Streptomycin



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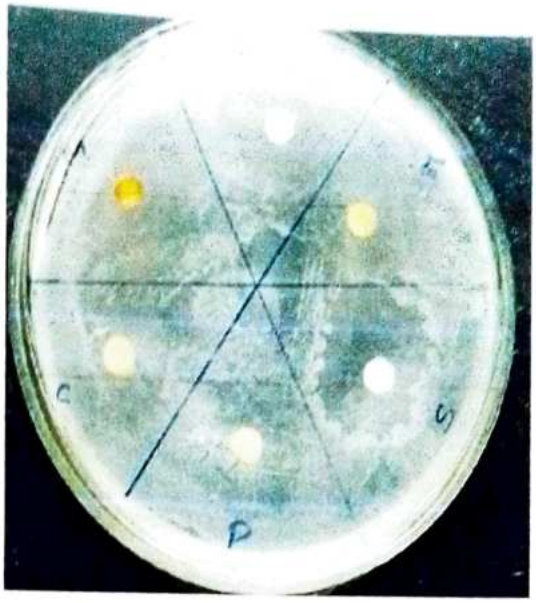
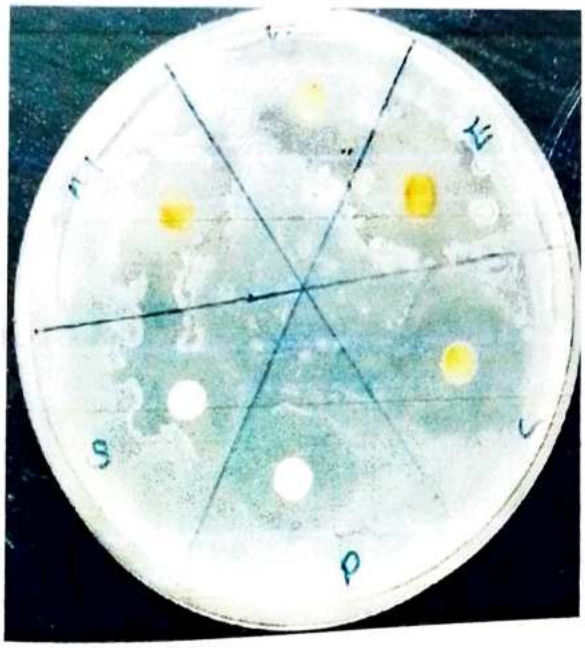


B



C

A. *Cassis fistula* leaf , B. *Cassia siamea* leaf, C. *Peltophorum petrocarpum* leaf.

E-Ethanol, M-Methanol, P-Petroleum ether, C-Chloroform, S-Streptomycin, W-Water

Table 5 : Antibacterial activity of *Cassia fistula* leaf extracted with different solvents against human pathogen

Zone of inhibition (mm)							
S. No	***Organism***	**Ethanol**	**Chloroform**	**Methanol**	**Petroleum ether**	**Water**	**Streptomycin**
1	*Bacillus substils*	9	7	4	5	NS	8
2	*Escherichia coli*	7	7	9	9	9	11
3	*Serratia marcescen*	5	NS	12	NS	8	13

Control = Streptomycin (100 µg/ml)

Plant extract = 2.5 mg/ml (effective concentration)

NS = NO Sensitivity

Table 6: Antibacterial activity of *Cassia siamea* leaf extracted with different solvents

		Zone of inhibition (mm)					
S.No	Organisums	Ethanol	Chloroform	Methanol	Petroleum ether	Water	Streptomycin
1	*Bacillus substilis*	6	7	4	4	5	5
2	*Escherichia coli*	5	6	6	5	5	8
3	*Serratia marcescen*	11	8	12	7	9	11

Control = Streptomycin (100 µg/ml)

Plant extract = 2.5 mg/ml (effective concentration)

NS = NO Sensitivity

Table 7 : **Antibacterial activity of *Peltophorum ptrocarpum* leaf extracted with different solvents against human pathogen**

		Zone of inhibition (mm)					
S.No	Organisums	Ethanol	Chloroform	Methanol	Petroleum ether	Water	Streptomycin
1	*Bacillus substilis*	NS	NS	NS	NS	4	4
2	*Escherichia coli*	4	7	6	NS	5	5
3	*Serratia marcescen*	12	12	6	9	9	8

Control = Streptomycin (100 µg/ml)

Plant extract = 2.5 mg/ml (effective concentration)

NS = NO Sensitivity

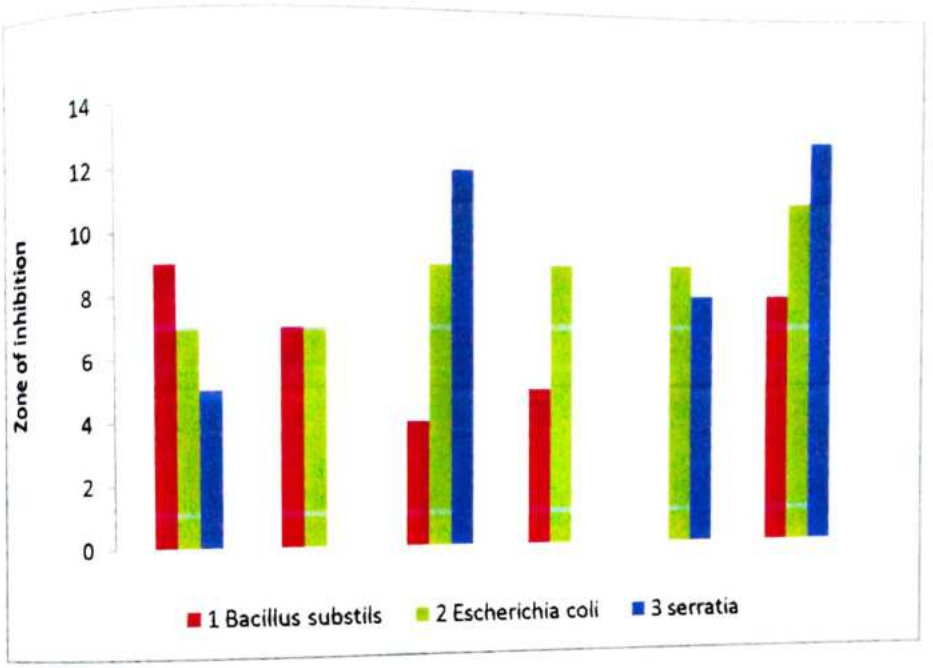


Fig. 4 : Different solvents were used as positive control, exhibitited no inhibition.

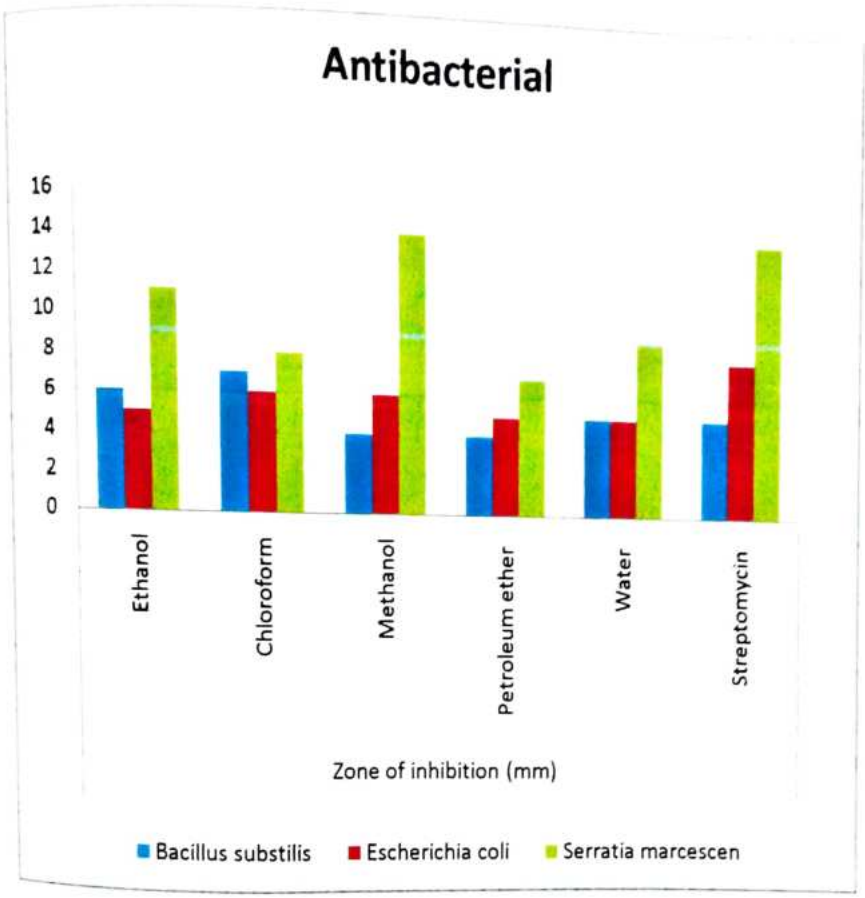


Fig. 5 : Different solvents were used as positive control, exhibitited no inhibition

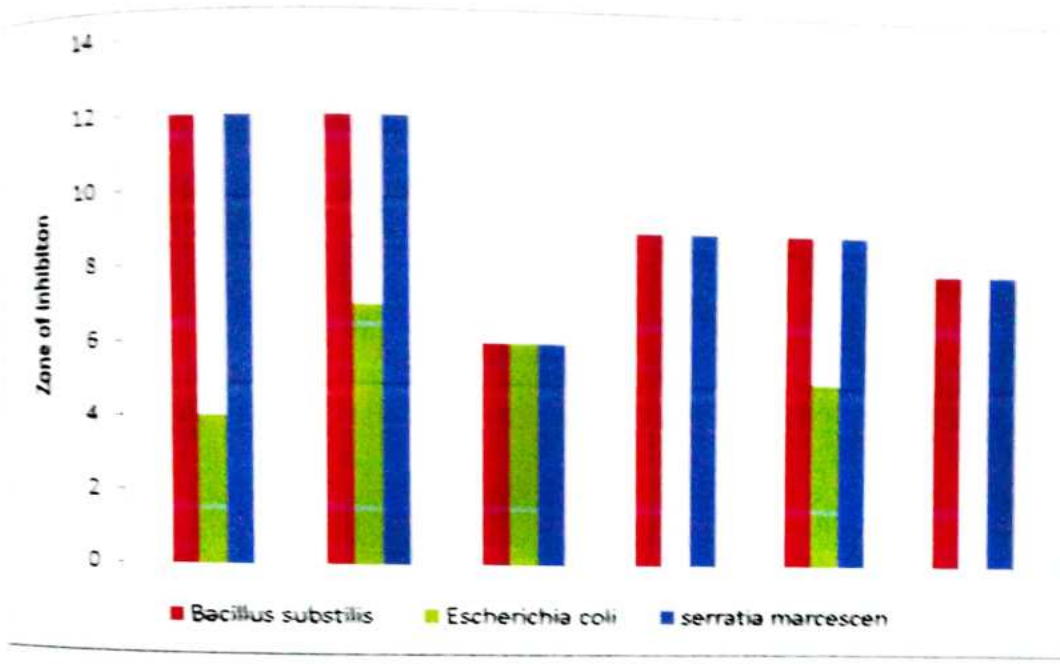


Fig. 6 : Different solvents were used as positive control, exhibitited no inhibition

(12 mm). Similarly ethanolic extract of *C. siamea* inhibited the growth of *Serratia marcescens* by showing11 mm of inhibition zone. *E. coli was* seemed to be more sensitive to petroleum ether, aqueous and methanolic extract of *C. fistula* by showing 9 mm of inhibition zone. The results obtained from the study concurred with earlier reports (Abo *et al*., 1999; Perumal Samy *et al*., 1998). Perumal Samy *et al*., 1998 reported moderate antibacterial activity of *C. fistula* against a wide spectrum of bacteria such as *E. coli* and *B. subtilis*. However, concentrate of *C. siamea* obtained from methanol and petroleum exhibited less inhibitory activity against *B. subtilis* and *E. coli* and the zone of inhibition ranged between 4 to 6 mm (Table 7). Chloroform and petroleum ether leaf extracts of *C. fistula* was no inhibitory activity against *Serratia marcescens*. The study indicated that among the bacterial organism tested *S. marscens* seemed to be more susceptible to all extracts. Ethanol, petroleum ether, methanol and chloroform extracts of *P. petrocarpum* was found to have no inhibitory activity of *Bacillus subtilis*. 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 all the plants used in the present study could be effectively processed to be utilized as a source for antibacterial therapeutic drug preparations. Rizvi *et al*., 2009 observed that *Cassia* species had a significant activity against Gram positive microorganisms. Abo *et al*., 1999 also found that leaves extracts of *C. fistula* have considerable antimicrobial activity. Vasudevan *et al*., 1996 reported that methanolic extracts inhibited Gram positive bacteria more than Gram negative species.

Fourier Transform Infrared Spectrophotometer (FTIR)

Fourier Transform Infrared Spectrophotometer (FTIR) is perhaps the most powerful tool for identifying the types of chemical bonds (functional groups) present

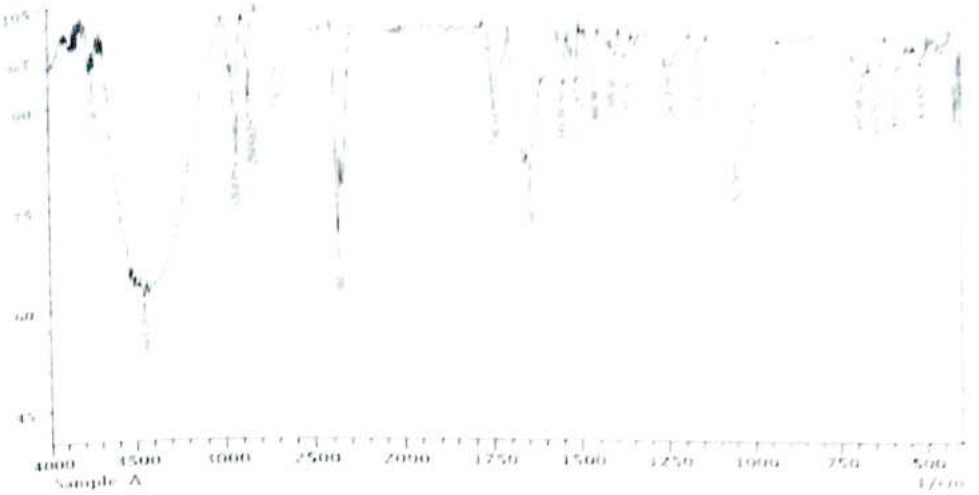


Fig. 10 : FT-IR spectrum of Cassia fistula leaf

Table 9 : FTIR spectral qualities interpretation of comparative shift in functional of critical value(Cassia fistula leaf)

Frequency range cm^{-1}	Compound type
3738.75	O-H stretch
3445.59	N-H stretch
2920.03	C-H stretch
2849.63	C-H stretch
2701.12	C-H stretch
2360.71	O=C=O stretch
1740.64	C=O stretch
1642.27	C=-N stretch
1545.84	N-O stretch
1511.12	N-O stretch
1460.98	C-H bending
1395.40	C-H bending
1372..26	S=O stretch
1242.07	S=O stretch
1161.07	C-O stretch
1052.10	C-O stretch
687.58	C-I stretch
655.75	C=C bending
593.07	C-L stretch
518.82	C-L stretch

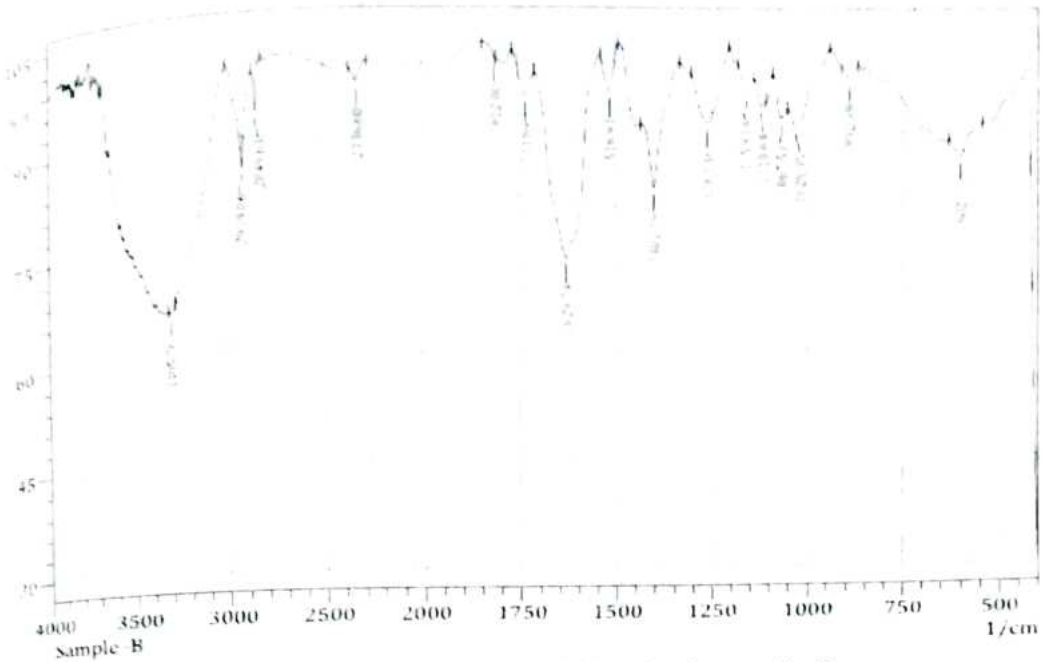


Fig 11 : FT-IR spectrum of Cassia siamea leaf

Table 10 : FT-IR Spectral qualities interpretation of the comparative shift infunctional peaks of critical value (*Cassia siamea* leaf)

Frequency range cm^{-1}	Compound type
3305.76	H stretch
2919.06	C-H stretch
2849.63	C-H stretch
2336.60	O=C=O stretch
1812.00	C=O stretch
1733.89	C=O stretch
1629.74	C=C stretch
1516.91	N-O stretch
1401.19	S=O stretch
1261.36	C-N stretch
1159.14	S=O stretch
1118.64	C-O stretch
1067.53	C-O stretch
1028.95	C-O stretch
892.98	C=C bending
662.71	C-Br stretch

FTIR analysis in plant

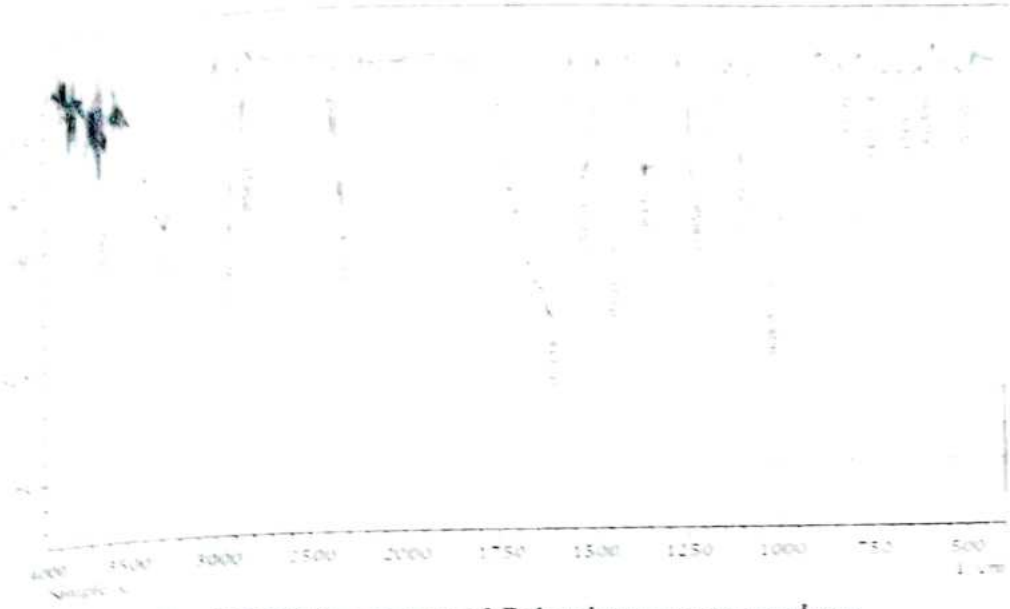


Fig: FT-IR Spectrum of *Peltophorum petrocarbum*

Table 11 : FTIR spectral qualities interpretation of the comparative shift in functional ppeaks of critical value (*Peltophorum petrocarbum*)

Frequency range cm^{-1}	Compound type
3644.25	O-H stretch
3272.01	O-H stretch H- bonded
2920.90	C-H stretch
2850.59	C-H stretch
2332.74	O=C=O stretch
1613.34	C=C stretch
1532.34	N-O stretch
1451.33	C-H bending
1369.37	S-O stretch
1230.50	C-N stretch
1111.89	C-O stretch
1020.95	C-N stretch
832.23	C-Cl stretch
764.72	C-H bending
674.07	C=C bending
622.00	C-Br stretch
517.85	C-Br stretch

compounds. The wavelength of light absorbed is characteristic of the chemical bond as can be seen in the annotated spectrum. By interpreting the infrared absorption spectrum, the chemical bonds in a molecule can be determined. FTIR were carried out identify the possible biomolecules responsible for antimicrobial and activity of *Cassia fistula*, *Cassia siamea* and *Peltophorum petrocarpum*. The FTIR spectrum of methanolic extract of *C. fistula* is presented in (Table 9 Fig. 7). The data on the peak values and the probable functional groups (obtained by FTIR analysis) present in the methanol extracts of *C. fistula* are represented in Table 9. The region of IR radiation helps to identify the functional groups of the active components present in extract based on the peaks values of the FTIR spectrum. When the extract was passed into the FTIR, the functional groups of the components were separated based on its peaks ratio. The results of FTIR analysis confirmed the presence of Alcohol, Aldehyde, Alkyne, Alkene, Amines and Ester. The absorbance bands analyses in bioreduction process are observed in the region between 400–4000 cm^{-1} were 3738.75, 3445.59, 2920.03, 2849.63, 2701.12, 2360.71, 1740.64, 1642.27, 1545.84, 1511.12, 1460.98, 1395.40, 1372.26, 1242.07, 1161.07, 1052.10, 687.58, 655.75, 593.07, 518.82 cm^{-1}. Major peaks were observed at 3738.75 cm1 that could be assigned to the 0-H stretching vibrations of O-H Alcohol. So the present study results indicate that the primary functional group present in *C. fistula* is O-H Alcohol. Methanolic extracts of *C. Seamea* were depicted in shown in Table 9. Methanolic extracts of *C. siamea* are represented in (Table 10). The representative spectrum of *C. siamea* showed absorption peaks located at 3305.76, 2919.06, 2849.63, 2336.60, 1733.89, 1629.74, 1516.91, 1401.19, 1261.36, 1159.14, 1118.64, 1067.53, 1028.95, 892.98, 602.71 cm^{-1}. So the present study results indicate that the primary functional group present in *C. siamea* is O-H Alcohol. In this analysis that revealed functional group components of

amino acids, amides, amines, carbonyl compounds, alkanes, aromatic carboxylic acid, alkyl halide through the FTIR analysis. Methanolic extracts of *Peltophorum pterocarpum* are represented in Table 11. The absorbance bands analyses in bioreduction process are observed in the region between 400–4000 cm^{-1} are 3644.25, 3272.01, 2972.99, 2850.59, 2332.74, 1613.34, 1532.34, 1451.33, 1369.37, 1230.50, 1111.89, 1020.95, 832.23, 764.72, 674.07, 622.00, 517.85 cm^{-1}. Strong peaks in the region of (3500-3200) cm^{-1} (O-H stretch) indicats phenolic active compounds(1500-1400) cm^{-1} (C-H Stretch) confirmed the presence of intermolecular hydrogen bonding OH, C-H alkanes, quaternary compounds, C=O aliphatic aldehydes, aromatic esters and (N-H stretch) primary amines. The results of the present study lead to conclude that compounds other than phenols, flavonoids and tannins, other chemical components like amines, aliphatic aldehydes, aromatic esters, cycloalkenes, heterocyclic, monosubstituted beneze etc., present in plants were responsible for the antibacterial and antifungal properties exhibited by respective plants.

Gas Chromatography-Mass Spectroscopy analysis

The results pertaining to GC-MS analysis led to the identification of 15 compounds from the GC fractions of the methanol extract of *Cassia fistula*. These compounds were identified through Mass Spectrometry attached with GC. The results were tabulated (Table 11 & Fig. 13). The gas chromatogram shows that the relative concentration of various compounds getting fractionated at their specific retention time. The heights of the percentage of peak area indicate the relative concentrations of the components present in the methanol extract of *C. fistula*. The maximum amount of bioactive compound found in the methanol extract of *C. fistula*. The compounds identified by GC-MS from the methanol extract of *C. fistula, Cassia siamea and*

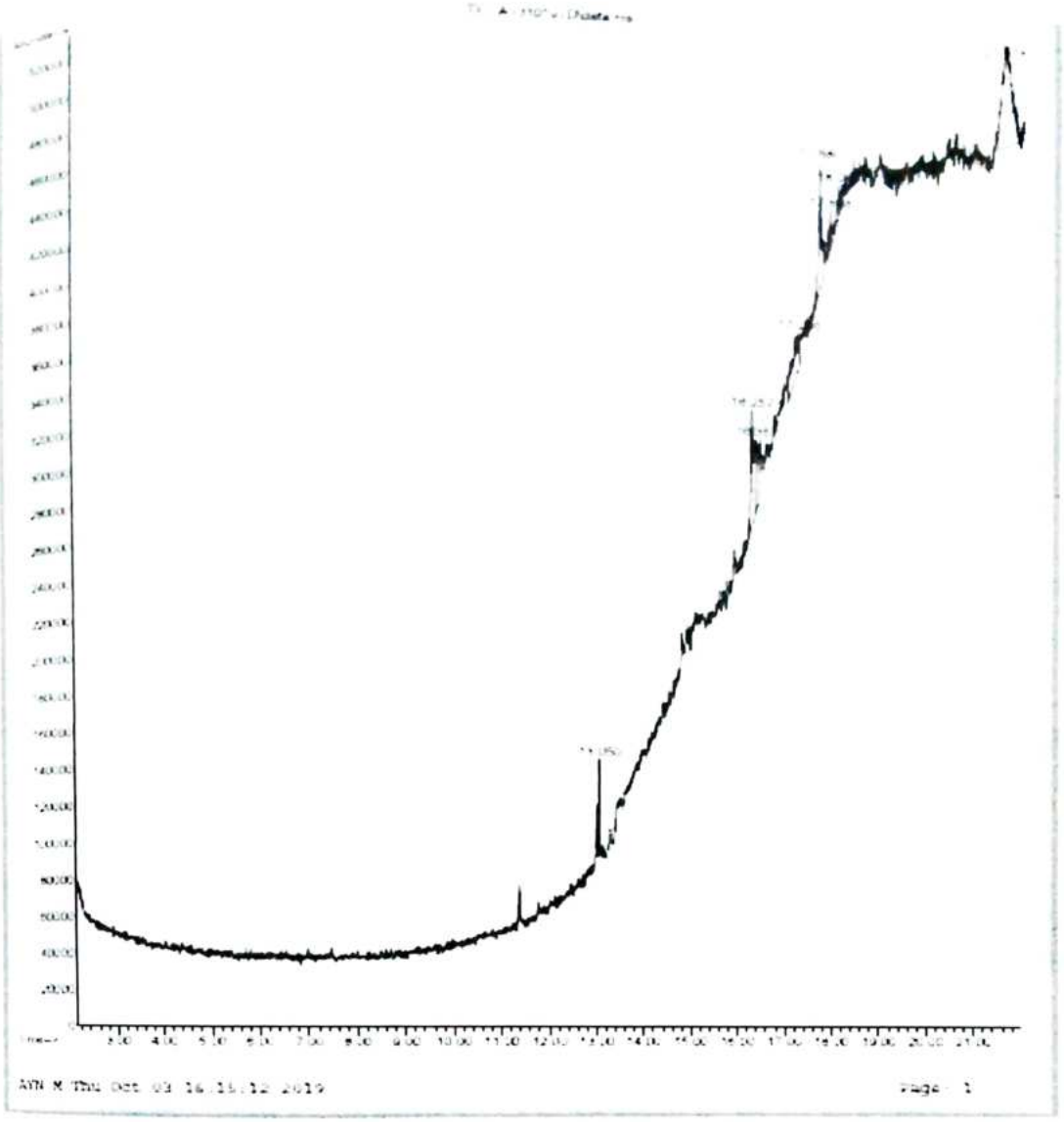


Fig. 13 : GCMS- Chromatogram of the methanol extract of *cassia fistula*

Table 12 : GC-MS analysis revealed the presence of bioactive compounds in the aerial parts of methanol extract of *Cassia fistula*.

Peaks	R/T	Compound Name	Area %	Bioactivity
1	13.050	6-11Octadecenoic acid, methyl ester, (Z)-	3.92	Antibacterial, Antiallergic, Nematicide and Antioxidant
2	16.357	Heptasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13-tetradecamethyl-,3-Methyl-2-phenylindole, Tetrasiloxane, decamethyl-	13.70	Preservative and Antimicrobial
3	16.257	Acetic acid, [4-(1,1-dimethylethyl)phenoxy]-, methyl ester, Cyclopentene-1-carboxylic acid, 4-[2-(diphenylmethyl)-2-propen-1-yl]-, methyl ester, Benz[b]-1,4-oxazepine-4(5H)-thione, 2,3-dihydro-2,8-dimethyl-	8.95	Anti-inflammatory and Antidiabetic activity
4	17.246	2,4-Cyclohexadien-1-one, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-,1,2-Benzisothiazol-3-amine tbdms, Benzo[h]quinoline, 2,4-dimethyl-	11.65	Antifouling,anti-diabetic, and anti-cancer
5	17.888	1-Dimethyl(butyl)silyloxybutane, Benzo[h]quinoline, 2,4-dimethyl-,1,2-Benzisothiazol-3-amine tbdms	14.80	Anti cancer
6	18.062	2-(Acetoxymethyl)-3-(methoxycarbonyl)biphenylene,Cyclobarbital, 2-Ethylacridine,	2.32	Anthelminitic and skin care
7	17.680	2-(Acetoxymethyl)-3-(methoxycarbonyl)biphenylene, 2,4-Cyclohexadien-1-one, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-,1,2-Benzisothiazol-3-amine tbdms	3.59	Used for softners and Anti cancer
8	21.578	Benzene, 2-[(tert-butyldimethylsilyl)oxy]-1-isopropyl-4-methyl-,Benzo[h]quinoline, 2,4-dimethyl-,1,2-Benzisothiazol-3-amine tbdms	41.08	Antimicrobial activity

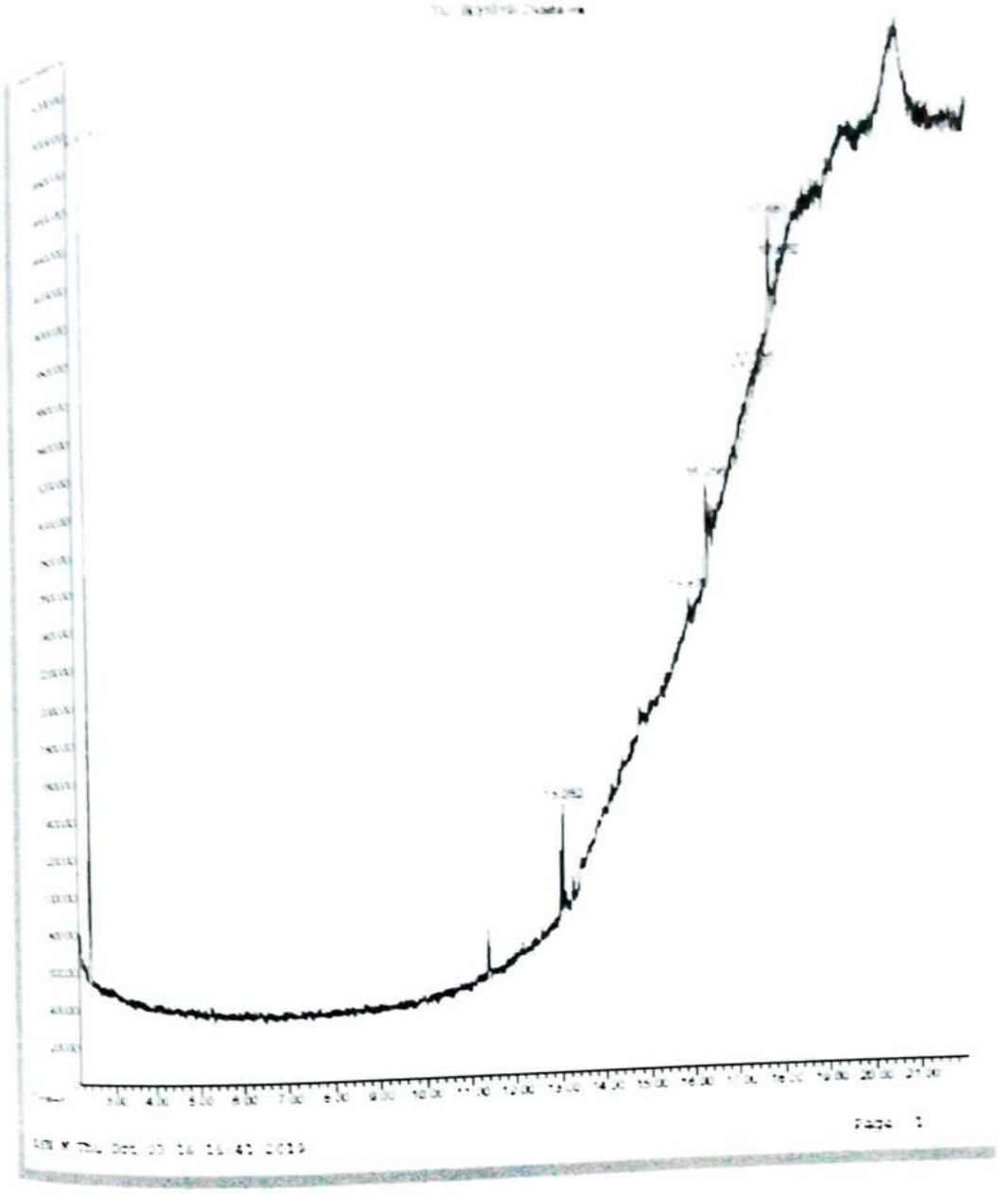


Fig. 14 : GCMS- Chromatogram of the methanol extract of *Cassia siamea*

Table 13 : GC-MS analysis revealed the presence of bioactive compounds in the aerial parts of methanol extract of *Cassia siamea*

Peaks	R/T	Compound Name	Area %	Bioactivity
1	2.413	Eucalyptol	29.80	Hypoglycaemic activity
2	13.052	9-Octadecenoic acid (Z)-, methyl ester, 8-Octadecenoic acid, methyl ester, (E)-, 11-Octadecenoic acid, methyl ester	5.49	Antimicrobial activity
3	15.876	1,2-Benzisothiazol-3-amine tbdms, Tetrasiloxane, decamethyl-,Tetrasiloxane, decamethyl-	5.64	Antimalarial activity and antifungal activity
4	16.256	1,2-Benzisothiazol-3-amine tbdms, 2-Ethylacridine, Hydrocinnamic acid, benzyldimethylsilyl ester	9.98	Anti- inflammatory activity and Antioxidant
5	17.291	1,2-Benzisothiazol-3-amine tbdms, Benzene, 2-[(tert-butyldimethylsilyl)oxy]-1-isopropyl-4-methyl-,5-Methyl-2-phenylindolizine	15.82	Antioxidant activity and cytotoxic activity, anti cancer
6	17.385	1,2-Benzisothiazol-3-amine tbdms, 2,4-Cyclohexadien-1-one, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-,1,2-Bis(trimethylsilyl)benzene	3.11	Antitumourals and antifungal activities
7	17.852	1,2-Benzisothiazol-3-amine tbdms, Cyclobarbital, Benzo[h]quinoline, 2,4-dimethyl-	13..90	Anticancer,larvicidal activity and analgesic and Anti-inflammatory
8	17.681	1,2-Benzisothiazol-3-amine tbdms, Cyclotrisiloxane, hexamethyl-,2,4-Cyclohexadien-1-one, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-	0.60	Antinociceptive and cytotoxic activities

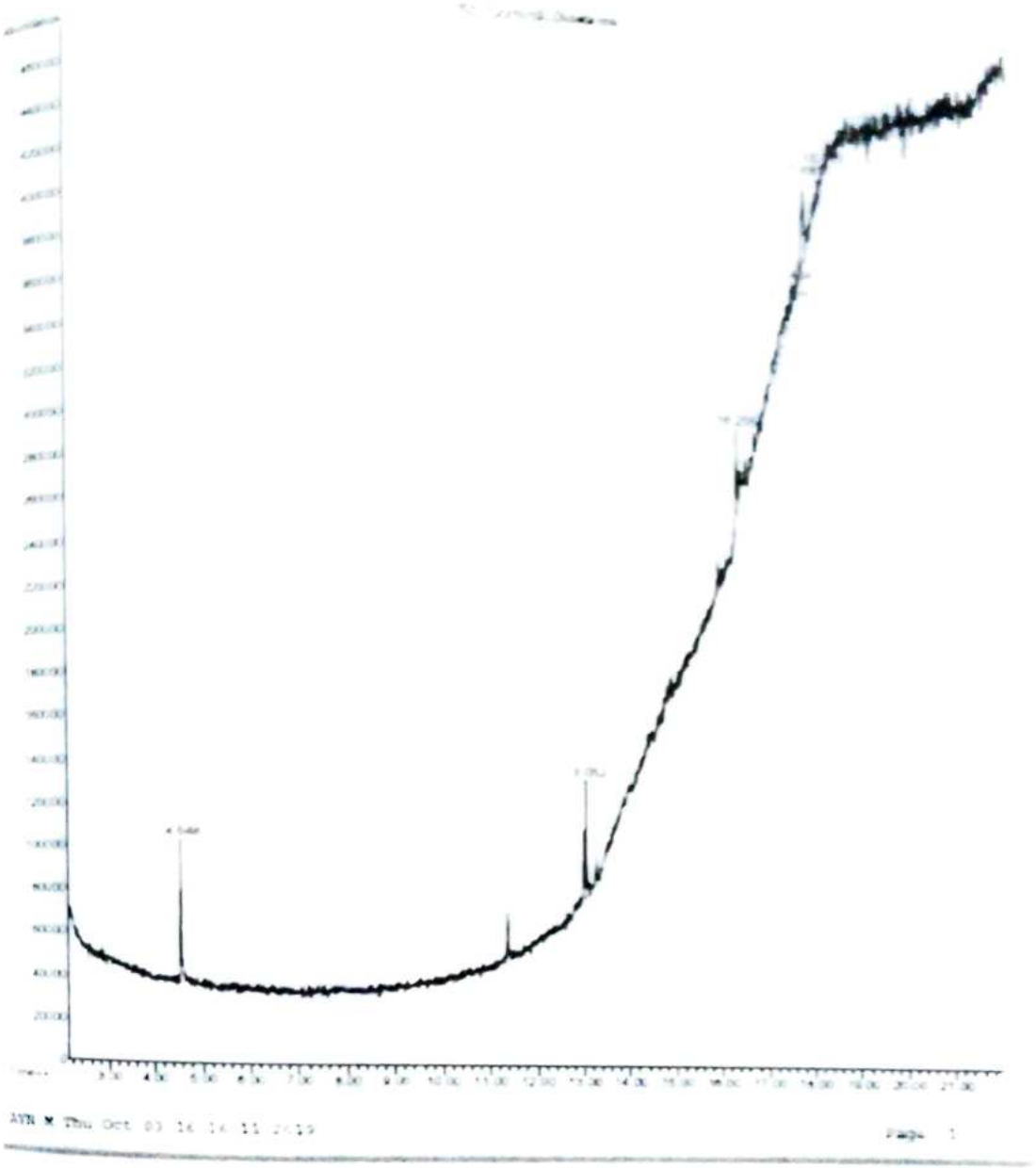


Fig. 15 : GCMS- Chromatogram of the methanol extract of *Peltophorum petrocarpum*

Table 14: GC-MS analysis revealed the presence of bioactive compounds in the aerial parts of methanol extract of *Peltophorum petrocarphum*

Peaks	R/T	Compound Name	Area %	Bioactivity
1	4.548	Anethole	16.03	Anti microbial activity
2	13.052	8,9,10 -Octadecenoic acid, methyl ester, (E)-	14.16	Anti-cancer, Anti-inflammatory, Antiandrogenic, irritatnt, insectifuge
3	16.255	1,2-Benzisothiazol-3-amine tbdms, Benz[b]-1,4-oxazepine-4(5H)-thione, 2,3-dihydro-2,8-dimethyl-1H-Isoindole-1,3(2H)-dione, 2-butyl-4,5,6,7-tetrahydro-	16.80	Antibacterial and Antifungal activities
4	17.375	,2-Bis(trimethylsilyl)benzene, 1,2-Benzisothiazol-3-amine tbdms, 1,2,4-Benzenetricarboxylic acid, 4-dodecyl dimethyl ester	23.27	Anti-inflammatory, Antioxitant, hypocholestrolemic
5	17.434	1,2-Benzisothiazol-3-amine tbdms, 2,4-Cyclohexadien-1-one, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-,5-Methyl-2-trimethylsilyloxy-acetophenone	3.71	Anticancer, Anti androgenic flavor,hemolytic
6	17.681	1,2-Benzisothiazol-3-amine tbdms, 5-Methyl-2-phenylindolizine, 1H-Indole-2-carboxylic acid, 6-(4-ethoxyphenyl)-3-methyl-4-oxo-4,5,6,7-tetrahydro-, isopropyl ester	23.77	Antimutagenic, Anti cancer activities
7	18.115	1,2-Benzisothiazol-3-amine tbdms, ,2-Bis(trimethylsilyl)benzene, 1H-Indole, 2-methyl-3-phenyl-	2.33	Anti bacterial and antifungal ,antipyretic activities

Peltophorum petrocarpum are medicinally valuable and possess various pharmaceutical applications.

GC-MS chromatogram of the methanol leaf extracts of *Cassia fistula* (Fig. 7) showed 8 peaks indicating the presence of 19 compounds. The chemical compounds identified in the extract of the leaf of *Cassia fistula* are presented in (Table 12). The GC-MS analysis revealed that the presence of 6-11Octadecenoic acid, methyl ester (3.92%), Heptasiloxane, 13-tetradecamethyl-,3-Methyl-2-phenylindole, Tetrasiloxane, decamethyl (13.70%), Acetic acid, [4-(1,1-dimethylethyl)phenoxy]-, methyl ester, Cyclopentene-1-carboxylic acid, 4-[2-(diphenylmethyl)-2-propen-1-yl]-, methyl ester, Benz[b]-1,4-oxazepine-4(5H)-thione, 2,3-dihydro-2,8-dimethyl (8.95%), 2,4-Cyclohexadien 1-one,3,5-bis(1,1-dimethylethyl)-4-hydroxy-,1,2-Benzisothiazol-3-aminetbdms, Benzo[h]quinoline, 2,4-dimethyl (11.65%), 1-Dimethyl(butyl) silyloxybutane, Benzo[h]quinoline, 2,4-dimethyl-,1,2-Benzisothiazol-3-amine tbdms (14.80%), 2-(Acetoxymethyl)-3-(methoxycarbonyl)biphenylene.Cyclobarbital, 2-Ethylacridine (2.32%), 2-(Acetoxymethyl)-3-(methoxycarbonyl)biphenylene, 2,4-Cyclohexadien-1-one, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-,1,2-Benzisothiazol-3-amine tbdms (3.59%), Benzene, 2-[(tert-butyldimethylsilyl)oxy]-1-isopropyl-4-methyl-, Benzo[h]quinoline, 2,4-dimethyl-,1,2-Benzisothiazol-3-amine tbdms (41.08%). Most of the constituents have found to show interesting biological activity aganints certain illness or pathogen. Methanol leaf oil extract of *Cassia siamea* (Figure 11) showed 8 peaks indicating the presence of 15 compounds. The chemical compounds identified in the extract of the leaf of *Cassia fistula* are presented in (Table13). The GC-MS analysis revealed that the presence of Eucalyptol (29.80%), 8,9,11-Octadecenoic acid, methyl ester (5.49%), 1,2-Benzisothiazol-3-amine tbdms, 2-Ethylacridine, Hydrocinnamic acid,

benzyldimethylsilyl ester (9.98%), 1,2-Benzisothiazol-3-amine tbdms, Benzene,2-[(tert-butyldimethylsilyl)oxy]-1-isopropyl-4-methyl-,5-Methyl-2-phenylindolizine (15.82%), 1,2-Benzisothiazol-3-amine tbdms, 2,4-Cyclohexadien-1-one,3,5-bis(1,1-dimethylethyl)-4-hydroxy-,1,2 Bis(trimethylsilyl)benzene (3.11%), 1,2-Benzisothiazol-3-amine tbdms, Cyclobarbital, Benzo[h]quinoline, 2,4-dimethyl- (13..90%), 1,2-Benzisothiazol-3-amine tbdms, Cyclotrisiloxane, hexamethyl-,2,4-Cyclohexadien-1-one, 3,5-bis(1,1-dimethylethyl)-4-hydroxy- (0.60%). Eucalyptol was proven to exhibit hypoglycaenicactivity. Octadecenoic acid and methyl ether was present in other plants. Exhibiting antimicrobial property. 1,2-Benzisothiazol-3-amine was proven to prevent cancer. Based on studies, some of the constituents revealed by GC-MS are biologically active compounds. They were proven to process pharmacological activities which may contribute to the healing potential of the plant. Methanolic leaf extract of *Peltophorum petrocarpum* (Fig. 12) showed 7 peaks indicating the presence of 19 compounds. The chemical compounds identified in the leaf of *Peltophorum petrocarpum* are presented in(Table 14) Anethole (16.03%), 8,9,10 -Octadecenoic acid, methyl ester, (E)- (14.10%), 1,2 Benzisothiazol-3-amine tbdms, Benz[b]-1,4-oxazepine-4(5H)-thione, 2,3-dihydro-2,8 dimethyl- 1H-Isoindole-1,3(2H)-dione, 2-butyl-4,5,6,7-tetrahydro- (16.80%), 2-Bis(trimethylsilyl)benzene, 1,2-Benzisothiazol-3-amine tbdms, 1,2,4-Benzenetricarboxylic acid, 4-dodecyl dimethyl ester(23.27%), 1,2-Benzisothiazol-3-amine tbdms, 2,4-Cyclohexadien-1-one, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-,5-Methyl-2-trimethylsilyloxy-acetophenone(3.71%),1,2-Benzisothiazol-3-amine tbdms, 5-Methyl-2-phenylindolizine, 1H-Indole-2-carboxylic acid, 6-(4-ethoxyphenyl)-3-methyl-4-oxo-4,5,6,7-tetrahydro-, isopropyl ester(23.77%), 1,2-Benzisothiazol-3-amine tbdms, .2-Bis(trimethylsilyl)benzene, 1H-Indole, 2-methyl-3-phenyl-(2.33%).

From this study it is concluded that the presence of phytocompounds in there plants extracts suggers that of these compounds in pharmacological activity.

SUMMARY AND CONCLUSION

Plant derived substances has obtained greater attention in the recent years to prevent and cure human diseases as they are considered to be more bio-friendly. It is generally estimated that over 6000 plants in India are in use in traditional, folk, and herbal medicine, representing about 75% medicinal needs of the third world countries. Phyto-chemical investigations of crude plant extracts shows the presence of active principles in the plant parts like bark, leaves, flowers, roots, fruits, seeds etc. Phyto-chemicals are non-nutritive plant chemicals that have protective or disease preventive properties. Plant produces these chemicals to protect itself but research works demonstrates that many phytochemicals can protect humans against diseases. Knowledge of the chemical constituents of plants is desirable because such information will be of value for the synthesis of complex chemical substances. In the present work, qualitative phytochemical value, antibacterial, FTIR and GC-MS analysis was carried out in *Cassia fistula*, *Cassia fistula* and *Peltophorum petrocarpum*.

The preliminary phytochemical studies revealed that the leaf extracts of *Cassia fistula* was showing the leaf of *Cassia siamea* maximum presence of alkaloids, proteins and steroids in all solvents. *Cassia siamea* have a various chemical group such as proteins, steroids and terpenoids were showing maximum presence in all extracts. Phenols and anthraquinone were absent in all extracts. Flavonoids were absent in chloroform and aqueous extracts but highly present in ethanolic and petroleum ether extracts. Tannin was adequately present in methanolic and ethanolic extracts but completely absent in chloroform and aqueous extracts.

The present study revealed that the various alcoholic and aqueous extracts of leaf of *Peltophorum petrocarpum* contained alkaloids, cardiac glycosides, flavonoids, glycosides, phenols, resins, saponins, steroids, tannins, terpenoids. It is found in large amounts in all extracts of *Peltophorum petrocarpum* except aqueous extract. Saponins, present in plants, have been suggested as possible anti-carcinogens. The study revealed that *Cassia fistula* and *Cassia siamea* leaf extract possessed very low quantity of flavonoid. It was found that *Peltophorum petrocarpum* leaf extract was endowed with higher amount of flavonoid than other plants. Flavonoids are useful in reduce body heat and remarkable activity in cancer prevention and anticancer activities. *P. petrocarpum* was found to have more tannin than other plant. The *C. fistula* maximum showed lower value. Tannins are useful in stimulating growth in children, anti ulcer activities. *C. siamea* obtained from methanol and petroleum exhibited less inhibitory activity against *B. subtilis* and *E. coli* and the zone of inhibition ranged between 4 to 6 mm Chloroform and petroleum ether leaf extracts of *C. fistula* was no inhibitory activity against *Serratia marcescens*. The study indicated that among the bacterial organism tested *S. marscens* seemed to be more susceptible to all extracts. Ethanol, petroleum ether, methanol and chloroform extracts of *P. petrocarpum* was found to have no inhibitory activity of *Bacillus subtilis*. 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 all the plants used in the present study could be effectively processed to be utilized as a source for antibacterial therapeutic drug preparations.

The FTIR spectrum was used to identify the functional group of the active components based on the peak value in the region of infrared radiation. It confirmed

the presence of compounds with fuctional groups such as primary alcohol, aldehye, amines, alkanes, and ester were found in all plant extracts.

The gas chromatogram shows that the relative concentration of various compounds getting fractionated at their specific retention time. The compounds identified by GC-MS from the methanol extract of *C. fistula*, *Cassia siamea* and *Peltophorum petrocarpum* are medicinally valuable and possess various pharmaceutical applications.

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PHYTOCHEMICAL ANALYSIS, IN VITRO PROLIFERATION AND ANTIBACTERIAL ACTIVITY OF STEMODIA VISCOSA ROXB. (SCROPHULARIACEAE)

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By

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CERTIFICATE

This is to certify that this dissertation entitled, **"Phytochemical analysis, *in vitro* proliferation and antibacterial activity of *Stemodia viscosa* Roxb. (Scrophulariaceae)"** submitted by **G. Mary Stephy Reg.No. 18APBO05** to ST.MARY'S COLLEGE (Autonomous), THOOTHUKUDI in partial fulfillment for the award of the degree of **"Master of Science in Botany"** is done by her under my supervision. It is further certified that this dissertation or any part of this has not been submitted elsewhere for any other degree.

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DECLARATION

I do here by declare that this dissertation entitled, **"Phytochemical analysis, *in vitro* proliferation and antibacterial activity of *Stemodia viscosa* Roxb. (Scrophulariaceae)"** submitted by me in partial fulfilment for the award of the degree of **'Master of Science in Botany'**, in the result of my original and independent work carried out under the guidance of **Dr. E. Daffodil D Almeida, M.Sc., SET, Ph.D.,** Assistant Professor, Department of Botany, St. Mary's College (Autonomous), Thoothukudi and it has not been submitted elsewhere for the award of any other degree.

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INTRODUCTION

Plants are boon of our life. Each and every animal needs plants for their day to day life. Without plants we can't survive but without us plants must live. Our mother earth contains a tremendous amount of resources on her body. Of which, plants are one of the most important, inspiring and wonderful source because which is the mother of all the resources. For its aesthetic beauty habit, would beautify all things surrounded its habitat. Maximum all plants have medicinal property, so they are used in the therapeutic field.

India is one of the 12 mega biodiversity centers having about 10% of the world's biodiversity wealth which is distributed across 16 agro-climatic zones. Our country is rich in biodiversity and is endowed with many useful plants. Out of 2,50,000 plant species, weeds constitute about 250 species which are prominent in agricultural and non-agricultural system (Cowan, 1999). These weeds possess non nutritive plant chemicals that contain disease protective and disease preventing compounds, therefore which acts against various microorganisms. Many of the weeds contain therapeutic property, so that is used in the field of medicine.

The medicinal plants are one of the exclamatory resources of novel pharmaceuticals. They are the most important thing which acts as a source of life saving drugs for the majority of the world's population. In India, herbal medicine has been the basis of treatment for various diseases and physiological condition in traditional system such as Ayurveda, Unani and Siddha. The goal of "Health for All" by WHO can't be achieved without herbal medicine. Approximately 90% of the ingredient used in Ayurveda, Unani, Siddha and Homeopathy medicine are plant based over in modern Allopathic medical system has 25% of its formulation from herbal medicine (Washimkar, *et al*., 2016).

In recent decades, the plant kingdom has been considered as the important source of potential drugs which are easily available, safe, and inexpensive and rarely show side effects (Yadav and Agarwala, 2011). Based on estimations of the World Health Organization, more than 80 percent of people still rely mostly on traditional drugs such as plants for treating their aliments (Arunkumar and Muthuselvam, 2009). So, nowadays, several studies have been carried out to provide scientific support for the effectiveness of herbal medicines.

Stemodia viscosa (Scrophulariaceae) is one of the common aromatic weed in agricultural fields of India. The volatile oil and essential oil were extracted from this plant. It is one of the medicinal plants and used to heal wounds, diabetes, cold, flu, etc. and it possesses antilipidemic activity. For this reason, many countries cultivated *S. viscosa* as economically and extract volatile oil from this plant. The fragrant leaves of this herb are placed in pillows to induce a restful sleep, or crushed and mixed with fat to make a rubbing medicine to treat cold and flu symptoms. It is used as an aboriginal healing rub along with olive oil and beeswax (Mammen *et al*., 2012). The chemical investigation of this genus is restricted to five species from which flavanoids, diterpenes, and diterpenes derivatives with a rare tetracyclic skeletal, named stemodane were isolated (Rodrigues *et al.,* 2010).

The volatile oil of *S. viscosa* was extracted by using dried whole plant. The yield of the extracted volatile oil of the whole plant was 1.5%. The oil was light greenish yellow in colour with a pleasant clove like odour, which on analysis was found to contain both mono and sesquiterpenoids in almost equal amounts. (Mammen *et al.,* 2012). The wound contracting ability of *S. viscosa* ointment (5% w/w) was found significant than the control. The plant extract of *S. viscosa* was orally supplemented in diabetic mice for a

period of 15 days; the extract were reduced the severity of the diabetic condition. (Karthigaiselvi and Rameshwari, 2018).

Plant volatile oils are generally isolated from nonwoody plant material by distillation methods, usually steam or hydrodistillation, and are variable mixtures of principally terpenoids, a variety of low molecular weight aliphatic hydrocarbons, acids, alcohols, aldehydes, acyclic esters and/or lactones. Terpenes are amongst the chemicals responsible for the medicinal, culinary and fragrant uses of aromatic and medicinal plants (Dorman, 1999).

Medicinal plants are intrinsic components of traditional medical systems in treating and preventing an array of ailments. For this health giving activity, our ancient people conserved that and gave it to their future generation; but many people of our generation, don't consider about conserving these plants. Apart from conservation, we destroy the plants and use that empty land for construction work. For this attraction of urbanization, we ruin all the plants present around their desiring places. Finally, it leads to drought, ground water loss, water shortage, global warming, etc. These natural calamities are giving us a warning to conserve the plants. In our modern scenario plant conservation is one of the most important things to survive our earth. Plant biotechnology provides a large number of tools and great ideas to more efficiently conserve plants. *In vitro* culture of plants is one of the most important techniques of plant biotechnology and good *ex situ* methods to conserve the plants.

The culture of plant cells or plant tissues in a synthetic culture medium under controlled aseptic conditions is known as tissue culture. It is also called *in vitro* culture. The medium of tissue culture provides all minerals and growth hormones necessary to the growing cells. The controlled conditions give the culture a suitable microenvironment for the cell growth, proliferation and morphogenesis. A plant contains lakhs of tissues. Each

and every cell has totipotency (i.e.) the ability of a cell to generate a new organism or part.

Plant tissue culture refers to growing and multiplication of cells, tissues and organs of plants on defined solid or liquid media under aseptic and controlled environment. A whole plant can be regenerated from a small tissue or plant cells in a suitable culture medium under controlled environment. The plantlets so produced are called tissue culture raised plants. These plantlets are a true copy of the mother plant and show characteristics identical to the mother plant. (Bhoite and Palshikar, 2014).

Infectious diseases and food borne illnesses can cause severe health effects and can even lead to death among the residing population, especially in the developing regions of the world. The continual emergence of antibiotic resistant microorganisms has prompted researchers' world over to search for new antimicrobial agents that are more effective against the resistant microbial pathogens (Gislene *et al.,* 2000; Thaller *et al.,* 2010). Actually volatile oils inhibit the microbial contamination. The plant *S. viscosa* also contains volatile oils, and I assumed this volatile oil is also used to resist the microorganisms.

However very little information has been published on this plant and there is no scientifically proven data to show whether S. *viscosa* has antimicrobial activity or not. The tissue culture study of *S. viscosa* is not yet done and this plant contains many medicinal properties. Many people didn't know about this plant and its therapeutic activity. Therefore I have undertaken the present study to explore the phytochemicals, *in vitro* proliferation and antibacterial activity of different extracts of *S. viscosa*.

SCOPE AND OBJECTIVES

Plants are marvellous pool of bioactive compounds. These phytochemicals are attracting the pharmaceutical industries to innovate new drugs. The most important and major issue of medical field is allopathic medicine. Those medicine exhibits raw chemicals, and those chemicals may affect the organs of our body but phytochemicals does not promote any side effects. My current study is useful to explore the active and useful phytochemical components of *Stemodia viscosa*.

Plant tissue culture is one of the emerging fields of biotechnology because of its high potential to develop improved crops, desired plants and mass proliferation within the controlled, aseptic and limited environment. With the advances are made in *in vitro* culture technology, it is possible to regenerate and conserve species of any plants in the laboratory.

Bacterial adhesion and proliferation is a serious and increasing concern in everyday life and is responsible for significant damage in several industries. Therefore, new strategies for controlling bacteria activity are urgently needed one for our society and phytochemicals constitute a very promising approach. My present study is very useful to overcome bacterial issues by using phytochemicals.

With this backdrop, the current study is designed to have the following objectives,

i. Collection of plant materials of *Stemodia viscosa* Roxb. from agriculture field of Kulayankarisal, Thoothukudi.
ii. Qualitative investigation of phytocomposition in petroleum ether, acetone, chloroform, methanol and aqueous extracts of stem and leaves of *S. viscosa*.
iii. Quantitative estimation of total phenolics, flavonoid and tannin using different extracts of stem and leaves of *S. viscosa*

iv. FTIR analysis of stem and leaves of *S. viscosa*.

v. Micropropagation of *S. viscosa* by using nodal explants.

vi. Comparing the activity of different growth hormones on shoot and root proliferation.

vii. Screening of antibacterial activity of methanol, acetone and aqueous extracts of stem and leaves of *S. viscosa.*

Secondary metabolites or phytochemicals, the major ones present in a plant, apparently function as defence (against herbivores, microbes, viruses or competing plants) and signal compounds (to attract pollinating or seed dispersing animals). They are thus important for the plant's survival and reproductive fitness. Phytochemicals aid to maintain excellent health and fighting against diseases. Phytochemicals now being described as functional constituents and neutraceuticals. The health beneficial effects of the plant extracts has been credited to antioxidant, antimicrobial, anticancer, antiulcerative and antidiabetic properties. At present the plant extracts are still widely being used in ethnomedicines in the world (Mahesh and Satish, 2008).

Phenolic compounds are well known phytochemicals found in all plants. They consist of simple phenols, benzoic and cinnamic acid, coumarins, tannins, lignins, lignans and flavonoids. Substantial developments in research focused on the extraction, identification and quantification of phenolic compounds as medicinal and/or dietary molecules have occurred over the last 25 years. Organic solvent extraction is the main method used to extract phenolics. Chemical procedures are used to detect the presence of total phenolics, while spectrophotometric and chromatographic techniques are utilized to identify and quantify individual phenolic compounds. (Khoddami *et al*., 2013).

PHYTOCHEMICAL ANALYSIS OF SCROPHULARIACEAE

Kakpure and Rothe (2012) qualitatively analyzed the phytochemicals of whole plant powder of *Striga asiatica* (L.) O. Ktze in six different extracts (i.e. petroleum ether, benzene, chloroform, acetone, ethanol and water). The extracts showed the prominently presence of phytoconstituents like carbohydrates, cardiac glycosides, alkaloids,

flavonoids, tannin, phenolics, steroids, coumarins and saponin. However anthroquinone glycosides and quinones are totally absent in all extracts.

Acharya *et al.* (2013) screened the phytochemicals of an ethno medicinal plant *Limnophila rugosa* Roth. (Merr) using ethanol and aqueous extracts. The extracts were subjected to various tests for alkaloids, flavonoid, steroids, protein, tannins, carbohydrate, chlorophyll (Phase test) and cyanogenetic glycosides in order to identify the main phytoconstituents of the plant. The study revealed that the ethanol extract contains glycosides, little amount of alkaloids and flavonoids where as the aqueous extract is rich in glycosides.

Phytochemical screening of the dried plant extract of *Lindenbergia indica* Vatke. showed the presence of alkaloids, proteins, carbohydrates, cardiac glycosides, saponins, triterpenoids, tannins and phenols. (Singh *et al.,* 2013)

Shakeri and Farokh (2015) reported phytochemical evaluation and antioxidant activity of *Verbascum sublobatum* Murb. leaves. In their studies, dried and powdered leaves were extracted with 70% methanol and then partitioned by chloroform, ethyl acetate, and butanol. The ethyl acetae fraction showed the strongest DPPH radical scavenging activity among the three fractions and was subjected to separation and identification. The separation and purification process were performed using various chromatographic methods. Structural elucidation was carried out on the basis of FTIR, NMR and UV data. The isolated compounds which had flavonoid structure, were identified as apigenin and luteolin.

Wankhar *et al.* (2015) studied phytochemicals screening and antimicrobial efficacy of *Scoparia dulcis* Linn. against clinical isolates. Phytochemicals screening of S*coparia dulcis* extracts reveals the presence of alkaloids, flavonoids, phenols, terpenoids, tannins and saponins.

Pawar *et al.* (2016) analyzed phytochemical and physicochemical constituents of *Bacopa monnieri* (L) extracts according to the pharmacopoeial method. The antimicrobial activity of *Bacopa monnieri* (L) was also investigated. Phytochemical investigation of *Bacopa monnieri* (L) revealed the presence of various important secondary metabolites such as carbohydrates, proteins, amino acids, steroids, glycosides, flavonoids, alkaloids and tannins in methanolic, ethanolic and aqueous extracts.

Phytochemicals and *in vitro* bioactivity of *Scrophularia umbrosa* rhizome were studied by Nikkhah *et al.* (2018). They investigated the phytochemical analysis of methanol, Dichloromethane, and n-Hexane extracts of rhizome as well as total phenol and total flavonoid contents (TPC and TFC). *In vitro* β-hematin formation assay and DPPH method were applied for analyzing antimalarial and free-radical scavenging activities of the extracts, respectively. The formation of hemozoin has been proposed as an ideal drug target for antimalarial screening programs. The results showed that n-hexane and methanol extracts of rhizome had no significant inhibitory effect on heme biocrystallization whereas the Dichloromethane extract of rhizome showed moderate antimalarial activity in comparison with chloroquine. GCMS data showed that volatile portions of Dichloromethane and n-Hexane extracts from *Scrophularia umbrosa* contained a few identifiable compounds. Moreover, fractions 20% and 40% methanol-Water of methanol extract of *S. umbrosa* displayed moderate to strong free radical scavenging activity which showed a positive relation between phenolic and flavonoid contents and free radical scavenging activity. Based on the results, the fractions of methanol extract were evaluated by 1HNMR for predicting the groups of natural compounds and interfacing of chemical and biological assessments.

Nahannu *et al.* (2018) studied phytochemicals of the ethanolic leaves and root extract of *Scoparia dulcis*. Preliminary phytochemical analysis of the extract revealed the

presence of tannins, saponins, alkaloids, flavonoids, terpenoids and phenols. Cardiac glycosides, anthraquinones, and reducing sugars were absent in this investigation.

PHYTOCHEMICAL ANALYSIS OF STEMODIA SP.

Silva *et al.* (2009) studied new volatile constituents from leaves of *Stemodia trifoliata* (Link.) Reichb. The leaf essential oils of *Stemodia trifoliata* collected at the same month of two different years, were analized by GCMS and GC-FID. A total of 22 volatile components represented by sesquiterpenes and diterpenes was identified. β-Caryophyllene (9.4-15.4%) and caryophyllene oxide (6.2-9.0%) were the major compounds identified in the sesquiterpene fraction, while the diterpenoids 6α-acetoxymanoyl oxide (13.9-23.2%) and 6α-hydroxymanoyl oxide (25.1-29.7%) were the main constituents of the diterpene fraction.

PHYTOCHEMICAL ANALYSIS OF STEMODIA VISCOSA

Mammen and Daniel (2012) studied Volatile oils and flavones of *Stemodia viscosa* Roxb. They discussed *S. viscosa* as an aromatic weed used as a medicine for cold and flu and as a healing rub, is analysed for its volatile oils and flavonoids. The whole plant yielded 1.5% of a light greenish yellow volatile oil consisting of β-caryophyllene (37.1%), endo-fenchol(31.8%) and p-mentha-1-(7)-8- diene (19.58%)as major components. The minor constituents were α- humulene (5.88), α- cadinene (3.19%) and Δ3- carene (1.74%). The flavones identified were scutellarein, 4'-OMe sculellarein and 7,4'- dimethoxy scutellarein. β-Caryophyllene being anti-inflammatory and scutellarein considered a potential therapeutic agent for ischemic cerebrovascular disease, the plant can be used as a source material for these valuable phytochemicals.

Karthigaiselvi and Rameshwari (2018) studied the pharmacological effects of silver nanoparaticle of *Stemodia viscosa* - aqueous plant extract on albino wistar rats**.** The plant extract 40 ml was added to 10 ml of 10mM silver nitrate aqueous solution and kept

at room temperature for synthesizing silver nanoparaticle. Diabetes was induced in overnight fasted mice by single intra-peritoneal injection of 55 mg/kg of streptozotozin (STZ), freshly dissolved in 0.1M cold citrate buffer, pH 4.5. After five days of STZ administration, blood was collected and plasma glucose levels were determined. For wound healing activity, a control ointment base was formulated without any drug content. Two creams were formulated by using 5% extract were incorporated in 100gm of cream base. The observation of the percentage wound contraction were made on 4th , 8th , 12th , 16th and 22nd day of post wounding days. All the values were statistically analyzed by unpaired student test comparing with control. The administrations of plant extract to STZ induce diabetic mice, significantly lowered the plasma glucose level as compared to diabetic control. The study is further, extended to analyze lipid profile which was significant that streptozotocin induced mice was found to be higher with 67.05 mg/dl than the normal control mice. The ointment formation were prepared from the plant extract the results given in that wound contracting ability of *Stemodia viscosa* ointment (5% w/w) was found significant than the control. *Stemodia viscosa* exhibit a remarkable wound healing and anti-lipedmic activity.

FTIR ANALYSIS OF SCROPHULARIACEAE MEMBERS

Badola and Negi (2017) screened FTIR spectrum of *Verbascum thapsus* by using leaves extract shows peaks at about 3435,2915,2328,1614,1384 and 1108cm-1. Peak at 3435cm-1 is indicative of O-H group due to presence of alcohols, phenols, carbohydrates etc. C-H bond of alkyl group showed peak at 2915cm-1. Peaks at 2328cm-1 and 1614cm-1 is due to N-H bond of amino acids. Peaks at 1384cm-1 corresponded to C-N stretching vibration of amide group. C-O stretch assigned to alcohols represented by peak at 1108cm-1.

IN VITRO PROPAGATION OF SCROPHULARIACEAE MEMBERS

Plant biotechnology is an emerging field which inculcates us for its useful things in our day to day life. In recent years, plants are under dangerous condition and it is the time to refill all the living organisms in our earth for saving of our life. Plant biotechnology has many branches to conserve the plants but micropropagation of plants is one of the good methods to regenerate a plantlet within a small area with suitable medium under aseptic condition.

Micropropagation is the practice of rapidly multiplying stock plant material to produce a large number of progeny plants, using modern plant tissue culture methods. It is a method of vegetative propagation conducted in the laboratory condition and it has a significant impact on plant breeding, horticulture and medicine. It is the ever-ready tool for specialization in hybridization either by sexual or asexual means. It is a suitable method for obtaining a large quantity of genetically homogenous and healthy plant material which can be used for planting. (Pierik, 1987). This technique is an alternative method of propagation as there is an increase in the propagation rate of plants, availability of plants throughout the year, protection of plants against pests and pathogens under controlled conditions and the availability of uniform clones and uniform production of secondary metabolites. (Bajaj *et al.,* 1988).

Garg and Rangaswamy (1984) investigated an organ regeneration in cultures of vegetative explants of an antidiabetic species *Scoparia dulcis* by using modified Nitsch's medium. The effects of casein hydrolysate, 2,4-D, and NAA were studied on the regeneration ability of the explants. All explants differentiated roots or shoot buds, or both roots and shoot buds or leafy shoots. The hypocotyl showed maximal and the apical leaf segment minimal regetative ability. On 4 out of the 10 media tested, the leaf

segments and hypocotyls produced a slight callus. Embryoid differentiation was never expressed by any of the explants in any of the treatments.

Wolf and Timko (1991) established *in vitro* root culture of *Striga asiatica* (L.) Kuntze. *Striga* roots cultured in a Gamborg B5 salts medium containing indole-3-acetic acid (22.8 μM) increased in size and proliferated lateral roots. They demonstrated that cultured lateral roots are capable of haustorial formation within 24 hours of treatment with a haustorial initiation factor 2,6-dimethoxy-*p*-benzoquinone (2,6-DMBQ). Cultured root tips developed haustoria when in contact with a solid medium that contained 2,6-DMBQ. The solidity of the medium was observed to influence the extent of haustorial development. Haustoria formed on cultured roots appeared morphologically similar to those formed by *Striga* radicles within a similar time period and were 5–10 folds larger. They also showed that root cultures can be used as an explant source for regeneration studies. Callus, derived from root culture explants, was induced to form either shoots or roots. The optimal concentrations of the growth regulators N^6-(2-isopentenyl) adenine and indole-3-acetic acid for shoot formation were 49 μM and 2.85 μM, respectively, whereas the best root regeneration medium contained 5.4 μM 1-naphthaleneacetic acid and 8.8 μM 6-benzylaminopurine.

Tiwari *et al.* (2001) comparatively studied cytokinins on *in vitro* propagation of *Bacopa monnieri.* A range of cytokinins have been investigated for multiple shoot induction with node, internode and leaf explants. Of the four cytokinins (6-benzyladenine, thidiazuron, kinetin and 2-isopentenyladenine) tested thidiazuron (6.8 μM) and 6-benzyladenine (8.9 μM) proved superior to other treatments. Optimum adventitious shoot buds induction occurred at 6.8 μM thidiazuron where an average of 93 shoot buds were produced in leaf explants after 7 weeks of incubation. However, subculture of leaf

explants on medium ontaining 2.2 μM benzyladenine yielded a higher number (129.1) of adventitious shoot buds by the end of third subculture. The percentage shoot multiplication (100%) as well as the number of shoots per explant remained the high during the first 3 subculture cycles, facilitating their simultaneous harvest for rooting. *In vitro* derived shoots were elongated on growth regulator-free MS medium and exhibited better rooting response on medium containing 4.9 μM IBA. After a hardening phase of 3 weeks, there was an almost 100% transplantation success in the field.

Alderete *et al.* (2006) established an *in vitro* micropropagation protocol for *Mecardonia tenella.* For the *in vitro* establishment of *M. tenella*, nodal segments were disinfected by standard methods using ethanol/sodium hypochloride and cultured on hormone free MS medium, supplemented with a mixture of antibiotics and an antifungal. In order to study the hormonal requirements of the species, nodal segments were cultured on basal MS supplemented with antibiotic/antifungal mixture and the following concentrations of BAP and NAA (mg/L): 0.0; 0.25; 0.5 and 1.0. These plant regulators were tested in all possible combinations. *In vitro* plants growing in hormone-free medium were used as explant source. The best results were obtained in the treatments containing 0.25 and 0.5 mg/l BAP with a multiplication rate of 32 shoots per explant. The regenerated shoots rooted spontaneously. When transferred to the greenhouse, the *ex vitro* plants grew and flowered normally.

Hassan *et al.* (2009) established an efficient protocol for *in vitro* clonal propagation of the perennial medicinal herb *Scoparia dulcis* L. Apical and axillary buds of young sprouts from selected plants were used as explants. Best shoot induction was observed on MS basal medium supplemented with 0.1 mg/l BAP, in which 94% of the explants produced 12 shoots per culture. Repeated subcultures in the same medium, it resulted rapid shoot multiplication with 16 shoots per culture. The half strength MS

medium with 0.5 mg/l IBA +0.5 mg/l NAA the highest percentage (85.20) and maximum number (13.40) of roots were initiated within four weeks of culture. For acclimatization and transplantation, the plantlets in the rooting culture tubes were kept in normal room temperature for 7 days before transplanting in pots where plantlets were reared for three weeks. The survival rate of regenerated plantlets was 85%.

Karthikeyan *et al*. (2009) observed direct regeneration and *in vitro* flowering of *Scoparia dulcis* L. Single node explants were inoculated on basal MS medium containing 3% (w/v) sucrose, supplemented with different concentrations and combinations of 6-benzylaminopurine (BAP), kinetin (KN), indole-3-acetic acid (IAA), indole-3-butyric acid (IBA) and Naphthalene acetic acid (NAA) for direct plant regeneration. Maximum numbers of shoot (~22) were observed on the medium containing 0.5 mg/l BAP and 0.25 mg/l IAA after four weeks of culture. Regenerated shoots were separated and rooted on half strength MS medium supplemented with 0.5 mg/l of IBA alone for three weeks. Simultaneous regeneration of shoots and roots and *in vitro* flowering were achieved from the nodal explants on MS medium supplemented with 0.5 mg/l KN and 2.0 mg/l IAA. Well-developed complete plantlets were transferred on to specially made plastic cup containing soil rite. Acclimatized plantlets were successfully grown in garden soil.

Sood and Chauhan (2009) developed a low cost micropropagation technology for an endangered medicinal herb (*Picrorhiza kurroa*) of North-Western Himalayas. Axillary shoot tips cultured on MS +IBA (2 mg L-1) + KN (3 mg L-1) + sucrose 3% (w/v) + agar-agar 0.8% (w/v) was the best medium for multiple shoot formation with 86.3% shoot apices forming multiple shoots. The sucrose was replaced with table sugar and agar-agar was omitted completely. Out of 6 low-cost media combinations tested, MS liquid medium supplemented with Indole-3-Butyric Acid (IBA) (2 mg L-1) + kinetin (KN) (3 mg L-1) + table sugar 3% (w/v) was found to be the best with 27 shoots/explant. Seventy percent

shoots formed roots on half strength MS salts supplemented with IBA (3 mg L-1) + table sugar 3% (w/v) + agar-agar with an average of 5.6 roots per shoot. The study resulted in the identification of a low-cost medium combination for rapid multiplication of *P. kuura* with a potential that the technology can be up-scaled to a large-scale production.

Rout *et al.* (2011) and Asha *et al*. (2013) standardized an efficient protocol for *in vitro* clonal propagation of *Bacopa monnieri* L. The best callus induction (71±2.2%) was found in MS medium supplemented with 2.0 mg l -1 BAP + 0.5 mg l -1 NAA from leaf explant. Combination of 2.0 mg l -1 BAP + 0.5 mg l -1 NAA and 2.0 mg l -1 BAP + 0.5 mg l -1 IAA gave the most effective for shoot regeneration from callus. The elongated shoots rooted in ½ strength MS medium supplemented with different concentrations of auxins (NAA, IAA and IBA). IAA (6.5±0.57) was more suitable for root induction when compared to NAA and IBA (5.1±0.32 and 4.7±0.44). The success of plant tissue culture for *in vitro* culture of *B. monnieri* was encouraged by acclimatization of the plantlets in the field conditions. About 86% plantlets survived under field conditions.

Sakthi and Mohan (2012) reported micropropagation and plant regeneration from leaf and node explants of *Scoparia dulcis.* In this work large-scale micropropagation of *Scoparia dulcis* was achieved on Murashige and skoog (MS) medium supplemented with 2, 4-dichloro phenoxy acetic acid and 6-benzylamino purine (1.5 mg/L each) which was essential for culture establishment and callusing. The maximum numbers of shoots were produced in the combination of BAP and IAA (1.5 mg/L each). Multiple shoot proliferation was achieved through five subcultures of the isolated shoots without any decline. Transfer of shoots (4-5 cm) in to MS solid and liquid medium favoured rooting in 4 weeks and rooted plants (9 cm) were hardened and established (70-85%).

Mehta *et al.* (2012) reported *in vitro* shoot regeneration of *Bacopa monnieri* (L.) using Cyanobacterial media. In this studies they highlights the recent development and

novel achievements made for the multiple shoots regeneration of *B. monnieri* in Cyanobacterial medium as well as in MS medium. Shootlets were regenerated from nodal explants of stem through auxiliary shoot proliferation. In Cyanobacterial culture medium *B. monnieri* survived for long period of time and multiple shoot initiation was observed in 2mg/l Kn. When *B. monnieri* was inoculated in MS medium supplemented with different concentrations of PGR's, maximum no. of shoots were observed in 0.5 mg/l BAP + 2.0 mg/l Kn and 0.5 mg/l Kn+1.0 mg/l BAP.

Karamian and Ghasemlou (2013) achieved plant regeneration via somatic embryogenesis and organogenesis in *Verbascum speciosum* Schard. Two types of calli, embryogenic and non-embryogenic, were induced from mature embryo explants on Murashige and Skoog (MS) medium supplemented with different concentrations of benzyl adenine (BA) and α-naphthalene acetic acid (NAA). In order to further proliferate the somatic embryoids, the yellow and friable embryogenic calli were transferred on MS medium containing 0.5 mg-1 charchol and 0.1 or 1 mg-1 2,4-dichlorophenoxy acetic acid (2,4-D) or into MS medium containing 60 g-1 sucrose, 50 mgl-1 casein hydrolysate (CH), 0.5 mg-1 kinetin (Kin), 5 mg-1 2,4-D and 0.5 mg-1 charchol. Among the 3 tested media, MS medium containing 0.1 mg-1 2,4-D and 0.5 mg-1 charchol was more effective for proliferation of embryonic calli. Somatic embryos were transferred to hormone free MS medium for maturation and shoot regeneration. In addition, shoots and roots regenerated from non-embryogenic calli in hormone free MS medium or containing NAA and BA. Shoot buds were obtained from non-embryogenic calli and they were transferred to MS medium supplemented with 1 mg-1 BA or Kin for further growth and multiplication. Regenerated plants then were potted and maintained in the greenhouse.

Rahman *et al*. (2013) established an *in vitro* propagation method of *Paulownia tomentosa* Steud. Through the induction of adventitious shoots in explants derived from

selected mature trees. Shoot proliferation was induced from shoot apices and lateral bud explants of *P. tomentosa* cultured on MS (Murashige and Skoog 1962) medium containing different concentrations of BAP, NAA and Kinetin either alone or in various combinations. Results showed that 2.5 mg/L BAP + 0.5 mg/L NAA showed highest 84% shoot induction with multiple shoots of 7.4 shoots per explants and 5.6 cm longest shoot, while 3.0 mg/L KIN + 0.5 mg/L NAA showed (76%) shoot induction with 6.5 shoots per explants and 5.02 cm longest shoot. *In vitro* grown shoots were cultured to root on half strength MS medium containing either of 3 auxins, namely, IAA, IBA or NAA at concentrations of 1.5, 1.0 or 0.5 mg/L. Regarding rooting development, addition of 0.5 mg/L IBA to half strength MS medium gave 98% rooting percentage. Successful transplanting was obtained when rooted plantlets were transferred to a mixture of soil, sand and compost (1:1:1). Plantlets were transferred to a tray containing soil, sand and compost and covered by polythene sheets. After two weeks they were transplanted individually in the small poly bags and kept in open place with indirect sunlight, where 75% of the plants survived.

Haque and Ghosh (2013) studied micropropagation, *in vitro* flowering and cytological studies of *Bacopa chamaedryoides*, an ethno-medicinal plant. Shoot-tips and nodal segments explants were inoculated on Murashige and Skoog basal medium containing 0.7% agar, 3.0% sucrose and different concentrations and combinations of cytokinins and auxins. Optimum multiplication was achieved on medium containing 6-benzyl-aminopurine (2.0 mg L–1) and indole-3-acetic acid (0.2 mg L–1). Shoot-tips proved to be a better explant in having a high rate of shoot multiplication (18.7 ± 0.17) in comparison to nodal segments (15.1 ± 0.18) in the same medium. *In vitro* rooting of multiplied individual shoots was achieved on half strength Murashige and Skoog medium supplemented with 50% of '*Aloe vera* gel', with a maximum of 18.3 ± 0.17 roots. Up to

66.7% of these multiplied shoots induced healthy flowers *in vitro* on Murashige and Skoog medium containing low concentration of 6-benzyl-aminopurine (0.2 mg L–1). *In vitro* produced flowers contained 96.54 % viable pollen, more or less same as the field grown mother plants. Micropropagated plants have shown normal diploid 2n = 22 chromosomes, same as that of the mother plant. These micropropagated plants were successfully established in soil after hardening them in submerged condition, with an 84% survival rate. In total, 88.9% of the survived plants flowered and fruited normally after 50 days of field transfer. More than 85% field-grown regenerated plants developed normal fruits and viable seeds.

Kaur *et al.* (2013) studied *in vitro* propagation of *Bacopa monnieri* (L.) Wettst. In this experiment nodal explants containing preformed axillary bud were used as the starting material. Surface sterilized explants were aseptically cultured on MS medium supplemented with different plant growth regulators. Best results of axillary bud induction were observed on medium containing 1.0 mg/l BAP wherein 100% bud break was achieved. Optimal results of further shoot multiplication were also obtained on same media combination and shoots were periodically subcultured alternately on media containing 0.5 mg/l BAP and basal MS medium without any PGR. Half strength MS media containing different concentrations of auxins were used for *in vitro* rooting of shoots. Medium supplemented with 1.0 mg/l IBA proved to be most efficient for development of healthy root system. Rooted plantlets were subsequently hardened, acclimatized and successfully established in field with 100% survival rate.

Kumari *et al.* (2014) propagated a memory vitalizer Bacopa monneiri (L.). through culture of nodal and apical shoot apex in MS (Murashige and Skoog, 1962) basal medium supplemented with various concentrations of 6-Benzyle amino purine (BAP), Kinetin, either alone or in combinations with IBA. Both BAP and KN at 0.5 mg/l

concentration were found most suitable Cytokinin with respect to multiple shoot induction. At this concentration, the mean number of shoots on nodal explants was 18.0 where as it was 15.0 in shoot tip explants on subculture in the same composition of the medium. However, in case of KN, at the same concentration the mean number was 16 in the nodal explants and 12 in the shoot tip explants. When both the Cytokinin were used together, no promising result was obtained. This was also true for the supplementation of IBA. Well-developed shoots raised in vitro were excised and used for rooting in MS basal medium supplemented with various concentration of IBA.95% explants rooted in the medium supplemented with 0.25 mg/l IBA where the mean number of roots was 12.0 and average length 5.6 cm after 4 weeks. Well rooted plants were carefully taken out from the medium and washed properly with glass distilled water to remove the agar adhere with the roots. They were planted in the rooting pot containing 1:1:1 autoclaved vermiculite sand and soil. Humidity of the chamber (polybag) was maintained through water soaked sponge platform. Half strength liquid MS medium was used for irrigation on alternate day.82% plantlets survived in this condition while survival rate in the field was 72%.

Lalabadi *et al*. (2014) performed *in vitro* micro-propagation of Tashnedari (*Scrophularia striata)* which is an endangered medicinal plant. In their experiment, effects of BAP, 2, 4-D, BA and IAA hormone were evaluated in MS medium on callus induction and regeneration of this plant. Explants were prepared from stem. Factorial experiment was done as completely randomized design with three replications. Results showed that the combination of BAP (1.5 mg/l) and 2, 4-D (1.5 mg/l) was the best treatment for callus induction (= 52.22) and BA (0.5 mg/l) and NAA (1 mg/l) was recorded as the best treatment for regeneration (= 46.66). Callus fresh weight was measured for shoot explant. And hormonal combination of BAP (3.5mg/l) and 2, 4-D (1.5mg/l) had the highest increase in fresh weight of callus (1.09 g).

Shtereva *et al.* (2014) investigated the effect of genotype and culture medium on the *in vitro* germination and development of plantlets from seeds of 6 different *Paulownia* genotypes (*P. tomentosa*, hybrid lines *P. tomentosa* × *P. fortunei* (Mega, Ganter and Caroline), *P. elongata* and hybrid line *P. elongata* × *P. fortunei*). Nodal and shoot tip explants were used for micropropagation of *Paulownia* genotypes by manipulating plant growth regulators. The highest germination percentage for all genotypes was obtained for seeds inoculated on medium supplemented with 50 mg L GA3 (MSG2). On Thidiazuron containing media, the explants of hybrid line *P. elongata* × *P. fortune* exhibited the highest frequency of axillary shoot proliferation following by *P. tomentosa* × *P. fortunei.* The results are discussed with the perspective of applying an improved protocol for *in vitro* seed germination and plantlet formation in several economically valuable *Paulownia* genotypes.

Koul *et al.* (2014) reported an efficient, cost effective protocol by using leaves as explants for *in vitro* propagation of *Bacopa monnieri.* Leaves of five different accessions of *B.monnieri* collected from various regions of North India were cultured on agar gelled Murashige & Skoog (MS) & Gamborg's (B5) media without the addition of any expensive nutritional supplements (plant growth regulators, growth additives). De novo shoot initiation from leaf explants was observed after 20 days of inoculation accompanied by rooting in the form of a single tap root in all the accessions in both the media tested. Maximum shoots/explant (5.5±0.65), leaves/explant (17.9±0.75) and roots/explants (6.3±0.65) were obtained for leaves of Accession BM003 cultured on MS media. Through this study they conclude that MS media is superior over B5 media for *in vitro* shoot multiplication and plantlet regeneration of *B. monnieri*.

Mohanta and Sahoo (2014) studied an *in vitro* culture of highly valuable medicinal plant *Bacopa monnieri* (L.) Penn. for rapid and mass multiplication. Nodal

segments containing axillary buds were surface sterilized with 0.1% solution of mercuric chloride for 5 min and were inoculated aseptically on culture medium. *In vitro* clonal multiplication methods and the elite clones were observed that, MS basal+0.5 mg/l IAA and MS basal+ 0.5 mg/l NAA shown the best results for culture initiation and axillary shoot proliferation. For rooting MS+ Agar 7 g/l, Sugar 20g/l and MS+Agar 8g/l were found to be the best medium in terms of shoot/root ratio and number of shoots. Compact globular callus was best initiated and proliferated on MS+0.5 mg/l BAP+1 mg/l 2,4 D and medium with best regeneration in MS+1.0 mg/l BAP+1 mg/l IAA. The experimentation was made successful with 71% survival plantlets producing regeneration of the *Bacopa monnieri* L. Penn. for mass cultivation.

Helena *et al.* (2015) eamined an *in vitro* regeneration of *Picrorhiza kurroa* Royal. ex Benth. for *ex situ* conservation and sustainable utilization. Different combinations of phytohormones based on usual practices of plant tissue culture were examined for callus induction and subsequently regeneration. Maximum callus induction was observed in MS supplemented with 0.5 mg L-1 TDZ + 0.3 mg L-1 IBA and 0.5 mg L-1 TDZ + 0.5 mg L-1 IBA in leaf and stem explants respectively. Callus derived from leaf and stem was transferred in shoot induction medium where maximum shoot frequency was observed in 1.0 mg L-1 BA + 0.75 mg L-1 KN in leaf derived callus and 1.0 mg L-1 BA + 1.0 mg L-1 Kinetin in stem derived callus respectively. Shoots thus obtained were transferred for induction of roots.

Nagarajan *et al.* (2015) performed an *in vitro* mass propagation of *Bacopa monnieri* (Linn.) Wettst from nodal explants - a multipurpose medicinal plant. Nodal explants of *Bacopa monnieri* were cultured on MS basal medium supplemented with different concentrations of BAP and KIN ranging from 0.5-2.5 µM/L for multiple shoot induction. Two cytokinins tested, BAP was found to develop in shoot multiplication and

higher number of shoots from the nodal explants when compared to KIN. Higher number of shoot was produced from all the concentrations of both BAP and KIN. The highest frequency (100%) of shoot induction and maximum number of shoot (6.4±1.94) was observed on 2.0 µM BAP with shoot length of 5.02±0.20 c.m. as well as in KIN the highest frequency (100%) of shoot induction and maximum number of shoot (5.4±1.51) was observed on 1.5 µM KIN with shoot length of 5.14±0.28 c.m. The isolated shoots were transferred to MS basal medium supplemented with different concentrations of IBA and NAA for root induction. The rooted plantlets were successfully transferred in soil through hardening and established in the field.

Shekhawat *et al.* (2015) generated an *in vitro* regeneration protocol of *Russelia equisetiformis* through nodal shoot segments cultured on Murashige and Skoog medium (MS) supplemented with different concentration and combination of cytokinins and auxins. Cent percent bud breaking response with maximum number of shoots (6.0) was observed on MS medium augmented with 1.0 mg/L 6-benzylaminopurine (BAP). Shoots multiplication further enhanced by repeated subculturing of *in vitro* shoots on MS medium with 1.0 mg/L BAP + 0.5 mg/L Kinetin (Kn) and additives. Maximum 13.2 shoots with 7.8 cm length were differentiated. Healthy shoots were rooted with high frequency (97%) on half strength MS medium containing 1.0 mg/L indole-3 butyric acid (IBA) with maximum number of roots (5.73). The *in vitro* regenerated plantlets were hardened in greenhouse and transferred to nursery with 85% survival rate.

Sheikh *et al.* (2015) established *in vitro* callus induction protocol of *Bacopa monnieri* (Linn.) Pennell. through young leaf, mature leaf, nodal and internodal segment culture using Murashige and Skoog medium supplemented with different combinations of phytoharmones. Of all the explants mature and young leaf were found to be best explant for callus induction. The MS medium supplemented with 0.8mg/l 2,4-D and 0.3 mg/l

kinetin was the best medium for callus induction in mature leaf. The very simple and effective protocol developed can be used for the large scale cultivation of this very important medicinal herb.

Behera *et al.* (2015) reported an efficient plant regeneration protocol through two stage culture of nodal segment for a valuable medicinal plant, *Bacopa monnieri*. Multiple shoots with large number of shoot buds were induced from nodal explant on Murashige and Skoog's (1962) (MS) medium fortified with (1.0-5.0 mg L-1) N6- benzyladenine (BA). Of the different concentrations of BA tested, 3.0 mg L-1 BA offered the best result, where the nodes swelled and an average of 6.5 shoots with numerous buds/node were recorded. The swollen nodes, cultured on MS + 3.0 mg L-1 BA, were sub-cultured either on MS or MS supplemented with 1.0 mg L-1 GA3 medium. The best result (114.2 shoots/ node) with an average shoot length of 6.4 cm was observed on MS media supplemented with 1.0 mg L-1 GA3. Cent per cent acclimatization of healthy *in vitro* regenerated shoots was obtained by *ex vitro* rooting in plastic pots containing garden soil, which saves time and tissue culture cost by abolishing the *in vitro* rooting step.

Stanilova *et al*. (2015) preserved the Bulgarian endemic *Verbascum davidoffii* (Scrophulariaceae) by means of *in vitro* propagation. As a first step, seed germination has been studied. In this experiment, seed germination was poor on the basal MS medium: only 1 seedling was obtained from 100 seeds for a period of 6 weeks; and no seed germinated on MS medium supplemented with 1 mg/l Kin. The stimulation of the process by seeds soaking in 0.35% solution of gibberellic acid for 22 hours increased the germination rate up to 61 % and 18 % for the two media, respectively. The effect of the gibberellic acid was strong even if applied for only 2 h, and the concentration of kinetin was better when supplemented in 10-fold less concentration. Seed stratification with low temperature at 6°C for a month prior to cultivation had additional effect on germination

which depended on the presence of Kinetin in the medium. *In vitro* seedlings with several leaves and roots were potted in soil substrate of soil mixture, sand and coconut fiber (2:1:1), and easily *ex vitro* adapted into the ambience of the laboratory phytotron, under controlled temperature, light, and humidity variations.

Mahajan *et al.* (2016) developed a protocol for *in vitro* mass multiplication of *Picrorhiza kurroa* Royle ex. Benth. Result of this study revealed that highest frequency of shoot regeneration was achieved on Murashige and Skoog's basal medium supplemented with 1.0mg/l BAP, 0.5 mg/l Kn and 1.0mg/l GA3, while the best rooting was observed in MS medium supplemented with 2.5 mg/l IBA. MS medium supplemented with 3.0 mg/l 2,4-D resulted in highest frequency of embryogenic callus. Callus inoculated on MS media supplemented with BAP and IAA resulted in both shoot and root formation while the callus on MS media supplemented with NAA and IBA resulted only root formation. The somatic embryos were established from callus on MS medium supplemented 2.5 mg/l 2,4- D after four weeks. MS medium containing 1.0 mg/l BAP and 1.0 mg/l GA3 resulted into shoots from well developed somatic embryos.

Mondal (2017) carried out *in vitro* pollen germination of *Scoparia dulcis* L. to study the role of different nutrients like sucrose, boric acid, different salts like calcium nitrate, potassium nitrate and magnesium sulphate. Flowers open in the morning (06:30-07:30 hrs.) after which anther dehiscence takes place. Maximum 97 % pollen germination along with 1183 µm pollen tube development was observed in 5 % sucrose solution supplemented with 100 ppm boric acid. Among the salts, maximum 52 % pollen germination along with 325 µm pollen tube development was observed in 300 ppm calcium nitrate solution. Pollen grains which were collected during anthesis (08:00-09:00 hrs.) showed the best results.

Mehta (2017) studied an effect of plant growth regulators on callus multiplication and *in vitro* plant regeneration in *Bacopa monnieri* L. *B. monnieri* has very high morphogenic potential. Its leaf explants respond very readily to treatment with auxins and cytokinins. Indefinite number of plantlets were regenerated from leaf margin, without intervening callus on Murashige & Skoog (MS) medium supplemented with indole-3-acetic acid (IAA) and kinetin (KN). Green embryogenic callus with indefinite number of micro shoots were developed on naphthalene acetic acid (NAA) supplemented medium when 2,4-dichlorophenoxyacetic acid (2,4-D) grown stem-derived callus was sub-cultured. During present investigation, stem (internode, node) explants exhibited callus induction, whereas, leaf explant showed a tendency to regenerate shoot and/or roots. *In vitro* grown plantlets were transferred to polypots containing sterile cocopeat and then series of steps were carried out for acclimatization. Survival rate was 100% when acclimatized plantlets were transferred to soil. Micro-propagated plantlets were without any morphological abnormalities.

Saiju *et al.* (2018) comparatively studied the growth statistics of two species of *Paulownia* and optimization of rooting methods. Growth comparison was performed by measuring shoot length in *in vitro* condition. Among two species of *Paulownia* - *Paulownia tomentosa* (Thunb.) Steud and *Paulownia fortuneii* (Seem.) Hemsl., the growth rate of *P. tomentosa* was found to be 0.355 cm/week while that of *P. fortuneii* was found to be 0.637 cm/week in *in vitro* conditions in MS medium supplemented with 0.1 mg/l NAA and 1mg/l BAP. Optimization of rooting methods was also performed, in which, sand rooting was found to be easier and more effective than *in vitro* rooting. Dipping the plantlets in 1 mg/l of NAA was found to produce longer and denser roots than lower or higher concentrations during sand rooting.

ANTIBACTERIAL ACTIVITY OF SCROPHULARIACEAE

The plants have capability to synthesize phytochemicals, which provide as plant defence mechanisms against microrganisms, herbivores and insects (Policegoudra *et al.* 2010). The plant origin antimicrobial substances were not linked with vast pharmaceutical activity to cure so many diseases (Gupta, 2003). Medicinal plants exhibted a rich source of antimicrobial substances. Although plant have been analysed for antimicrobial properties yet so many plants have not been adequately evaluated (Mahesh and Satish, 2008).

Khana *et al.* (2010) studied the antibacterial efficacy of *Bacopa monnieri* leaf extracts against pathogenic bacteria. Five different concentrations (500 µg, 1, 2, 5, 10, and 15 mg/mL) of crude leaf extracts of were tested for antibacterial efficacy against seven Gram positive and 11 Gram negative bacteria. The sensitivity of plant fractions was tested using the disk diffusion method. Maximum activity was revealed by ethyl acetate and methanol extracts, followed by aqueous, benzene, and petrol extracts.

Ibrahim *et al.* (2013) evaluated the chemical composition, antimicrobial activity of the essential oil of the flowers of *Paulownia tomentosa* (Thunb.) Steud. The volatile oil of the fresh flowers was isolated by hydrodistillation method. The antibacterial activity of the oil was tested against *Bacillus subtilis* NRRL B-543, *Staphylococcus aureus* NRRL B-313and *Escherichia coli* NRRL B-210. Moreover, the minimum inhibitory concentration (MIC) was calculated and resulted with 55 %, which revealed that *P. tomentosa* oil constituents have antimicrobial activity and could be used as a source of pharmaceutical materials required for the preparation of new therapeutic and antimicrobial agents.

Wankhar *et al.* (2015) studied the antimicrobial activity of *Scoparia dulcis.* In this study the plants were tested by broth dilution method ranging from 8mg/mL to 256mg/mL and the minimum inhibitory concentration (MIC) were determined when there

is positive or negative growth of the tested microorganism on the nutrient agar plates. In this particular study methanol extract displayed a better MIC when compared to aqueous against both gram positive, gram negative bacteria and fungus and this could have attributed to its potent extraction capacity.

Dulger *et al.* (2015) described antimicrobial potential of the leaves of common mullein (*Verbascum thapsus* L.) on microorganisms isolated from urinary tract infections by using disk diffusion method and microdilution method. Air dried leaves of the plant were extracted using 95% ethanol. The extracts showed strong antimicrobial activity against *Escherichia coli*, *Enterococcus faecalis* and *Candida albicans* with inhibition zones of 19.2, 16.8 and 16.2 mm, with MIC's and MBC's or MFC's of 32(64), 64(128) and 64(128) µg/mL, respectively. Also, the extracts exhibited moderate activity against the other test microorganisms.

Prakash *et al.* (2016) studied the antibacterial activity of *Verbascum thapsus.* The methanolic and acetone leaf extracts was determined *invitro* against medically important pathogens such as *Escherichia coli*, *Yersinia pestis, Bacillus cereus, Pseudomonas aeruginosa, Listeria monocytogenes* and *Staphylococcus aureus* following agar-well diffusion method using different concentrations (25%, 50%, 75% and 100%). Methanolic leaf extract was found to be more effective against selected pathogenic bacterial *spp*. as compared to acetone leaf extract. Further the leaf extract of both plants inhibited gram-positive bacteria more efficiently than gram negative bacteria.

Biva *et al*. (2019) screened the antibacterial performance of terpenoids from the Australian plant *Eremophila lucida*. The n-hexane fraction of the crude acetone extract of the leaves exhibited antibacterial activity against *Staphylococcus aureus*. Isolation led to the known compounds cembratriene, (3Z, 7E, 11Z)-15-hydroxycembra-3,7,11-trien-19-oic acid, the sesquiterpenoid, farnesal and the viscidane diterpenoid, 5_-hydroxyviscida-

3,14-dien-20-oic acid. The purified compounds were tested for antibacterial activity with 2 and 3 showing moderate antibacterial activity against Gram-positive bacteria.

ANTIBACTERIAL ACTIVITY OF STEMODIA VISCOSA

Karthigaiselvi *et al.* (2016) evaluated the antimicrobial activity of *Stemodia viscosa*. The results of this study revealed that the AgNPs of *S. viscosa* was found to be most effective against *Staphylococcus aureus* with 27 mm in diameter, followed by *Pseudomonas aeruginosa* with 25mm in diameter. In this study suggests that this plant is used for the development of antimicrobial drug.

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EXXPERIMENTAL DESIGN OF THE PRESENT STUDY

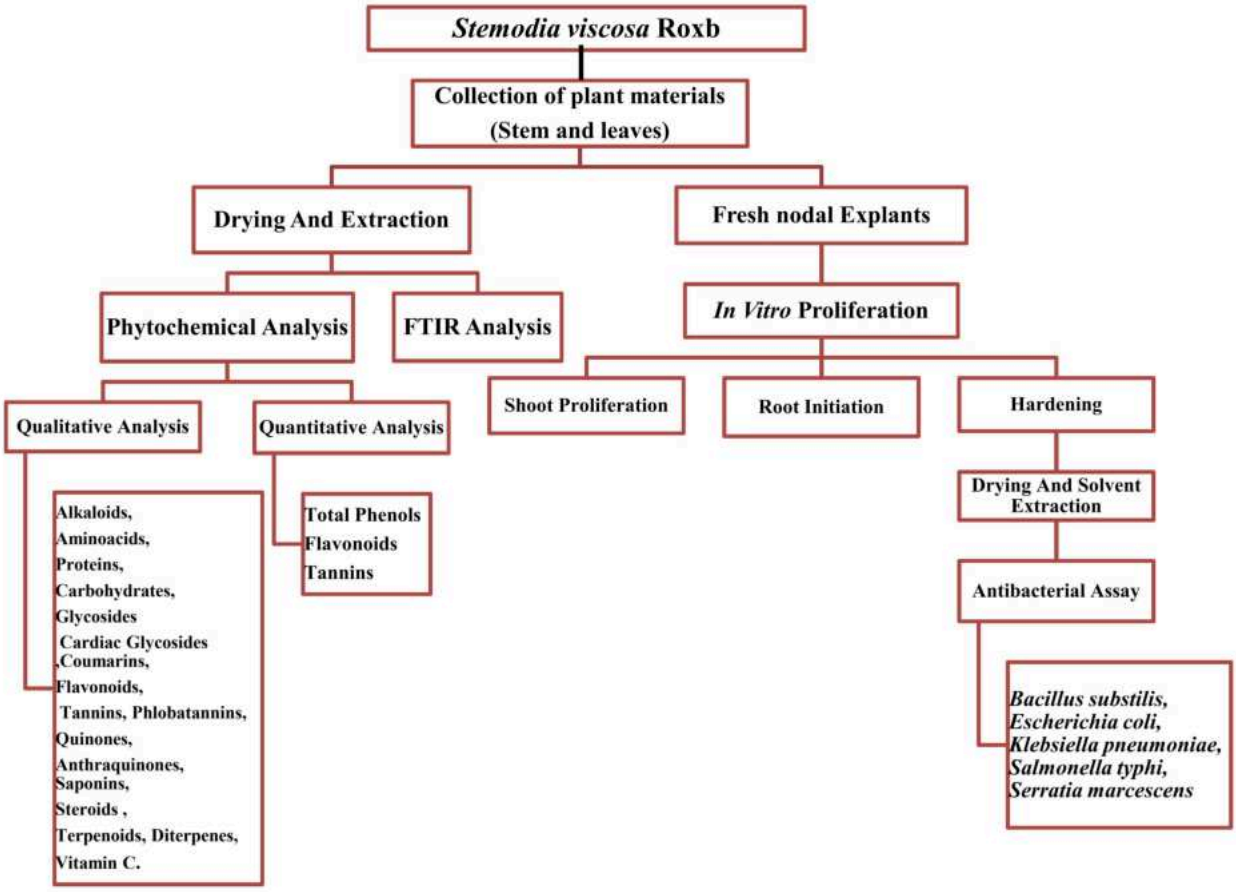

Plate 1: Morphological features of *Stemodia viscosa* Roxb.

a. Habit; b. Side view of flower; c. Flower central close up view; d. Young fruit; e. Mature fruit

SYSTEMATIC POSITION OF STEMODIA VISCOSA ROXB.

Class - Dicotyledons

Sub-Class - Gamopetalae

Series - Bicarpellate

Order - Personales

Family - Scrophulariaceae

Genus - *Stemodia*

Species - *Stemodia viscosa* Roxb.

Common name : Sticky blue rod, Pintye.

Vernacular name: Kokkurali

DISTRIBUTION:

S. viscosa distributed in tropical and subtropical regions of the world. It is the common weed of agricultural fields.

BOTANICAL DESCRIPTION:

Branched, aromatic, viscidly pubescent herb, wiith tap root system. hence its name. Stem aerial, erect, herbaceous, quadrangular and reaching upto 60 cm. height, viscous. Leaves exstipulate, sessile, simple, oblong, amplexicaul, opposite decussate, base auriculate, margin serrate, apex acute, reticulate and 4 cm. long. Flowers solitary, axillary, bracteate, bracteolate, pedicellate, hypogynous, complete, zygomorphic, bisexual, cyclic. Sepals 5, polysepalous, valvate, persistent. Petals 5, bilipped (upper 3 lobes and lower 2 lobes), gamopetalous, imbricate blue. Stamens 4, didynamous, epipetalous, polyandrous, filaments short, anthers dithecous, basifixed, introrse, opening on longitudinal slits. Ovary bicarpellary, syncarpous, bilocular, ovules numerous in each

locule, minute, ovules on axile placentation, nectariferous disc is present; style short,simple; stigma bifid. Fruits dry, dehiscent, capsule. Seeds numerous, minute, dicotyledonous, endospermic, extremely fine and dust like in nature **(Plate 1)**.

COLLECTION OF PLANT MATERIALS

The field grown *Stemodia viscosa* (Scrophulariaceae) were collected from agricultural field of Kulayankarisal village in Thoothukudi District. For phytochemical analysis mature and healthy plants were collected and for *in vitro* proliferation 30 days old, fresh and healthy explants (nodal regions with axillary bud) were collected from the field. The collected plants were described by using Flora of the Presidency of Madras (Gamble, 2004)

PHYTOCHEMICAL PROFILE

Solvent extraction

Stem and leaves of *Stemodia viscosa* were harvested and allowed to dry under shade condition. The dried material was then ground to a fine powder. Approximately 8 g of ground material was dissolved in 100 ml of different solvents (petroleum ether, acetone, chloroform, methanol and water). The solution was filtered, the crude extract was retained. The resultant liquid was used for qualitative phytochemical tests.

Qualitative phytochemical tests

Phytochemical analyses of the crude extracts (petroleum ether methanol, chloroform and acetone) and negative control (water) were conducted in accordance with standard protocols (Harbourne, 1973; Trease and Evans, 1978; Philip *et al*. 2011; Damodaran and Manohar, 2012). All tests were replicated twice. Detailed methodology are as follows:

i. Detection of Alkaloids

Hager's test:

One ml of extract was mixed with one ml of Hager's reagent (1 g of picric acid in 100 ml distilled water). The formation of a yellow precipitate indicated the presence of alkaloids.

ii. Detection of Amino acids

Ninhydrin test:

Two drops of Ninhydrin reagent were added to two ml of dilute extract. A deep purple colour change indicated the presence of amino acids.

iii. Detection of proteins

Xanthoproteic Test:

The extracts were treated with few drops of conc. Nitric acid. Formation of yellow colour indicates the presence of proteins.

iv. Detection of Carbohydrates

Extracts were dissolved individually in 5 ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.

Molisch's test:

Molisch's reagent (15 g α-naphthol dissolved in 100 ml ethanol) was added to five ml crude plant extract. A brownish red colour reaction indicated the presence of polysaccharides.

v. Detection of glycosides:

Extracts were hydrolysed with dil. HCl, and then subjected to test for glycosides.

Modified Borntrager's Test:

Extracts were treated with ferric chloride solution and immersed in boiling water for about 5 minutes. The mixture was cooled and extracted with equal volumes of

benzene. The benzene layer was separated and treated with ammonia solution. Formation of rose pink colour in the ammonical layer indicates the presence of anthranol glycosides.

vi. Detection of Cardiac glycosides

Keller-Killiani test:

5 ml of plant extract was mixed with 2 ml glacial acetic acid. Two drops of ferric chloride were added, followed by the addition of concentrated sulphuric acid such that the acid remains underneath. The presence of glycosides was indicated by the formation of a brown ring at the junction of the two layers and a blue green ring at the upper surface.

vii. Detection of Coumarins

1 ml of 10% NaOH was added to 1 ml extract. The development of a yellow colour indicates a positive reaction for coumarins.

viii. Detection of Flavonoids

Lead acetate test:

A 10% lead acetate solution (three ml) was added to two ml dilute extract. The formation of a white precipitate indicated the presence of flavonoids.

ix. Detection of tannins

Lead acetate test

To 1 mL of the extract, 2 drops of lead sub acetate solution was added. A coloured precipitate indicates the presence of tannins.

x. Detection of Phlobatannins:

Few drops of 2% hydrochloric acid were added to 1ml of the extract. Appearance of red colour precipitate indicates the presence of phlobatannins.

xi. Detection of Quinones

1 ml concentrated sulphuric acid was added to equal amount of extract. The development of a red precipitate indicated the presence of quinones.

xii. Detection of Anthraquinones

A few drops of 2% hydrochloric acid were added to one ml of extract. The formation of a red precipitate indicates the presence of anthraquinones.

xiii. Detection of Saponins

Foam test:

The extract (5 ml) was diluted with distilled water to 20 ml. The solution was shaken in a graduated cylinder for 15 minutes. The presence of saponins in the extract was identified by the formation of a persistent two cm foam layer.

xiv. Detection of Steroids

Lieberman-Burchard test:

The extract was dissolved in water and then treated with chloroform. The liquids were separated using separating funnel. The chloroform portion was collected and then divided into 2 portions and was used for the test.

A few drops of acetic anhydride was added to the filtrate in a test tube, then followed by the addition of conc. sulphuric acid by the wall of the test tube. The formation of brown ring at the junction indicates the presence of phytosterols.

xv. Detection of Terpenoids

Salkowski's test:

5 ml of crude extract was mixed with 2 ml chloroform. Concentrated sulphuric acid (3 ml) was then carefully added to the mixture forming distinct layers. The presence of terpenoids was indicated by the formation of a reddish brown colour at the interface between the two solutions.

xvi. Detection of diterpenes

Copper acetate Test:

Extracts were dissolved in water and treated with 3-4 drops of copper acetate solution. Formation of emerald green colour indicates the presence of diterpenes.

xvii. Detection of Vitamin C

1 ml of sample extract were mixed with few drops of Dinitrophenyl hydrazine following the addition of 1 ml concentrated H_2SO_4. Appearance of yellow precipitate indicates the presence of Vitamin C.

Quantitative analysis

Estimation of Flavonoids:

The total flavonoid content in the sample was estimated by the method of Chang (2002). A volume of 0.25 ml of the sample was diluted to 1.25 ml with distilled water. 75 µl of 5% sodium nitrite was added and after six minutes 0.15 ml of aluminium chloride solution was added. 0.5 ml of 0.1M NaOH was added after 5 min and made up to 2.5 ml with distilled water. The solution was mixed well and the absorbance was read at 510 nm along with standard quercetin at 5 - 25 µg concentration. The results are expressed as mg of flavonoids as quercetin equivalent / gm of dried sample.

Total Phenolic Content (TPC):

Total phenolic content of extract was determined according to the Folin-Ciocalteau method of Slinkard and Singleton (1977) with some modifications. Briefly, 0.1 ml of extract (200, 600 and 1000 µg/ml), 1.9 ml distilled water and 1 ml of Folin-Ciocalteau's reagent were seeded in a tube, and then 1 ml of sodium carbonate was added. The reaction mixture was incubated at 25 °C for 2 h and the absorbance of the mixture was read at 765 nm. The sample was tested in triplicate and a calibration curve with six data points for catechol was obtained. The results were compared with catechol

calibration curve and the total phenolic content of sample was expressed as mg of catechol equivalents per gram of extract.

Total Tannins Content (TTC):

Tannins were determined by the method of Peri and Pompei (1971). 1 ml of the sample extracts of concentration 1mg/ml was taken in a test tube. The volume was made up to 1ml with distilled water and 1 ml of water serves as the blank. To this 0.5 ml of Folin's phenol reagent (1:2) followed by 5ml of 35% sodium carbonate was added and kept at room temperature for 5 min. Blue colour was formed and the colour intensity was read at 640 nm. A standard graph (gallic acid - 1 mg/ml) was plotted, from which the tannin content of the extract was determined. The total tannin content was expressed in mg/g of extract.

FTIR ANALYSIS

A little powder of plant specimen was mixed with KBr salt, using a mortar and pestle, and compressed into a thin pellet. Infrared spectra were recorded as KBr pellets on a Thermoscientific Nicot iS5 iDl transmission, between 4000- 400 cm-1(Kareru *et al.* 2008).

***IN VITRO* CULTURE OF STEMODIA VISCOSA**

Culture Medium

The nutrient medium consists of inorganic salts, carbon source and organic supplements. In addition, vitamins and growth regulators are also added to the medium. In the present study, the basal medium consists of the mineral salts and organic nutrients of Murashige and Skoog (1962) salts with B5 vitamins are used. The basal medium is supplemented with various concentrations and combinations of different growth regulators.

Growth regulators

The present study the hormone concentration was used in μM (micromole) per liter. These growth regulators were used as supplement to the basal medium individually as well as in different combinations.

Cytokinins : **BAP** (6-benzylaminopurine)

KIN (6-furfurylaminopurine)

Auxins : **IBA** (Indole butyric acid)

NAA (Naphthalene acetic acid)

The present study was performed in the basal medium with MS salts, B5 vitamins, 3% sucrose and 0.8% agar.

The basal medium was variously supplemented with factorial combinations of different growth regulators ranging from 2 – 8 μM/L BAP or KIN alone for shoot multiplication. After adding all the supplements (various concentrations of different hormones) to the basal medium, the pH of the medium was adjusted to 5.8. The molten medium was dispensed in culture tubes or conical flasks and was tightly capped with cotton plugs.

Sterilization of Culture Medium and Glassware's

The culture medium containing high concentration of sucrose supports the growth of several microorganisms. These microbes generally grow much faster than the explants and finally spoil the culture. So it is very essential to maintain a complete aseptic environment inside the culture tube. Therefore, the culture medium, glassware's, forceps and scalpels was sterilized by using autoclave. After sterilization, the culture tubes left free until agar in the medium become solidified. Then the tubes were transferred to inoculation chamber for inoculation.

Sterilization of explants

The explants were taken from the field grown mature plants. The nodal explants with axillary bud and leaf explants were surface sterilized by rinsing in running tap water for 30 minutes. Then they were washed in an agitated solution of liquid detergent for 5 minutes and followed by distilled water for 2-3 times for removing the traces of liquid detergent. After thorough washing, the materials were taken in to the laminar air flow chamber where they were disinfected with 70% alcohol for 30-60 seconds followed by 0.1% mercuric chloride for 3-5 minutes. Finally, the materials were thoroughly rinsed with sterile distilled water for 4-5 times to remove the traces of mercuric chloride.

Inoculation Procedure

Before starting inoculation all the requirements such as culture tubes containing media, bunsen burner, sterile water, glassware, 70% ethanol, 0.1% mercuric chloride and explants, were placed in the laminar air flow chamber. The platform surface of the chamber was swapped with 70% alcohol. After swapping the chamber with 70% alcohol, the UV light was switched on for 30 minutes. After 30 minutes, the UV light was switched off and the white fluorescent light was switched on. Before inoculation, hands were rinsed with absolute alcohol. The instruments were sterilized by dipping in absolute alcohol followed by flaming and cooling.

The inoculation was carried out in the vicinity of flame. The surface sterilized explants were aseptically transferred to the respective culture media in the Laminar Flow Chamber. The explants were taken out from beaker and at the same time the cotton plug of the culture tube was slightly opened in front of the bunsen burner flame, the explant was kept it in the medium and immediately covered with cotton plug. The explants with nodal regions were inserted in the medium vertically and the leaf explants were inserted

in the medium horizontally. Cultures were transferred to fresh media with the same hormone concentration at 4 week intervals.

Culture Conditions

The cultures were maintained in a culture room at 25±2°C under 16 hr photoperiod with a light intensity supplied by cool white fluorescent tubes. These growth conditions were referred to as standard culture conditions for *in vitro* studies.

Culture Maintenance

The nodal explants regions, were initially cultured on MS solid medium in test tubes. After 4 weeks, the initiated shoot multiples were subcultured on MS basal medium fortified with the same growth regulator concentrations and combinations or whichever is the best for further multiplication. To facilitate higher number of shoot formation, the explants were also subcultured on conical flasks which can provide more space and more medium for growth and multiplication. (Jahirhussain *et al.* 2016.)

Rooting

In vitro raised shoots of 2 cm and above were excised from the culture tube or culture bottle and subcultured into MS medium fortified with 3% sucrose (w/v) and 0.8% agar (w/v). The medium was further supplemented with different concentrations (2- 8 µM/L) of IAA or IBA. The root number and length were measured in each culture medium.

Hardening and Acclimatization

Plantlets with well-developed roots were dislodged from the culture medium and roots were washed gently under running tap water to remove the adhering medium. Plantlets were transferred to plastic cups (10 cm diameter) containing autoclaved garden soil, farmyard manure and sand (2:1:1). Each plantlet was irrigated with distilled water every 2 days for 2 weeks followed by tap water for one week. The potted plantlets were

initially maintained under culture room conditions (3 weeks) and later transferred to normal laboratory conditions (2 weeks).

The potted plantlets were initially covered with porous polyethylene sheets to maintain high humidity and were maintained inside the culture room. The relative humidity was reduced gradually. After 30 days the plantlets were transplanted to the field under shade for 3 weeks and then transplanted to the soil for further growth and development.

ANTIBACTERIAL ASSAY

Preparation of extracts for antibacterial activity

Stem and leaves of *Stemodia viscosa* grown by *in vitro* technique was dried and extracted successively with methanol, acetone and water. All the extracts were filtered through Whatman No: 1 filter paper and stored for future use.

Collection of microorganisms

Stock cultures of bacteria such as *Escherichia coli, Bacillus substilis, Klebsiella pneumoniae, Salmonella typhi, Serratia marcescens* were obtained from Research Laboratory, Department of Botany, St.Mary's College (Autonomous), Thoothukudi, Tamil Nadu.

Preparation of media

The growth media employed in the present study included nutrient broth and nutrient agar.

Nutrient broth is composed of

Beef extract – 3.0 g.

Peptone – 5.0 g.

Distilled water – 1000 ml.

Nutrient agar is composed of

Beef extract – 3.0 g.

Peptone – 5.0 g.

Agar – 15.0 g.

Distilled water – 1000 ml.

The medium was adjusted to pH 7.4 and sterilized by autoclaving at 15 lbs pressure (121^{o}C) for 15 minutes.

Sub culturing of microorganisms

The pure culture of microorganism was maintained on nutrient agar slants by frequent sub culturing. The culture was stored at 4^{o}C.

Preparation of inoculums

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 incubated overnight at 37^{o}C. These overnight cultures were used as inoculums.

Antibacterial activity

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

Qualitative analysis

The preliminary phytochemical screening of petroleum ether, acetone, chloroform, methanol and water extracts of stem of *Stemodia viscosa* revealed the presence of proteins, carbohydrates, cardiac glycosides, coumarins, flavonoids, tannins, quinones, steroids, terpenoids, diterpenes and vitamin C and similar solvent extracts of leaves of *S. viscosa* divulged the presence of proteins, carbohydrates, cardiac glycosides, coumarins, flavonoids, tannins, phlobatannins, quinones, steroids, terpenoids, diterpenes and vitamin C **(Table 1 & 2)**.

Quantitative analysis

Though wide range of photochemicals are present in *Stemodia viscosa*, phenolic compounds only effectively contributed to the antibacterial activity. So the total phenolics, flavonoids and tannin content of selected plants were estimated and illustrated in the **table 3 and figure 1**.

Aqueous extract of *S. viscosa* leaves possess more amount of total phenolic content (1.674±0.012 g $100g^{-}1$) than stem (1.159±0.011 g $100g^{-}1$).

Total flavonoid content of aqueous extract of S. viscosa was presented in the Figure 4. It was evident that the total flavonoid content of leaves (0.676±0.0009 g $100g^{-}1$) is more than that of stem flavonoid content (0.665±0.002 g $100g^{-}1$).

The aqueous extract of leaves contain more tannin content (5.482±0.009 g $100g^{-}1$) than the stem (3.033±0.014 g $100g^{-}1$).

Table 1. Qualitative phytochemicals analysis of *Stemodia viscosa* Roxb. stem

Tests	Petroleum ether	Acetone	Chloroform	Methanol	Water
Alkaloids	-	-	-	-	-
Amino acids	-	-	-	-	-
Proteins	-	-	-	+	+
Carbohydrates	+	-	+	-	+
Glycosides	-	-	-	-	-
Cardiac glycosides	+	-	+	-	-
Coumarins	-	+	+	-	+
Flavonoids	+	+	+	+	-
Tannins	-	-	-	+	+
Phlobatannins	-	-	-	-	-
Quinones	+	+	+	-	+
Anthraquinones	-	-	-	-	-
Saponins	-	-	-	-	-
Steroids	+	+	+	+	-
Terpenoids	-	-	-	-	+
Diterpenes	-	+	-	+	-
Vitamin C	-	+	+	+	+

Table 2. Qualitative phytochemicals analysis of *Stemodia viscosa* Roxb. leaves

Tests	Petroleum ether	Acetone	Chloroform	Methanol	Water
Alkaloids	-	-	-	-	-
Amino acids	-	-	-	-	-
Proteins	-	+	-	-	+
Carbohydrates	+	-	+	-	+
Glycosides	-	-	-	-	-
Cardiac glycosides	-	+	-	-	-
Coumarins	-	+	+	+	+
Flavonoids	+	+	-	+	-
Tannins	-	+	-	+	+
Phlobatannins	-	+	-	-	-
Quinones	-	+	-	-	+
Anthraquinones	-	-	-	-	-
Saponins	-	-	-	-	-
Steroids	+	-	+	-	-
Terpenoids	+	-	+	+	+
Diterpenes	-	+	-	+	-
Vitamin C	+	+	+	+	+

Table 3. Total phenolics, flavonoid and tannin content of stem and leaves of *Stemodia viscosa* Roxb.

S. No	Sample	Total phenols (g 100g-1)	Total flavonoids (g 100g-1)	Total tannins (g 100g-1)
1.	Stem	1.159±0.011	0.665±0.002	3.033±0.014
2.	Leaves	1.674±0.012	0.676±0.0009	5.482±0.009

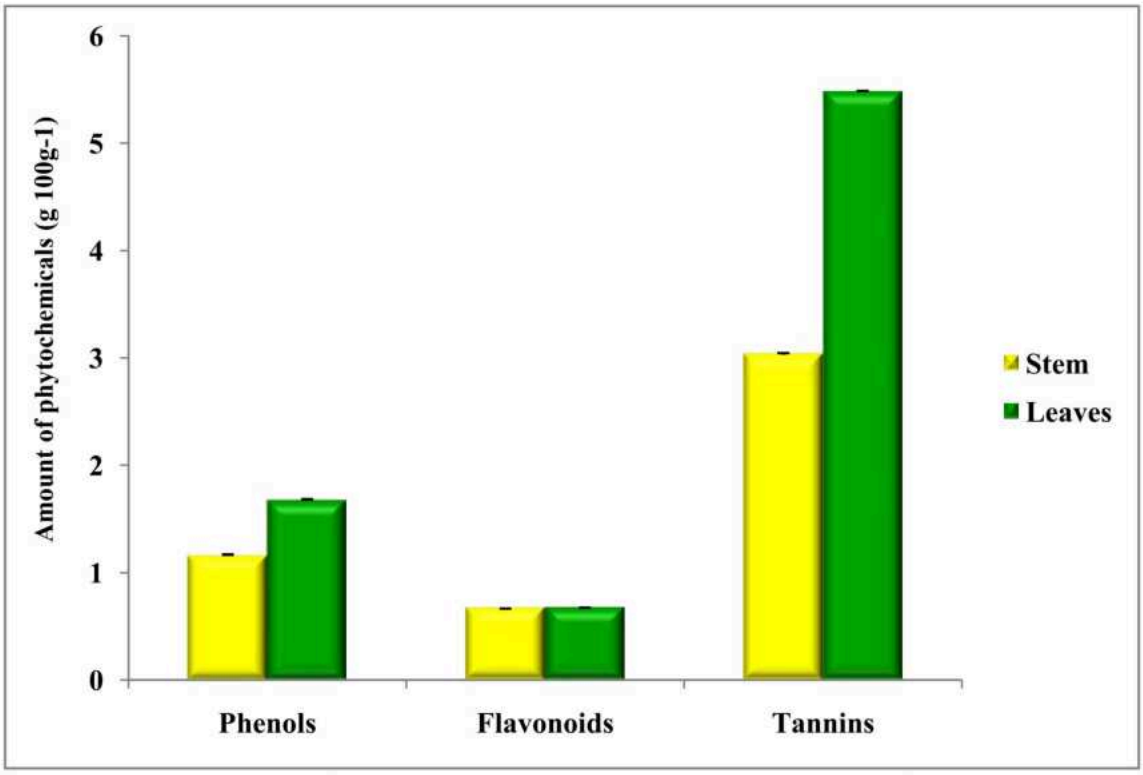

Figure 1: Total phenolics, flavonoid and tannin content of stem and leaves of *Stemodia viscosa* Roxb.

On the whole the aqueous extract of *S. viscosa* leaves contained more amounts of total phenolics, flavonoids and tannin content than the stem aqueous extract.

FTIR analysis

The FTIR analysis was carried out to predict the functional groups present in the methanolic extracts of stem and leaves of *Stemodia viscosa*. The results of FTIR spectral studies were presented in **Table 4 & 5** and **Figure 2 & 3.**

From the spectral data, presence of C-Br, C-C, =C-H, C-Cl, C-F, C-N, C=C, C=O, C-H, O-H and N-H were identified. These bonding are responsible for the presence of alkyl halide, alkyne, alkane, halo compound, conjugated alkene, amine, cyclic anhydride, ester and carbonyl compound in the stem of *S. viscosa*.

The spectral data revealed the presence of C-I, C-Br, C-Cl, =C-H, C-Cl, C-O, C-N, O-H, C=O, N-H and O=C=O in the leaves of *S. viscosa*. These bonding are responsible for the presence of alkyl halide, alkene, halo compound, ester, alcohol, amine, phenol, alkane, aromatic compounds, carbonyl compounds and carbon dioxide.

***In vitro* proliferation**

Nodal explants of *S. viscosa* were cultured on MS basal medium supplemented with different concentrations of selected cytokinins BAP and KIN were ranging from 2 to 8µM/L for shoot multiplication. Multiple shoots were initiated within 10 days of inoculation. Maximum number of shoots was observed in 20 to 30 days. The *in vitro* raised 2 cm and above plants were excised, which is subcultured on MS basal medium supplemented with different concentrations of selected auxins and the IAA and NAA concentration were ranging from 2 to 8µM/L for root proliferation. The data in respect of shoot induction frequency, number of shoots and length of shoots on different

Table 4. FTIR analysis of stem extracts of *Stemodia viscosa* Roxb.

S.No	Peak Value (cm^{-1})	Functional Group
1.	517.85	Alkyl Halide C-Br
2.	601.75	Alkyl Halide C-C
3.	654.79	Alkyne
4.	676.97	Alkene =C-H
5.	753.15	Alkyl Halide C-Cl
6.	833.19	Halo compound C-Cl
7.	894.91	Symmetric CH stretching
8.	1029.92	Alkyl Halide C-F
9.	1117.67	Carboxylic acids
10.	1161.07	Isopropyl
11.	1247.86	Alkyl Halide C-F/ Amine C-N/ Ether C-O
12.	1319.22	Alkyl Halide C-F/ Amine C-N
13.	1401.19	Alkane –C-H
14.	1434.94	Alkane –C-H
15.	1511.12	Tetrazoles C-H
16.	1548.73	Cis amides (band absent)
17.	1605.63	Conjugated alkene C=C
18.	1745.46	Carbonyl C=O/ Ester C=O/ Cyclic Anhydride
19.	2308.63	Amino acids (in hydrochlorides)
20.	2349.14	Sulphinic acid ($-SO_2H$)
21.	2379.03	Sulphinic acid ($-SO_2H$)
22.	2926.78	Alkane C-H/ Carbonyl O-H
23.	3332.76	Alcohol O-H (H-bonded)/ Amine N-H

Table 5. FTIR analysis of leaves extracts of *Stemodia viscosa* Roxb.

S.No	Peak Value (cm^{-1})	Functional Group
1.	468.67	Alkyl Halide C-I
2.	517.85	Alkyl Halide C-Br
3.	648.04	Alkyl Halide C-Cl
4.	677.93	Alkene =C-H
5.	774.37	Halo compound C-Cl
6.	914.2	C-H out-of-plane bending
7.	1010.63	Ester C-O
8.	1114.78	Alcohol C-O/ Ether C-O
9.	1170.71	Amine C-N/ Ether C-O
10.	1236.29	Amine C-N/ Ether C-O
11.	1338.51	Phenol O-H
12.	1370.33	Alkane –C-H/ Phenol O-H
13.	1453.26	Aromatic C=O
14.	1511.12	Tetrazoles C-H
15.	1548.73	Cis amides (band absent)
16.	1604.66	Amine N-H
17.	1657.7	Aromatic compound C-H
18.	1679.88	Aromatic compound C-H
19.	1748.35	Carbonyl C=O
20.	2309.6	Carbon dioxide O=C=O
21.	2872.77	Azoles N-H
22.	2933.53	Azoles N-H
23.	3282.62	Alcohol O-H (H-bonded)/ Amine N-H
24.	3628.82	Alcohol O-H (free)

concentrations of each hormone on nodal explants were presented in **table 6 & 7** and **plate 2**.

Shoot proliferation from nodal explants

The nodal explants were initially grown on MS basal medium supplemented with BAP or KIN alone in different concentrations ranging from 2 to 8µM/L. Of these two cytokinins, KIN was found to induce more number of shoots when compared to BAP. Among different concentrations of KIN, the MS basal medium supplemented with 8µM/L KIN showed the highest number of shoots (14.33±2.29) per explant and mean shoot length is 1.84 cm.

Maximum numbers of shoots were produced from all the concentrations of KIN than BAP. The highest frequency (100%) of shoot induction and maximum number of shoot (14.33±2.29) was observed on 8µM/L KIN with shoot length of 1.84±0.25 cm. Next to 8µM/L KIN, BAP 8µM/L and KIN 4µM/L showed high frequency of shoot induction with maximum number of shoot (9.63±1.41 and 9.5±1.77 respectively) and mean shoot length (1.56±0.13cm and 1.62±0.24cm respectively).

Root initiation from nodal explants

The *in vitro* raised plants, which grew upto 2 cm and above were excised from culture tubes and subcultured on the MS basal medium with different concentrations of auxins (IAA or NAA ranging from 2 to 8 µM/L). Of these two auxins, IAA was found to induce more number of roots when compared to NAA. Among different concentrations of IAA, the MS basal medium supplemented with 6µM/L IAA showed the highest number of roots (4.83± 0.91) per plant and mean root length is 2.83± 1.36 cm.

Table 6. Effect of different concentrations of cytokinins on shoot induction from the nodal explants of *Stemodia viscosa* Roxb.

BAP	KIN	Percentage of response	Number of shoots	Shoots length
2µM	-	85	5.86±0.89	1.43±0.43
4 µM	-	70	4.17±0.75	0.77±0.11
6 µM	-	70	4.0±0.89	0.83±0.08
8 µM	-	90	9.63±1.41	1.56±0.13
-	2 µM	85	8±0.82	1.25±0.80
-	4 µM	90	9.5±1.77	1.64±0.24
-	6 µM	80	6.86±1.35	1.0±0.41
-	8 µM	100	14.33±2.29	1.84±0.25

Table 7. Effect of different concentrations of auxins on root induction from the nodal explants of *Stemodia viscosa* Roxb.

IAA	NAA	Percentage of response	Number of roots	Roots length
2µM	-	80	1.03 ± 0.99	1.26 ± 1.11
4 µM	-	90	3.06 ± 1.33	2.56 ± 1.56
6 µM	-	100	4.83 ± 0.91	2.83 ± 1.36
8 µM	-	90	3.36 ± 1.24	2.50 ± 1.27
-	2 µM	80	1.41 ± 1.03	1.65 ± 0.70
-	4 µM	85	2.13 ± 0.97	2.20 ± 0.84
-	6 µM	95	4.10 ± 1.15	2.80 ± 0.47
-	8 µM	90	3.20 ± 1.29	2.13 ± 0.68

Higher numbers of roots were produced in all the concentrations of IAA than NAA. The highest frequency (100%) of root induction and maximum number of roots (4.83 ± 0.91) were observed on 6µM/L IAA with root length of 2.83 ± 1.36 cm. Next to 6µM/L IAA, NAA 6µM/L induced high frequency of root proliferation (95%) with maximum number roots (4.10 ± 1.15) and mean root length (2.80 ± 0.47 cm).

Antibacterial activity

The antibacterial activities were carried out by disc diffusion assay using acetone, methanol and water extracts of both stem and leaves of *Stemodia viscosa*. The zone of inhibition of various extract values were compared to the standard antibiotic values. All extracts of stem and leaves of *S. viscosa* showed antibacterial activity against one gram positive bacterium (*Bacillus substilis*) and four gram negative bacterial strains (*Escherichia coli, Klebsiella pneumoniae, Salmonella typhi* and *Serratia marcescens*). The results were presented in the **figure 4 & 5 and plate 3 & 4**.

The acetone extracts of *S. viscosa* stem showed maximum inhibitory activity (18 mm) against *Bacillus substilis* than methanol and aqueous extracts. Methanol extracts of *S. viscosa* stem (19 mm) possessed higher inhibitory activity against *E. coli* which is followed by acetone extracts (18 mm). When compared other solvents acetone extract (7 mm) of stem showed maximum inhibitory activity against *K. pneumoniae.* This is higher than the standard streptomycin also. Acetone and methanolic extracts showed maximum inhibitory activity (8 mm) against *S. typhi* than water extract and streptomycin. Maximum inhibitory activity against *S. marcescens* was observed in methanolic extract (12 mm) of *S. viscosa* stem than other solvents.

Methanolic extract of S. viscosa leaves inhibited growth of *B. substilis* and *E. coli* to the maximum (8 mm and 16 mm respectively) than other solvents and streptomycin.

Aqueous extract of S. viscosa leaves showed maximum inhibitory activity against *K. pneumonia* and *S. typhi* (16 mm and 7 mm respectively). Methanol extracts of *S. viscosa* leaves (19 mm) possessed higher inhibitory activity against *S. marcescens* when compares to other solvents and streptomycin.

In spite of increasing use of herbal medicines, there is still a major need of research data in the field of medicinal plant. Henceforth, the current study has been carried out to discover the efficiency of *Stemodia viscosa* against bacterial infection and their ability to proliferate under *in vitro* condition.

Qualitative analysis

Phytochemicals are responsible for medicinal activity of plants. These are non-nutritive chemicals that protect human from various diseases. Phytochemicals are basically divided into two groups that are primary and secondary metabolites based on the function in plant metabolism. Primary metabolites comprise of common carbohydrates, amino acids, proteins and chlorophylls while secondary metabolites consist of alkaloids, saponins, steroids, flavonoids, tannins and so on. (Jigna and Sumitra 2007; Kumar *et al.,* 2009). Phytochemical constituents are the basic source for the establishment of several pharmaceutical industries. These constituents are playing a significant role in the identification of crude drugs (Savithramma *et al.,* 2011).

The current study reavealed that stem and leaves of *Stemodia viscosa* possess different phytochemicals as mentioned in the result. So, it can be effectively used for the treatment of various diseases.

Quantitative analysis

Phenolic compounds are widely distributed in all plants have been reported to exert multiple biological effects (Canini *et al.,* 2007). The phenolic compounds are one of the largest and most ubiquitous group of plant metabolites. A number of studies have focused on the biological properties such as antiapoptosis, antiaging, anticarcinogen, antiinflammation, antiantherosclerosis, cardiovascular protection and improvement of the

endothelical function as well as inhibition of andiogenesis and cell proliferation activity (Han *et al.*, 2007). Phenolic compounds have been extensively used in disinfections (Okwu 2001).

Flavonoids are a group of polyphenolic compounds which influence the radical scavenging, inhibition of hydrolytic and oxidative enzymes and also act as antiinflammatory agent (Frankel 1995). The flavonoids show antioxidant activity and their effects on human nutrition and health is considerable. The mechanisms of action of flavonoids are through scavenging or chelating process (Kessler *et al.,* 2003 and Cook and Samman 1996).

Tannins are complex moieties produced by majority of plants as protective substances; they have wide pharmacological activities and have been used since past as tanning agents. Tannins contribute property of astringency i.e. faster the healing of wounds and inflamed mucous membrane and have received considerable attention in the fields of nutrition, health and medicine largely due to their physiological activity such as antioxidant, antimicrobial and antiinflammatory properties (Killedar and More 2010).

Keeping these in mind, total phenolics, flavonoids and tannin content were quantitatively estimated in the present study that revealed that the higher amount of total phenolics, flavonoids and tannin content were observed in leaf than stem.

Bystricka *et al*. (2010) reported that concentration and dynamics of the polyphenol synthesis in plant organs depends on the plant species, type of organs and growth stage. Arash *et al.* (2010) comparatively analyzed the concentration of phenolic compounds in different plant parts of *Andrographis paniculata*. This study revealed that higher concentration of phenolic compounds was observed in leaves extracts compared to the stem extracts, which is the same in relation to our results.

FTIR analysis

Fourier transform infrared spectroscopy is a physicochemical analytical technique which provides a clear picture of the metabolic composition of leaves at a given time. FTIR is employed to elucidate the structure of unknown composition and the intensity of absorption spectra associated with molecular composition or content of respective chemical functional groups (Bobby *et al.,* 2012). It is possible to detect the minor changes in the primary and secondary metabolites in leaves by observing the IR spectra (Surewicz *et al.,* 1993). FTIR has been used to identify the complicated structures of plant secondary metabolites and in the characterization of bacterial, fungal and plant species (Hori and Sugiyama 2003; Yang and Yen 2002).

FTIR spectrum of stem and leaves of *S. viscosa* divulged the presence of alkyl halide, alkyne, alkane, halo compound, conjugated alkene, amine, phenol, cyclic anhydride, ester, aromatic compounds and carbonyl compound. Thus, the study exposed that the *S. viscosa* contain a considerable amount of secondary metabolites and it may considered in future to be used in human disease management.

***In vitro* proliferation**

In micropropagation technique, shoots are directly induced from the nodal explant with axillary buds where meristematic tissue is present. This technique is primarily used to produce pathogen free plantlets. Nowadays, it is widely used to get a mass propagation within a short period. Since the meristematic region is the very active site, the axillary buds are readily proliferated. The efficiency of shoot multiplication depends on plant growth regulators and types of explants (Velayutham *et al.,* 2005; Prakash *et al.,* 1994; Hu and Wang 1983).

In the present study nodal explants with axillary bud were taken as explants source. The nodal explants showed active site of positive morphogenetic response and readily developed multiple shoots. The propagation rate and morphogenetic response significantly varied to a greater extent according to the explant type. Shoot tips have always been preferred for *in vitro* studies because they can be handled easily and restore their regeneration potential over other explants. Some earlier findings showed that more number of shoots were produced from the nodal explants (Reddy *et al.,* 1998; Komalavalli and Rao 2000; Chang and Criley 1993 and Padmapriya *et al.,* 2011).

In many plants, multiple shoots were obtained from the shoot tips or axillary buds by administering BAP or KIN (Singh *et al.,* 2011; Krishnan *et al.,* 1995; Kannan and Jasrai 1996; Abubacker and Alagumanian 1999; Reddy *et al.,* 1998; Alagumanian *et al.,* 2014; Handique and Bora 1999; Alagumanian *et al.,* 2014; Alagumanian *et al.,* 2003 and Arumugam *et al.,* 1997).

The process of root formation is influenced by a number of internal and external factors. Among the internal factors, the most important role is ascribed to phytoharmones, especially the auxins. It is generally accepted that auxins have a certain role in the root initiation. Auxins control growth and development in plants, including lateral root initiation and root gravity response (Stefancic *et al.,* 2005). The effects of auxin group of hormones on rooting have been discussed in several studies. (Alvarez *et al.,* 1989; Stefancic *et al.,* 2005; Hussain and Khan 2004).

Antibacterial activity

The plants extracts, various organic fractions and essential oils were found to have significant antimicrobial activity and therefore can be used as a natural antimicrobial agent for the treatment of several infectious diseases caused by the studied

microorganism, which have developed resistance to antibiotics (Derwich *et al.*, 2011). The medical properties are not essentially limited to a single compound present in plant. But various phytocostituents combinely showed antimicrobial activity (Pasquale, 1984).

Various workers have shown that Gram positive bacteria are more susceptible towards plants extracts when compared to Gram negative bacteria (Yao and Moellering 1995 and Parekh and Chanda 2006). These differences may be attributed to the fact that the cell wall in Gram positive bacteria is of a single layer, whereas the Gram negative cell wall is multilayered (Zulfiker *et al.*, 2011). The presence of proteins or peptide may act directly on microorganisms on results in growth inhibition by distrupting cell membrane synthesis of essential enzyme. This may lead to inhibit the growth of pathogens taken into an account for the study (Devendra *et al.*, 2011).

Flavonoids, another constituent found in plants extracts and various organic fractions showed a broad range of biological properties like antimicrobial activity and antioxidant potential (Hodek *et al.*, 2002). Anthraquinones possessed antiinflammatory and bactericidal effects (Feroz *et al.*, 1993). Tannins were toxic to bacteria, fungi and viruses and inhibit their growth (Scalbert 1991). The earlier reports showed that growth of microorganism are inhibited very effectively by plants extracts due to the presence of phenolics and flavonoids (Mori *et al.*, 1987).

Hence, bactericidal effect of different extracts of stem and leaves of *S. viscosa* may be attributed to different phytochemicals like phenol, flavonoid, tannin etc. that present in them.

Thus, it is concluded that the investigated plant may be new source for antibacterial compound discovery for treating drug resistant human pathogens.

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Stemodia viscosa is one of the common aromatic weed present in the agricultural field. The plant materials were collected from the agricultural field of Kulayankarisal village, Thoothukudi. Since the volatile oil of this plant was already extracted and the wound healing activity have already been reported, the current study was intended to identify the phytochemicals present in *S. viscosa*; to quantify the phenol, flavonoids and tannins content; to proliferate the nodal explants under *in vitro* condition and to screen the antibacterial activity.

Phytochemicals are the intermediary components of the plant metabolism, which play a vital role to control many diseases and microorganisms. The qualitative analysis of *S. viscosa* revealed various phytochemicals and these components are useful to treat disease causing pathogens and ailments.

The quantitative analysis of present study exposed that both stem and leaves of S. viscosa contain more tannin content than total phenolics and flavonoids. The major activity of tannin is to heal wounds and it also acts on the inflammation, microorganisms and so on. Following to the tannin content, phenols placed as a major component. Phenolic components are widely used as disinfectant and it also used as a anticarcinogen, antiapoptosis, antiaging, etc.

The FTIR analysis has been used to identify the complicated structures of plant secondary metabolites (Hori and Sugiyama 2003). FTIR spectra showed the presence of the functional group in both stem and leaf extracts which have medicinal properties and can be used as antimicrobial, antiinflammation and antidiabetic agents.

In *in vitro* proliferation the nodal explants were cultured in the MS basal medium with different concentrations of KIN and BAP and the concentrations ranging from 2µM/L to 8µM/L for shoot induction. Of these two cytokines, KIN was found to develop in shoot multiplication and higher number of shoots from the nodal explants when compared to KIN. The highest frequency (100%) of shoot induction and maximum number of shoot (14.33±2.29) was observed on 8µM/L KIN with shoot length of 1.84±0.25 cm. Next to 8µM/L KIN, 8µM/L BAP and 4µM/L KIN showed high frequency (90%) of shoot induction maximum number shoots (9.63±1.41 and 9.5±1.77 respectively) and with the shoot length (1.56±0.13 and 1.62±0.24 respectively).

The initially grown nodal explants were subcultured on the MS basal medium supplemented with different concentrations of NAA and IAA and the concentrations ranging from 2µM/L to 8µM/L. The more number of root initiation was observed in IAA than NAA. Among different concentrations of IAA, the MS basal medium supplemented with 6µM/L IAA showed the highest number of roots (4.83± 0.91) per plant and mean root length is 2.83± 1.36 cm.

Hence this study suggests that, MS basal medium supplemented with 8µM/L of KIN and 8µM/L of IAA is more suitable for *in vitro* proliferation of *S. viscosa.*

The antibacterial activity of *S. viscosa* was tested by disc diffusion method. The dried plant samples were mixed in different solvents namely acetone, methanol and water. All extracts of stem and leaves of *S. viscosa* showed antibacterial activity against the human pathogenic bacterial strains namely *Bacillus substilis, Escherichia coli, Klebsiella pneumoniae, Salmonella typhi and Serratia marcescens.* In future, it could be targeted to prepare silver nanoparticles impregnated antibacterial drug.

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PHYTOCHEMICAL SCREENING AND ANTIBACTERIAL ACTIVITY OF GARDENIA GUMMIFERA (L.F) BAILL.

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CERTIFICATE

This is to certify that this dissertation entitled, **"Phytochemical Screening and Antibacterial Activity of *Gardenia gummifera* (l.f) Baill."** submitted by **M. Mixlin nithya Reg.No. 18APBO06** to ST.MARY'S COLLEGE (Autonomous), THOOTHUKUDI in partial fulfillment for the award of the degree of **"Master of Science in Botany"** is done by her under my supervision. It is further certified that this dissertation or any part of this has not been submitted elsewhere for any other degree.

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I do here by declare that this dissertation entitled "**Phytochemical Screening and Antibacterial Activity of *Gardenia gummifera* (l.f) Baill.**" Submitted by me in partial fulfilment for the award of the degree of **'Master of Science in Botany'**, in the result of my original and independent work carried out under the guidance of **M. Glory M.sc., M.Phil., Ph.D.,** Associate Professor, Department of Botany, St. Mary's College (Autonomous), Thoothukudi and it has not been submitted elsewhere for the award of any other degree.

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INTRODUCTION

INTRODUCTION

"When obscurities and legends are removed in the light of modern methods, the treatment with plant samples so dear to our ancestors are still capable of rendering their services".

-Henri LeclercPrecise De Phytotherapie

Plants are the basic source of knowledge of modern medicine. The basic molecular and active structures for synthetic fields are provided by rich natural resources. Plants alone have the capacity to synthesize their own food. The simple sugar formed as a result of photosynthesis serve as the building units of polysaccharides and the aminoacids as the building units of proteins. The photosynthetic products are essential for the growth and development of all living organisms and plants synthesis a further group of compounds called secondary metabolites. Though secondary metabolites are non-essential to life, they play an important role in the growth and development of the plants. Plants produce a series of secondary metabolites and store them in various organs like leaf, root, stem, flower, fruit, seeds etc. for immediate and future use. The secondary metabolites like tannins, saponins, flavonoids and alkaloids have more therapeutic values.

Phytochemistry is concerned with the enormous variety of organic substances that are elaborated and accumulated by plants. For a long period of time, plants have been a valuable source of natural products for maintaining human health, especially in the last decade, with more intensive studies for natural therapies. The use of plant extracts and phytochemicals, both with known and

unknown antimicrobial properties, can be of great significance in therapeutic treatments. The art of healing using plant extracts or their active principles as medicine began thousands of years ago. Now-a-days, there are large numbers of pharmaceutical industries throughout the world dealing with modern medicine and synthetic products, which may cause side effects. Herbal remedy is one of the trend in the field of biology today. In the last few years a number of studies have been conducted in different countries to prove such efficiency. Many plants have been used because of their antimicrobial traits, which are due to compounds synthesized as secondary metabolites by the plants for their defense purpose.

Antimicrobial drugs are designed to kill, or to prevent the growth of microorganisms such as bacteria, fungi and viruses. The history of antimicrobials begins with the observations of Pausteur and Joubert, who discovered that one type of bacteria could prevent the growth of another. They did not know at that time, the reason for why one type of bacterium failed to grow as the other bacteria was producing an antibiotic substance. Technically that kills, or prevents the growth of another microorganism. Of course, in today's common usage, the term antibiotic is used to refer almost any drug that cures a bacterial infection. The term antibiotic originally described only those formulations derived from living organisms but if now applied also to synthetic antimicrobials, mostly the antibacterial activities of various plant parts such as root, stem, leaf, flowers have been already carried out in many plants.

Drug discovery from medicinal plants used in different Indian medicinal system is a hot spot of research. A number of drugs were obtained from the plant

sources and several others have discovered by using natural substance as lead. National/ International research has discovered a number of drugs from plants which have been used in Indian traditional medicine since ancient time, like, vasicine from *Adhatoda vasica*, tylophorine from *Tylophora indica*, glycyrrhizin from Glycrrhiza glabra, curcumin from curcuma longa, aloin from *Aloe vera* and etc.(Sen and Charkraborty 2015). With all these challenges in drug discovery, today there is a revival of interest in plant drugs due to the current widespread belief that green medicine is safe and dependable than costly synthetic drugs (Parekh and Chanda,2006).There is a growing interest in correlating phytocompounds of a plant with its pharmacological activity (Vaidya, 1994). Scientists events started correlating botanical properties of plants with their pharmacological activity (Rawal *et al.*, 1999). In future, more co- ordinate multidimentional research aimed at correlating botanical and phytochemical properties to specific pharmacological activity is expected (Dahanukar *et al.*, 2000)

In the 21st century pollution, unhealthy life, environmental toxin increases the risk of diseases. The side effects, overuse/ misuses of allopathic drugs are also a major concern. In 2013, WHO developed and launched 'WHO Traditional Medicine Strategy 2014- 2023' and emphasized to integrate traditional and complementary medicine to promote universal health care and to ensure the quality, safety and effectiveness of such medicine (WHO 2013). Therefore, the world is looking for cost effective, easily available, better physiological compactable traditional systems of medicine and holistic approach to avert such problems and provide the basic health care to all.

SCOPE AND OBJECTIVE

SCOPE AND OBJECTIVES

The use of plants and plant products as medicines could be traced as far back as the beginning of human civilization. Medicinal plants are a source of great economic value all over the world. Nature has bestowed on us a very rich botanical wealth and a large number of diverse types of plants grow in different parts of the country. Herbal medicine is still the mainstay of about 75-80% of the whole population and the major part of traditional therapy involves the use of plant extract and their active constituents. Following the advent of modern medicine, herbal medicine suffered a setback, but during last two or three decades, advances in phytochemistry and in identification of plant compounds, effective against certain diseases have renewed the interest in herbal medicines. Nowadays multiple drug resistance has developed due to the indiscriminate use of commercial antibacterial drug commonly used in the treatment of various diseases. Mountains and hills are one of the rich source herbal wealth and are the gift of our country. India's biodiversity is rich and recognised as one of the world's top 12 mega diversity nation. About 45,000 plant species in India which are abundant in Eastern Himalayas, western Ghats and Easten Ghats. Western Ghats of our country bestowed on us with megadiversity . The plants were collected from the Maruthuval Malai, as it is the part of the Western Ghats.It extends for about a km, reaching a height of 800 feet at the highest point. It is about 11km. from Nagercoil. Maruthuva Malai is a holy hilly place that is entirely filled with several life-saving medicinal plants with medicine mixed free air, located just 7 km away from

Kanyakumari. Originally, the hills had a thick forest of vegetation with leopards, Cheetahs, Pythons, and poisonous snakes. More than 1000 medicinal herbs are there for curing many diseases.

Only a small percentage of plant species has been investigated phytochemically and even a smaller percentage has been properly studied in terms of their medicinal potential .Gardenia leaf has been good source of traditional medicine. Yet this species not explored. So fulfill this gap, the present work is undertaken with a view to analyse the phytochemical and antibacterial characters of the species of *G. gummifera* with greater medicinal potential.

Objectives

Keeping this in mind, the present investigation has been planned with the following objectives;

- Collection of *Gardenia gummifera* from Maruthuvazh Malai.

- Elucidation of macroscopic and microscopic characteristics of leaves of *Gardenia gummifera*.
- Estimating the secondary metabolites using standard method.
- Screening of biochemical constituents through standard tests.
- Finding the antibacterial activity from leaf extract .
- Analyzing the HPTLC profiles of leaf extract.
- Exploring the bioactive compounds by FT-IR spectroscopy and GC-MS analysis.

REVIEW OF LITERATURE

Prakash *et al.*, (2015) investigated phytochemical screening and antioxidant activity of *Adina cordifolia* leaf. The plant extracts were screened for presence of flavonoids, carbohydrate, alkaloid, saponin, phenol, tannins, phlobatannins, terpenoids, cardiac glycosides. Total flavonoid content, phenols content was estimated. Antioxidant activity was determined using nitric oxide scavenging assay, DPPH assay, hydrogen peroxide scavenging and ferric reducing methods, also MIC was calculated against a set of bacteria (*S. aureus, B. subtilis, E. coli, K. pneumonia*).

Ramalingum and Mahomoodally (2014) assessd the potential of *Vangueria madagascariensis* J. F. Gmelin (Rubiaceae) on α-amylase, α-glucosidase, glucose movement, and antimicrobial activity. The antioxidant properties were determined by measuring the FRAP, iron chelating activity, and abilities to scavenge DPPH, HOCl, ·OH, and NO radicals. Leaf decoction, leaf methanol, and unripe fruit methanol extracts were observed to significantly inhibit α-amylase. Active extracts against α-glucosidase were unripe fruit methanol, unripe fruit decoction, leaf decoction, and ripe fruit methanol, which were significantly lower than acarbose. Kinetic studies revealed a mixed noncompetitive type of inhibition. Leafmethanolic extract was active against *S. aureus* and *E. coli*. Total phenolic content showed a strong significant positive correlation (r = 0.88) with FRAP. Methanolic leaf extract showed a more efficient NO scavenging potential and was significantly lower than ascorbic acid. Concerning ·OH-mediated DNA degradation, only the methanol extracts of leaf, unripe fruit, and ripe fruit had IC50

values which were significantly lower than α-tocopherol. Given the dearth of information on the biologic propensities ofVM, this study has established valuable primary informationwhich has opened newperspectives for further pharmacological research.

Halilu *et al.*, (2012) studied antimicrobial and preliminary phytochemical studies of methanol extract of root bark of *Crossopteryx febrifuga* (Rubiaceae). It is one of the useful plants used in Hausa traditional medicine in North Western Nigeria. The phytochemical studies of the root bark of the plant was carried out using standard procedure. The was found tocontain: steroids, flavonoids, terpenoids, anthraquinones, cardiac glycosides, tannins, alkaloids and saponnins. The antimicrobial activity screening was carried out using both bacterial and fungal strains. The bacterial strains include: *Pseudomonas aeruginosa, Staphylococcus aureus, Eschericia coli*. The fungal strains Include*: Aspergillus fumigatus, Candida albicans, Aspergillus flavus* and *Aspergillus niger*. In general, the extract showed considerable activity on the bacterial species. It inhibited the growth of both gram positive and gram negative microorganism with zones of inhibition ranging from 7- 23 mm at concentrations of 50 µg/ml, 100 µg/ml and 200 µg/ml. The plant extract did not show significant activity on fungal strains. It inhibited the growth of *Aspergillus fumigatus* at 400 µg/ml and 500 µg/ml which produced zones of inhibition of 8 mm and 12 mm respectively at the stated concentration. It can be concluded that the activity showed by the methanolic extract of the plant is as a result of the phytochemicals present in the plant.

Zayed *et al.*, (2014), studied GC-MS analysis of phytochemical constituents in leaf extracts of *Neolamarckia cadamba*. This study was conducted to identify the phytochemical constituents of *N. cadamba* leaf extracts using gas chromatography mass spectrometry (GC-MS). Solvents with increasing polarities viz. hexane, petroleum ether, chloroform, ethyl acetate and methanol were used in this study. The solvent extracts were analyzed using GC-MS and the mass spectra of the compounds found in the respective extract were matched with the National Institute of Standards and Technology (NIST) library. A total of 26 compounds were identified and the major chemical constituents were n-hexadecanoic acid (44.88%), hexadecanoic acid ethyl ester (17.96%) and octadecanoic acid ethyl ester (11.71%). Some of the identified compounds have been reported to possess various biological activities such as antioxidant, antimicrobial, anesthetic, antiseptic, antidiabetic, hypocholesterolemic and etc. The results thus concluded that *N. cadamba* leaves possess various potent bioactive compounds and is recommended as a plant of phytopharmaceutical importance.

Kekuda *et al.*, (2017) studied antimicrobial, antiradical and insecticidal activity of *Gardenia gummifera* L. F. The leaf and fruit extracts inhibited all test bacteria. Marked antibacterial activity was displayed by fruit extract when compared to leaf extract. *S. Epidermidis* and *E. coli* were inhibited to highest and least extent by both extracts respectively. Fruit extract was found to exhibit higher antifungal effect when compared to leaf extract. Leaf extract and fruit extract exhibited highest inhibitory activity against *A. Niger* and *A. Flavus* respectively. Leaf and fruit extracts scavenged DPPH radical's dose dependently with an IC_{50}

value of 49.01µg/ml and 2.53µg/ml respectively. The scavenging of ABTS by leaf and fruit extracts was dose dependent and the IC_{50} value for leaf and fruit extract was 2.58µg/ml and 2.31µg/ml respectively. Fruit extract was shown to exhibit marked antiradical activity when compared to leaf extract. Leaf and fruit extracts exhibited dose dependent insecticidal activity in terms of larvicidal and pupicidal activity and the susceptibility of larvae and pupae to extracts was in the order II instar larvae>IV instar larvae>pupae. Fruit extract displayed marked insecticidal potential when compared to leaf extract. Overall, fruit extract of *G. Gummifera* exhibited marked antimicrobial, antiradical and insecticidal activity when compared to leaf extract. The plant can be used for developing agents/formulations effective against infectious microorganisms, oxidative stress and insect vectors that transmit dreadful diseases. The observed bioactivities could be ascribed to the presence of active principles which are to be isolated and characterized.

Sabbani *et al.*, (2016) evaluated a hepatoprotective activity with different fractions of *Gardenia gummifera* Linn. on paracetamol induced liver damage in rats. And the GGME fractionated based on polarity of solvents with toluene, ethanol, 2-butanone, n-butanol and petroleum ether. The substantially elevated serum enzymatic levels of Aspartate Aminotransferas (AST), Alanine Transaminase (ALT), Alkaline Phosphate (ALP) and total bilirubin were restored towards normalization significantly by the GGME in a dose dependent manner in paracetamol induced liver damage. The biochemical observations were supplemented with histopathological examination liver sections high protection against paracetamol induced hepatoxicity. Further investigation continued with

quantification of histopathological changes also supported the dose dependent protective effects of MEGG. MEGG significantly ($p \leq 0.05$) protected the above mentioned parameters to fall from the normal levels. LCMS analysis of MEGG revealed the presence of cardioprotective constituents such as erythrodiol, lupeol, epicatechin, β- sitosterol, asiatic acid, myricetin, oleanolic aldehyde, vernolic acid, chlorogenic acid and dicaffeoylquinic acid. Their results suggest that MEGG affords a dose dependant cardio protection against isoproterenol-induced myocardial infarction.

Vindhya *et al*., (2014) studied the preliminary phytochemical screening of *Gardenia latifolia* Ait. and *Gardenia gummifera* Linn. belonging to the family Rubiaceae by using successive extraction from petroleum ether, ethyl acetate, methanol, ethanol and aqueous. The extracts showed the presence of various phytocompounds like glycosides, phytosterols, fats and oils, phenols, resins, tannins, flavonoids, tannins and terpenoids.

Wong, *et al*., (2014) studied the phytochemical screening and antimicrobial potentials of *Borreria sps* (Rubiaceae). Successive hexane, acetone, ethanol and methanolic whole plant extracts of the *Borreria sps* were investigated for phytochemical screening and assessed for antimicrobial activity. Phytochemical analysis of *Borreria sps* extracts revealed the presence of phenolics, flavonoids and tannins. Among them, Borreria laevicaulis hexane extracts were found to be most effective showing the largest zone of inhibition against *Staphylococcus aureus* (22.15 mm) and *Candida albicans* (25.65 mm). Further studies indicated that the minimum inhibitory concentration of *B. laevicaulis* hexane extracts was

found to be 62.5 lg/ml against *S. aureus* and 250 lg/ml against *C. albicans* and the zone of inhibitions was significantly higher than nystatin (positive control). They provided new insights of the *B. laevicaulis* as a potential candidate for antimicrobial drug discovery using in vitro studies that might be useful to treat human infectious diseases and antibiotic resistant pathogens.

Kakad *et al.*, (2015) studied phytochemical, antibacterial and antifungal activities of leaf extract of *Morinda citrifolia* L. The antibacterial activity was tested against gram positive bacteria *Bacillus subtilis, Escherichia coli, Pseudomonas fluroscence* and *Salmonella typhi* using disc diffusion method. Methanol extract showed highest zone of inhibition in *B. subtilis*. The antifungal activity was tested against *Aspergillus niger, Candida albicans* and *Daedalea flavida*. Methanol extract showed highest zone of inhibition in *A. niger*. The leaf extract noticed phytochemicals such as tannin, phenol, alkaloid, flavonoids, glycosides, steroids and terpenoids. Both the bacterial and fungal strains were exhibited significant inhibition. Phenol and anthraquinone activity was also performed.

Mangalanayaki and Sivaneshwari *et al.*, (2016) investigated phytochemical screening and antioxidant activity of *Adina cordifolia* leaf. The plant extracts were screened for presence of alkaloids, Flavonoids, Phenols and in major proportion. Methanol extract was shown to be more effective against all the organisms followed by Ethylacetate and Hexane extracts. *Proteus vulgaris* (35mm) was found to be most sensitive organism followed by *Klebsiella pneumonia* (33mm) and *Staphylococcus aureus* (34mm). The present study concludes that the different

extracts of *M. tinctoria* leaves contain a broad spectrum of secondary metabolites and also exhibit antimicrobial activity against all the tested microorganisms.

Ngozi *et al.*, (2014) studied the preliminary phytochemical screening and nutritional compositions of *Morinda lucida* Benth and *Alstonia boonei* De Wild growing in Nsukka, Enugu state, south eastern Nigeria. The qualitative analyses of the plant parts showed the presence of all the tested phytochemicals in various levels ranging from trace (+) to very heavily present (++++). The quantitative estimates showed significant variations in their values in leaves, stems bark and roots in some phytochemical components. Results of the proximate also showed the contents of protein were 2.46 - 17.69 and 1.45-2.1; fats 0.49-1.88 and 0.84-1.4; carbohydrates 68.76- 81.63 and 17.81- 31.38; and ash 2.33-2.9 and 0.78- 2.75 in *M. lucida* and *A. Boonei* plant parts, respectively. Vitamins A, E and K and anti-nutritional factors, phytate and oxalate also varied in the leaves, stems bark and roots.

Abdullah *et al.*, (2012) investigated the huge diversity of Malaysian flora. The screening practice for phytochemical compound in them is essential to explore more natural sources to replace synthetic antibiotics, which generally have side effects such as hypersensitivity, immune-suppression and allergic reactions. Antibacterial activities have been detected in some of the Malaysian plants and most of the Malaysian medicinal plants have been screened for this property. However, so far no study has focus on Malaysian flowering plants yet. In this study, the extraction and determination of antibacterial property from 19 Malaysian flowering plants were conducted. The plants were extracted with

methanol, ethyl acetate, hexane and distilled water, individually at concentration of 0.1g mL-1. The extraction process condition was set to 300 rpm agitation for 10 h at room temperature. The crude extracts of each plant (5 mg/disc) were tested against *Bacillus subtilis* and *Escherichia coli* using agar disc diffusion assay method. The screening results showed that ethyl acetate extract of *Spathiphyllum cannifolium* ('peace lily') leaves possesses the highest antibacterial activity against *B. subtilis* with zone of inhibition of 25 mm. Most of the plant samples extracted with methanol and ethyl acetate have indicated positive activity toward *B. subtilis* growth. However, the hexane and distilled water extracts was ineffective to combat the *B. subtilis* growth. Unfortunately, all of the extracts were not active against *E. coli*. This study suggested that *S. cannifolium* is highly potential in antibacterial activity which can be further analyzed for the development of new antibiotic exclusively for gram positive bacteria.

The selection of crude plant extracts for screening programs has the potential of being more successful in initial steps than the sscreening oh pure compounds isolated from natural products discovered that active compounds include essential oils,flavonoids, triterpenoids and the other compounds of pfenolic nature or with free hydroxyl group which are classified as active antimicrobial compounds present in plants parts.Secondary metabolites of plants. i.e.,phenols,and related alcohol,tannin,flavonoid,glycosides and their derived glycenes,unshirated terpenes and alkaloids showed antibacterial activity (Mitschser, 1974 and Prabakaran *et al.*, 2005)

FT-IR analysis:

Bobby *et al.* (2012) undertaken FT-IR studies on the leaves of *Albizia lebbeck* benth. The results of *A. lebbeck* leaves FTIR analysis confirmed the presence of amide, alkynes, alkanes, carboxylic acids, alkenes, aromatics, aliphatic amines and alkyl halides compounds which shows major peaks at 3654.12, 3307.55, 2918.44, 2849.92, 1643.73, 1454.46, 1054.13 and 510.34 respectively. The dry ethanolic extracts of *A. lebbeck* leaves FTIR analysis results proved the presence of alcohols, phenols, alkanes, carboxylic acids, aromatics, ketones and alkyl halides compounds which shows major peaks at 3370.19, 2955.65, 2925.68, 2853.40, 1739.72, 1463.02 and 506.57 respectively.

Moses *et al.*, (2013) studied Fourier Transformer Infra-Red Spectrophotometer analysis of *Warburgia ugandensis* medicinal herb used for the treatment of diabetes, malaria and pneumonia in Kisii region, Southwest Kenya. Results of the FTIR Spectra of the hexane, dichloromethane, ethyl acetate and ethanol crude extracts revealed the presence of different functional groups as follows: OH stretching for hydroxyl (3460.2-3359.8 cm 1), C=O stretching for carbonyls (1751.2-1643.2 cm 1), C-O stretching for alcohols (1450.4 -1049.2 cm 1), carboxylic acid (1458.9-1242.1 cm 1), carboxylic anhydrides (1253.6-1049.2 cm 1), ethers (1242.1-1049.2 cm 1), esters (1234.4-1049.2 cm 1), C-N stretching for amines (1253.6-1049.2cm 1), N-H stretching for amines (3460.1-3359.8 cm 1), amides (3444.6-3359.8 cm 1), C=C stretching for aromatic (1643.2-1531.4 cm 1), C=N stretching for nitriles (2356.9-2252.7 cm 1), N=C stretching for isocyanides (2137.0 -2090.7cm 1), C-H stretching for alkyl (2981.7-2931.6 cm 1), C-H bending for alkyl (1377.1 cm 1), C-H bending for methyl (1458.1-1450.4 cm 1),

S=O stretching for sulphur derivatives 1384.8-1049.2 cm 1), N=O stretching for nitro compounds (1377.1-1330.8 cm 1), C-F stretching for organic halogens (1253.6-1049.2 cm 1), C-Cl stretching (736.8-621.0 cm 1), C-Br stretching (663.5 cm 1) and C-I stretching (663.5 cm 1). These findings indicate the presence of aldehydes, amines, amides, alcohols, phenols, aromatics, carboxylic acids and anhydride, esters and lactones, ethers, nitriles, isonitriles, nitro compounds, sulphur derivatives, quinones, organic halogen compounds and carbohydrates in *Warburgia ugandensis*. The medicinal value of the herb could be attributed to the presence of O-H, N-H, C-H, C=O, C-O, C-N, C=C, C=N, N=C, N=O and S=O bond stretching of the detected functional groups. The results confirm the presence of secondary plant metabolites *viz.*, alkaloids, saponins, tannins, flavonoids, steroids and terpenes, polyphenols and cardiac glycosides in the leaves of *Warburgia ugandensis*. The results confirm the presence of secondary plant metabolites *viz.*, alkaloids, saponins, tannins, flavonoids, steroids and terpenes, polyphenols and cardiac glycosides in the leaves of *Warburgia ugandensis*.

HTPLC analysis:

Patel *et al.*, (2012) studied the quantitative analysis through HPTLC, which revealed the presence of 2.74 % and 0.543% w/w of berberine and gallic acid in *Aloe vera* extract. HPTLC finger print scanned at wavelength 420nm for alcoholic extract of Pisonia aculeate leaf showed eleven polyvalent phytoconstituents and corresponding ascending order of RF values start from 0.03 to 0.93, in which highest concentration of the phytoconstituents was found to be 32.60% and with RF value 0.03 (Syed et al.,2013).

Devi *et al.*, (2013) reported that the HPTLC method applied to identify the plant of Ficus nervosa from other species and HPTLC fingerprint enables a particular plant to be identified and distinguished from closely related species. Karthikeyan *et al.*, (2013) examined *Leucas aspera* whole plant methanolic extract for their photochemical profile by high-performance thin layer chromatographic (HPTLC) method and reported two types of alkaloids, six types of flavonoids and two types of steroids.

HPTLC finger print of methanol leaf extract of *Azadirzchta indica* showed 16 peaks and the Rf values ranged in between 0.03 to 0.96. Similarly, ethyl acetate sub fraction confirmed 18 peaks and the RF value ranged in between 0.03 to 0.95 (Dalal *et al.*, 2015).

GC-MS:

Jayapriya and Shoba (2015) studied GC-MS analysis of bio-active compounds in methanolic leaf extracts of *Justicia adhatoda* (Linn.). GC-MS analysis of *J. adhatoda* leaf extracts revealed the existence of the major peaks presented in methanol were Amrinone (RT: 15.88); n- Hexadecanoic acid (RT: 16.33); Phytol (RT: 17.81); 9, 12, 15-Octadecatrienoic acid, (Z, Z, Z) - (RT: 18.04). From this study it is obvious that *J. adhatoda* leaf extracts contains many biologically active compounds and also it gives a detailed insight about the phytochemical profile which could be exploited for the development of plant based drugs and Insecticides.

Yakubu *et al.*, (2017) studied the Gas Chromatography-Mass Spectrometry (GC-MS) analysis of aqueous extract of *Daniellia oliveri* stem bark. The extract

was obtained using 1:4 (w/v) of the pulverized stem bark in distilled water. Gas Chromatography-Mass Spectrometry Analysis was carried out on a Perkin Elmer Turbo Mass Spectrophotometer while measurement of peak areas and data processing were carried out by Turbo-Mass- OCPTVS-Demo SPL software and spectrums of the components were compared with the database of spectrum of known components stored in the gas chromatography-mass spectrometry library. The phytochemical constituents identified are some fatty acids such as oleic acid, fatty acid methyl esters such as 1-(hydroxymethyl)-1, 2-ethanediyl ester and some volatile organic substances such as 1, 1, 1, 4-Tetramethyl-4-chloro-4-vinyl-1, 4-disilabutane. The presence of these compounds justifies the use of some parts of the plant for various elements in folklore and can be advised as a plant of phytopharmaceutical and industrial importance.

Shanthamani and Ulagi *et al.*, (2018) studied antimicrobial Extracts of *Benkara malabarica* (Lam.) *Triveng* (BM) and *Tarenna asiatica* (L.) Kuntze ex K. Schum (TA) was screened for their *in vitro* antimicrobial activity by agar disc diffusion method. The antimicrobial activity of Petroleum ether, chloroform, methanol and acetone extracts of the leaves of these plants were studied using five bacterial cultures and the five fungal cultures as test organisms. Acetone extracts of BM and TA were found to be more effective for antibacterial against *S.paratypi* and *P.aeruginosa* and antifungal against *M.rubes*, respectively when compared to other extract of BM and TA and it reveals the presence of alkaloids and steroids respectively which suggests that these phytoconstituent may be responsible for their antimicrobial activity.

Venugopalan *et al*.. (2017) studied the preliminary phyto chemical analysis and acute oral toxicity study of Nadi hingu niryasa (*Gardenia gummifera*). Acute oral toxicity study was done preliminary to fix the particular dose for further studies. Photochemical analysis of Nadi hingu shows the presence of Alkaloids. Carbohydrates. Flavanoids. Phenols. and Resin. The study showed Loss on Drying- 5.05%. Total ash is 4.03%. Water soluble ash value is 59%. Acid insoluble ash value is 0.30%. Water soluble extractive value is 8.43%. Alcohol soluble extractive value is 59.97%. The LD50 is greater than 2000 mg/kg.

Secondary metabolites produced by the plants constitute a source of bioactive substances and now-a-days scientific interest has increased due to the search for new form of medicine. This is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action because there has been an alarming increase in the incidence of new and re-emerging infectious diseases. Another big concern is the development of resistance to the antibiotics in current clinical use. (Rajas 2003)

MATERIALS AND METHODS

Field survey and collection of samples

A preliminary field survey was carried out to locate in ecological set up of the mountain and distribution. Though this mountain is enriched with myriads of medicinal plants, literally survey indicated that plant like *Gardenia gummifera* was not explored for its medicinal potent. So the study has planned in collecting this species from the high elevation of the mountain.

Since the plant is endangered to Srilanka and India so the fresh leaves was collected in the plant without making any damage to the whole plant. The collected samples are brought to the laboratory and cleaned before drying. The dried samples were ground into powder and preserved in the glass bottles for future analysis.

Macroscopic studies

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 feature were described in technical terms and illustrated.

Organoleptic characters (Khandelwal, 2003)

Organoleptic characterization can be done by means of organs of sense which include 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 identify and degree of purity of the drug.

Microscopic studies (P. Sudhakar 2016)

The leaves are boiled and fixed in F.A.A. (Formaldehyde – Acetic acid – Alcohol), dehydrated through xylene – alcohol series embedded in paraffin wax. The sections were cut at 10 – 12 µm on Optica 1090A rotary microtome, stained with crystal violet and basic fuchsin combination and mounted in canada balsam. Epidermal peels were obtained by gently scraping and peeling by razor blade, were stained with saffranine and mounted in glycerine.

PHYTOCHEMICAL PROFILE

Solvent extraction

The leaves of *Gardenia gummifera* were washed under tap water and dried in air under shade at room temperature and converted to coarse powder and stored in airtight container. For physicochemical investigation 50 gm of dried powder was successively extracted with different solvents such as petroleum ether, chloroform ,acetone, methanol and water in increasing order of polarity using apparatus. After drying ,the respective extracts were weighed and percentage yield was determined and stored in airtight container.

Qualitative phytochemical tests

Phytochemical analyses on the crude extracts (methanol, chloroform, acetone and petroleum ether) and negative control (water) were conducted in accordance with standard protocols (Harbourne, 1973; Trease and Evans, 1978;

Philip *et al.*, 2011; Damodaran and Manohar, 2012). All tests were replicated twice. Detailed methodology was as follows:

i. **Detection of Alkaloids**

Hager's test:

One ml of extract was mixed with one ml of Hager's reagent (1 g of picric acid in 100 ml distilled water). The formation of a yellow precipitate indicated the presence of alkaloids.

ii. **Detection of Amino acids**

Ninhydrin test:

Two drops of Ninhydrin reagent were added to two ml of dilute extract. A deep purple colour change indicated the presence of amino acids.

iii. **Detection of proteins**

Xanthoproteic Test:

The extracts were treated with few drops of conc. Nitric acid. Formation of yellow colour indicates the presence of proteins.

iv. **Detection of Carbohydrates**

Extracts were dissolved individually in 5 ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.

Molisch's test:

Molisch's reagent (15 g α-naphthol dissolved in 100 ml ethanol) was added to five ml crude plant extract. A brown-red colour reaction indicated the presence of polysaccharides.

v. **Detection of Cardiac glycosides**

Keller-Killiani test:

5 ml of plant extract was mixed with 2 ml glacial acetic acid. Two drops of ferric chloride were added, followed by the addition of concentrated sulphuric acid such that the acid remains underneath. The presence of glycosides was indicated by the formation of a brown ring at the junction of the two layers and a blue-green ring at the upper surface.

vi. **Detection of Coumarins**

1 ml of 10% NaOH was added to 1 ml extract. The development of a yellow colour indicates a positive reaction for coumarins.

vii. **Detection of Flavonoids**

Lead acetate test:

A 10% lead acetate solution (three ml) was added to two ml dilute extract. The formation of a white precipitate indicated the presence of flavonoids.

viii. **Detection of tannins**

Ferric trichloride test:

2 ml distilled water was added to 1 ml of the extract and a few drops of 10% ferric trichloride. A dark green colour change indicated a positive result for the presence of phenols and/or tannins.

ix. **Detection of Quinones**

1 ml concentrated sulphuric acid was added to equal amount of extract. The development of a red precipitate indicated the presence of quinones.

x. **Detection of Anthraquinones**

A few drops of 2% hydrochloric acid were added to one ml of extract. The formation of a red precipitate indicates the presence of anthraquinones.

xi. **Detection of Saponins**

Foam test:

The extract (5 ml) was diluted with distilled water to 20 ml. The solution was shaken in a graduated cylinder for 15 minutes. The presence of saponins in the extract was identified by the formation of a persistent two cm foam layer.

xii. **Detection of Steroids**

Lieberman-Burchard test:

The extract was dissolved in water and then treated with chloroform. The liquids were separated using separating funnel. The chloroform portion was collected and then divided into 2 portions and was used for the test.

A few drops of acetic anhydride was added to the filtrate in a test tube, then followed by the addition of Conc. Sulphuric acid by the wall of the test tube. The formation of brown ring at the junction indicates the presence of phytosterols.

xiii. **Detection of Terpenoids**

Salkowski's test:

5 ml of crude extract was mixed with 2 ml chloroform. Concentrated sulphuric acid (3 ml) was then carefully added to the mixture forming distinct layers. The presence of terpenoids was indicated by the formation of a reddish- brown colour at the interface between the two solutions.

xiv. **Detection of diterpenes**

Copper acetate Test:

Extracts were dissolved in water and treated with 3-4 drops of copper acetate solution. Formation of emerald green colour indicates the presence of diterpenes.

xv. **Detection of Vitamin C**

1 ml of sample extract were mixed with few drops of Dinitrophenyl hydrazine following the addition of 1 ml concentrated H_2SO_4. Appearance of yellow precipitate indicates the presence of Vitamin C.

Quantitative analysis:

Total phenolic content: (Duan *et al.*, 2006)

In phenolic content the 100 mg of plant samples was homogenized with 10 ml of distilled water and filtered through a muslin cloth 1 ml of the filtrate was added to 1.5 ml of deionized water and 0.5 ml of 50% folin-ciocalteau reagent and the contents were mixed thoroughly. After 1min, 1 ml of 20% sodium carbonate solution was added mixed the control contained all the reagents except the sample. After 30 minutes of incubation at 37^0c,the absorbance was measured at 750 nm. Total phenolics were calculated as Gallic acid equivalent (GAE) per gram fresh weight.

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

In flavonoid content the 100 mg of plant material was homogenized with 10 ml of distilled water and filtered through a muslin cloth.0.5 ml of the extract was added with 2.5 ml of distilled water and mixed. After 6 minutes 0.15 ml $NaNo_2$,was added and again after 6 min 0.3 ml of 10% $AlCl_3$ was added. After 5 min 1 ml of 1M NaOH and 0.5 ml of water were added. Following through mixing of the solution the absorbance against blank was recorded at 510 nm. Quercetin

was used as standard and the results were expressed as my quercetin equivalents (QE) 1g fresh weight.

Estimation of Tannin (Peri 1971):

Tannins - phenolics were determined by the method of Peri and Pompei (1971). 1 ml of the sample extracts of concentration 1mg/ml was taken in a test tube. The volume was made up to 1ml with distilled water and 1 ml of water serves as the blank. To this 0.5 ml of Folin's phenol reagent (1:2) followed by 5ml of 35% sodium carbonate was added and kept at room temperature for 5 min. Blue colour was formed and the colour intensity was read at 640 nm. A standard graph (gallic acid - 1 mg/ml) was plotted, from which the tannin content of the extract was determined. The total tannin content was expressed in mg/g of extract.

HPTLC (High performance thin layer chromatography) analysis (Rao *et al.*, 2005)

In the past few decades, compounds from natural sources have been gaining importance because of vast chemical diversity that they offer. HPTLC (High performance thin layer chromatography) is one of the techniques for the qualitative, semi-qualitative and quantative phytochemical analysis of herbal drugs and formulations. This includes developing TLC finger print profiles and estimation of chemical markers and biomarkers. The major advantage of HPTLC is that several samples can be analyzed simultaneously using a small quantity of mobile phase.

Flavonoid profile

Samples given

STD Flavonoid standard as reference marker (Rutin)

Sample A *Gardenia gummifera* stem methanolic extract

Procedure

Extraction and Test solution preparation

The dried plant materials (1gm) are extracted with respective solvents (methanol) in soxhlet apparatus for 3 hrs. The content is cooled and concentrated using vacuum flash evaporator. The content is dissolved with 1 ml of respective solvents and centrifuged at 3000 rpm for 5 min. These solutions are used as test solution for HPTLC analysis.

Sample application

2 µl (sample 1 and 3) and 1 ml (sample 2 and 4) of test solution and 2 ml of standard solution are loaded as 5 mm band length in the 5 × 10 silica gel $60F_{254}$ TLC plate using Hamilton syringe and CAMAG LINOMAT 5 instrument.

Spot development

The samples loaded plate is kept in TLC twin trough developing chamber (after saturated with solvent vapour) with respective mobile phase (Flavonoid) and the plate is developed upto 90 mm.

Photo- documentation

The developed plate is dried by hot air to evaporate solvents from the plate. The plate is kept in photo - documentation chamber (CAMAG REPROSTAR 3) and captured the images at visible light, UV 254 nm and UV 366 nm.

Derivatization

The developed plate is sprayed with respective spray reagent (Flavonoid) and dried at 100c in hot air oven. The plate is photo- documented in visible light and UV 366 nm mode using photo- documentation (CAMAG REPROSTAR 3) chamber.

Scanning

After derivatization, the plate is fixed in scanner stage (CAMAG TLC SCANNER 3) and scanning is done at UV 366 nm. The peak table, peak display and peak densitogram are noted. The software used is win CATS 1.3.4 version.

Detection

Blue and violet coloured zone at visible light mode present in the given standard and sample track observer in the chromatogram after derivatization, which confirms the presence of steroid in the given sample.

Fourier Transform Infrared Spectrophotometer (FTIR) analysis of plant extracts (Ashokkumar and Ramaswamy,2014)

Fourier Transform Infrared Spectrophotometer (FTIR) is perhaps the most powerful tool for identifying the types of chemical bond (functional group) present

in compounds. The wavelength of light absorbed is characteristic of chemical bond as can be seen in the annotated spectrum. By interpreting the infrared absorption spectrum, the chemical bond in a molecule can be determined. Dried powder of ethanol extracts of the plant materials are used for FT-IR analysis. 10 mg of dried extract powder is encapsulated in 100 mg KBr pellet in order to prepare transluscent sample discs. The sample is loaded in FT-IR spectroscope (Thermo Scientific Nicolet 10, USA) with a scan range from 400 to 4000 cm^{-1} with a resolution of 4 cm^{-1}. The reference spectra are acquired for cleaned blank crystal prior to the presentation of sample replicate.

GC-MS analysis :

Leaf extract of *G. gummeifera* were analyzed for the presence of different compounds by gc-ms technique. GC analysis of the extracts was performed using a GC-MS (Model; Thermo GC-Trace Ultra Ver:5.0, thermo MS DSQ II) equipped with a DB-5-MS capillary standard non-polar column fused silica column (30m length X outside diameter 0.25 mm X internal diameter 0.25µm). For GC-MS detection, an electron ionization system with ionization energy of -70eV was used" (Jayashree *et al.*, 2015).

Helium gas was used as a carrier gas at a constant flow rate of 1ml/min and the sample injected was1µl; Injector temperature 250oC; Ion source temperature 200oC. The oven temperature was programmed from 70° to 260°C at the rate of 6°C/min, held isothermal for 1minutes and finally raised to 250°C at 10°C/min. Interface temperature was kept at 250oC. Total GC run time was 37.51 min. The

relative percentage of each extract constituent was expressed as percentage with peak area normalization.

RESULTS AND DISCUSSION

RESULTS AND DISCUSSION

Description of the plant

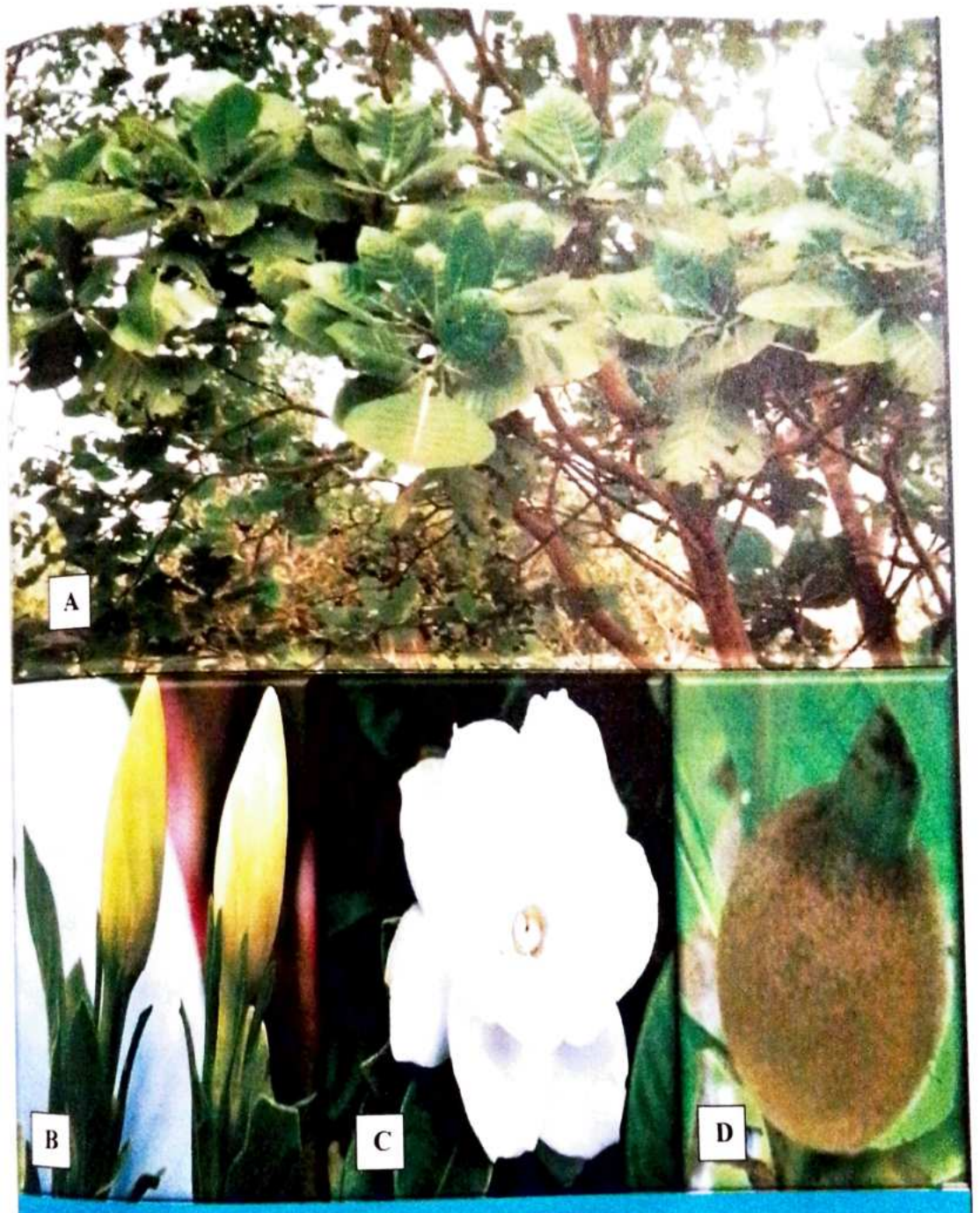
Gardenia gummifera is a small tree to 8 m high, bark greyish-brown, smooth; blaze dull yellow; exudation bright yellow resinous; branches and branchlets virgate, young parts pubescent. Leaves simple, opposite decussate; stipules intrapetiolar, connate; petiole stout, glabrous; lamina ovate, obovate or obovate-oblong, base obtuse, round or subcordate, apex acute or obtuse, margin entire, puberulent when young, glabrous when mature, shiny, coriaceous; lateral nerves 8-20 pairs, parallel, prominent, intercostae reticulate, prominent; domatia present. Flowers bisexual, white turns to yellow, axillary, solitary or in lax cymes; calyx truncate, lobes 5, triangular, puberulous without, acute; corolla cylindrical, lobes 5, puberulous without, obovate, obtuse; stamens 5, included; ovary inferior, 1-celled, 5-gonous, ovules many; style single, stigma 5 angled, fusiform. Fruit a berry, ellipsoid. This species can be helpful in treating digestive problems, including dyspepsia and diarrhea; or used as an astringent and expectorant for nervous conditions and spasms (Plate 1).

Microscopic studied

Leaf surface

Epidermal cells 5-7 sided, few 4 sided, mostly polygonal anisodiametric, few isodiametric, rarely polygonal linear, sides thick, mostly straight to curved, few curved to wavy, surface smooth, contents dense with calcium oxalate crystals in few. Stomata anomocytic and paracytic, subsidiaries 2-5, monocyclic, guard cells reniform. Unicellular conical hair distributed all over,

Plate 1: Morphological features of *Gardenia gummifera*.



A. A portion of tree; B. Flower buds of *G.gummifera*; C. Closeup view of *G.gummifera* flower; D. A Fruit.

more on primary and secondary veins, irregularly arranged, variously oriented.

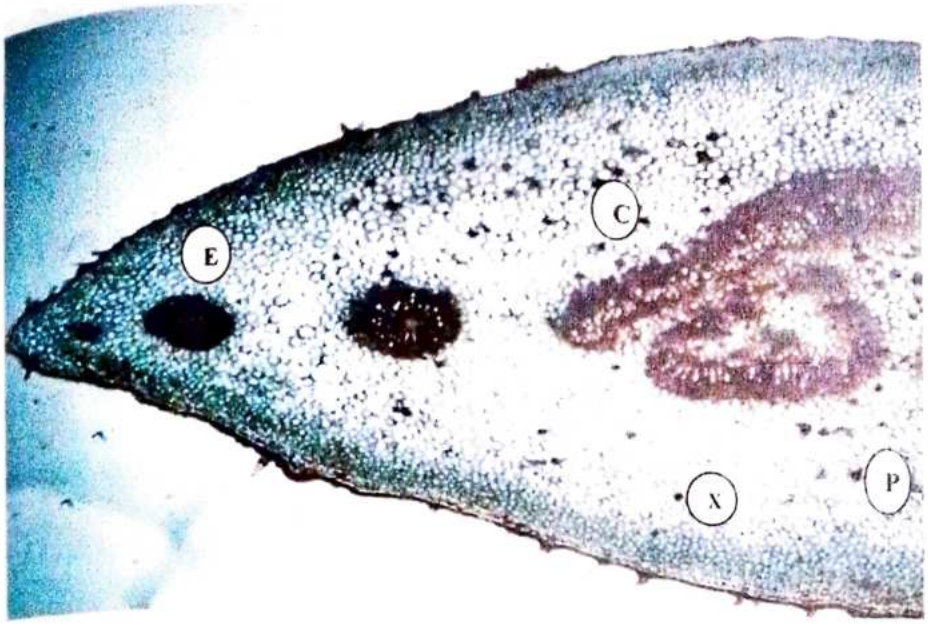
Transverse section of leaf:

In T.S. leaf is ribbed on either sides but prominently on abaxial at midvein. Epidermis 1 – layered, cells over the lamina mostly barrel shaped, few tabular and oval to circular, adaxially larger, walls thin, contents scanty; cuticle thick, covered over the surface (Plate 2).

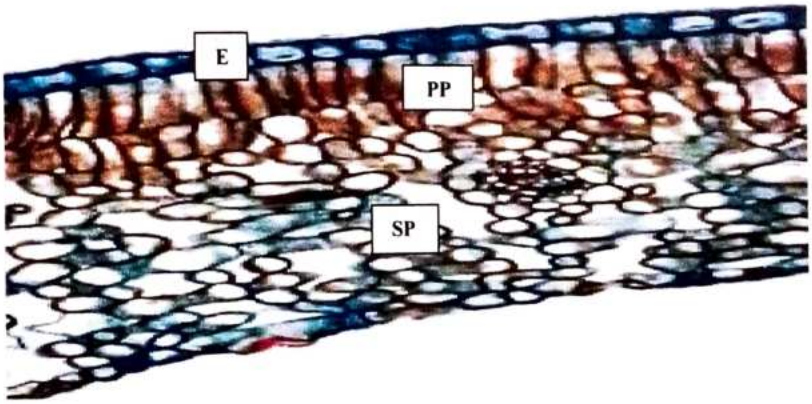
Mesophyll dorsiventral with palisade and spongy tissues. Palisade mostly 2 – layered, at some places upto 3 layered, closely packed with small intercellular spaces, interrupted at midvein, secondary and tertiary veins; cells columnar, cylindrical, perpendicular to epidermis; 16 – 33 (25) µm long and 8 – 16 (11) µm wide, walls thin, interspersed with sphaerocrystals and prism in few, contents dense with chloroplasts. Spongy tissue 3-5 layered, cells circular, oval to oblong, spherical, dumbbell shape, elongated cells 19-36 (26) µm long 11-19 (15) µm wide, isodiametric cells 8-19 (14) µm in diameter; cells loosely arranged in a reticulum with large intercellular spaces, interspersed with crystals and dense with chloroplasts in few.

Ground tissue of midvein contains collenchyma, parenchyma and sclerenchyma tissues. Sclerenchyma tissue is enclosing the central vascular cylinder and adaxial bundles. Collenchyma 6-9 layered in the adaxial ridge, 2-3 layered on the abaxial, angular or lamellar, contents scanty. Parenchyma 8-10 layered adaxially and 6 – 9 layered abaxially thick, cells polygonal, oval to circular and oblong, intercellular spaces narrow, with sphaeraphides in few. Sclerenchyma 2-3 layered, surrounding the central vascular cylinder.

Plate 2: Microscopic illustrations of *Gardenia gummifera* leaves



(1) T.S of ***Gardenia gummifera*** **Petiole [E-Epidermis; C- Cortex; X- Xylem; P- Phloem**



(2) T.S. of Leaf lamina [E-Epidermis; PP- Palisade Parenchyma; SP- Spongy Parenchyma]

(3) Stomata

Vascular tissue consists of predominantly vascular cylinder at centre enclosing the 3-4 central medullary bundles besides 2 adaxial bundles. Central vascular cylinder 792 – 962 (857) µm and adaxial vascular bundles 68 – 88 (79) µm in diameter; conjoint, bicollateral, endarch. Phloem bundles are enclosed by endodermis. Few of sclerenchymatous cells are attached to one or two bundles. The adaxial bundles are endarch, collateral, enclosed by sclerenchyma towards abaxial side. Xylem consists of trachieds, vessels, fibers and xylem parenchyma arranged in radial rows. Tracheary elements numerous, thick walled, polygonal 14 – 27 (20) µm in diameter. Xylem parenchyma in between tracheary elements. In L.S. tracheary elements showing mostly helical and reticulate few scalariform and annular. Xylem surrounded by phloem on either sides consists of phloem parenchyma, sieve cells, fibers and companion cells. Centrally the ground parenchyma inside vascular cylinder consists of polygonal to spherical cells, thin walled with small intercellular spaces.

Transverse section of petiole:

Subspherical, adaxially flat; covered by few unicellular conical hairs. Epidermis single layered, cells oval to spherical, barrel shaped, some cells containing crystals and chloroplasts in few, covered by thick cuticle. Collenchyma hypodermal, 4-8 layered, angular, contents slightly dense in some with crystals and chloroplasts in few. Parenchyma vertically 28-33 celled thick and laterally 24-27 celled thick, covering the entire hypodermal area beneath collenchyma, often interspersed with sphaeraphidalidioblasts (Plate 2).

Vascular tissue consists of a single wedge shaped vascular bundle at the centre and 2-4 small adaxial bundles on either sides at adaxial side. The central vascular bundle is laterally 518 – 604(559) µm long and vertically 270 – 388(346) µm wide, endarch, conjoint, bicollateral. Xylem is made of vessels/tracheids, xylem parenchyma and fibers; tracheary elements numerous, arranged in radial rows and few laterally aligned, interspersed by xylem parenchyma and few fibers in between; xylem is surrounded by phloem on both sides, consists sieve cells, companion cells, phloem parenchyma and phloem fibers.

Central vascular bundle is enclosed by a 1-3 layered sclerenchyma, continuous towards abaxial side. The smaller adaxial bundles are conjoint, collateral, enclosed by a sclerenchymatous sheath. A small phloem bundle is present one on each side of the central wedge shaped bundle.

Organoleptic characters

The organoleptic characters of G. gummifera leaf examined and presented in Table 1. The leaf powder of G. gummifera is light green in colour, with characteristic odour and slightly bitter taste.

Qualitative analysis

Qualitative analysis involves detection and identification of phytoconstituent from different solvent extracts of the leaf samples using standard test. In the present investigation, this analysis of leaves of *G. gummifera* was conducted by using various organic solvents such as petroleum ether, chloroform, acetone, water and methanol. The study revealed the presence of myriads of compounds in all the types of extracts tested (Table 2).The compounds dectected

Table 1. Organoleptic characters of leaves of *Gardenia gummifera*

S.No	Character	Leaf	Leaf powder
1.	Colour	Dark green	Light green
2.	Texture	Smooth	Smooth
3.	Taste	Slightly bitter	Slightly bitter
4.	Odour	Characteristic	Characteristic

Table 2. Qualitative Phytochemicals Analysis of *Gardenia gummifera* Leaf

Tests	Acetone	Chloroform	Petroleum ether	Methanol	Water
Alkaloids	-	+++	++	-	-
Amino acids	-	+++		-	+
Proteins	-	-	++	-	-
Carbohydrates	-	-	+	++	+
Glycosides	-	-	-	-	-
Cardiac glycosides	+	++	++	+++	+
Coumarins	+	+++	++	-	–
Flavonoids	-	++	+	-	+++
Tannins	+	-	++	+++	+
Quinines	-	-	+	+++	-
Anthraquinones	-	-	-	-	++
Saponins	-	+++	++	-	++
Steroids	-	-	+++	-	-
Terpenoids	-	++	-	+++	-
Diterpenes	-	+++	++	-	-
Vitamin C	+	+	-	+	+

includes flavanoids, tannins, phenolic compounds, terpinoids, saponins and glycosides. Of these, terpioides, flavanoids ,glycocides and proteins showed intensified colour complexes for their respective test. However, compounds like diterpens, saponins, coumarins, aminoacids were well extracted by chloroform because the result of these test showed highly intensified colour complex in chloroform extraction. In turn, the study has found petroleum ether is the best solvent to extract different types of phytochemicals. Some of the compound which is not reported through chlorofrom was found from petroleum ether. So, petroleum ether is considered as a powerful extractant. The study also suggest that aqueous medium is not suitable to dissolve so many non polar compounds such as coumarins, terpenoids, alkaloids, steroids .

Since the present invistigation has reported the presence secondary metaboilites through preliminary phytochemical screening , our further study was focused to quantify some of the compounds that were known for the therapeutic values.

Quantitative analysis

The quantitative phytochemical tests of leaves of *G. gummifera* were undertaken for phenolic compounds such as total phenol, flavonoids and tannin content. Table 3 showed the quantitative analysis results of cholorofrom extracts of *G. gummifera*. The results are extimation of these compound are present(Table 3 & Figure 1). For this comparative study, leaves extracts were showed more phenol

Table 3. Phytochemical analysis of *Gardenia gummifera* (L.f) Baill

S.NO	PHYTOCHEMICALS	AMOUNT OF PHYTOCHEMICALS (mg/g/DW)
1	Flavonoids	3.49±0.59
2	Phenols	8.44±0.38
3.	Tannins	1.04±0.07

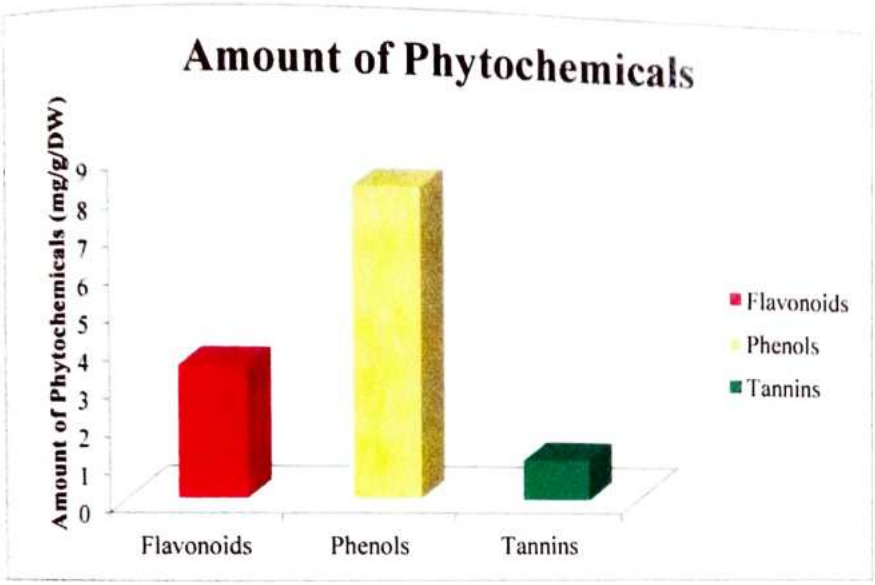


Figure 1. Phytochemical content of *Gardenia gummifera*

content (8.242 ± 0.38) than other phytochemicals. Next to this, flavonoids revealed choice content (3.49±0.59) than tannins (1.047±0.07).

Phenols

Analysis of total phenolic compound revealed the presence of remarkable quantity (8.242±0.38) . The amount of total phenols reported the present investigation is comparable with the studies of in other plants.

The vast majority of plant-based aromatic natural products are phenols. Phenols constitute a large class of compounds in which a hydroxyl group (–OH group) is bound to an aromatic ring. Numerous categories of these compounds exist, including the simple phenols, phenylpropanoids, flavonoids, tannins, and quinines (Cseke *et al.*, 2006). Phenolics are produced by plants mainly for their protection against stress.

Flavonoids

Flavonoids are diverse group of phytonutrients (plant chemical) found in almost all fruits and vegetables. Along with carotenoids, they are responsible for the vivid colors in fruits and vegetables. Flavonoids are the largest group of phytonutrients, with more than 6,000 types. They are powerful antioxitants with antiinflamatery and immune system benefits. In the present investigation on chloroform extract has reported (3.49±0.59) mg/g/dry weight (DW) of flavonoids. This is remarkably higher than the studies conducted in other plants (3.35±0.257[*Bacopa monneri*] Nikkhah et al., 2018). High amount of flavonoids in the study material confirms that it is a good choice of a plant can be used for

treating various aliment. A large number of studies have reported the impact of consuming flavonoids-rich foods on biomarkers of cardiovascular disease risk in healthy volunteers or at-risk individuals. Recent evidence suggests that some polyphenols in their purified form, including resveratrol, berberine and naringenin, have beneficial effects on dyslipidemia in humans and/or animal models. In a mouse model of cardiovascular disease, naringenin treatment, through correction of dyslipidemia, hyperinsulinemia and obesity, attenuated atherosclerosis (Mulvihill and Huff, 2010).

Tannins

Tannins are a polyphenolic biomolecules that bind to precipitate proteins and various other organic compounds including aminoacids and alkaloids. Analysis of total tannin compound revealed the presence of remarkable quantity (1.047±0.07) mg/g/dry weight (DW). The amount of total tannin reported the present investigation is comparably high, indicates its medical potential. Compare to other studies the amount of tannin reported in *G. gummifera* is substantially high. That is, 1.035±0.005 mg/g/dry weight in *G. gummifera* (S Manorama *et al.*, 2013). In medicine, especially in Asia (Japanese and Chinese) in natural healing process the tannin containing plant extracts are used as astringents, against diarrhoea, as diuretics, against stomach and duodenal tumours, and as anti-inflammatory, antiseptic, and haemostatic pharmaceuticals. It is also becoming clear that tannins often are the active principles of plant-based medicines.

Antibacterial activity of leaves extract

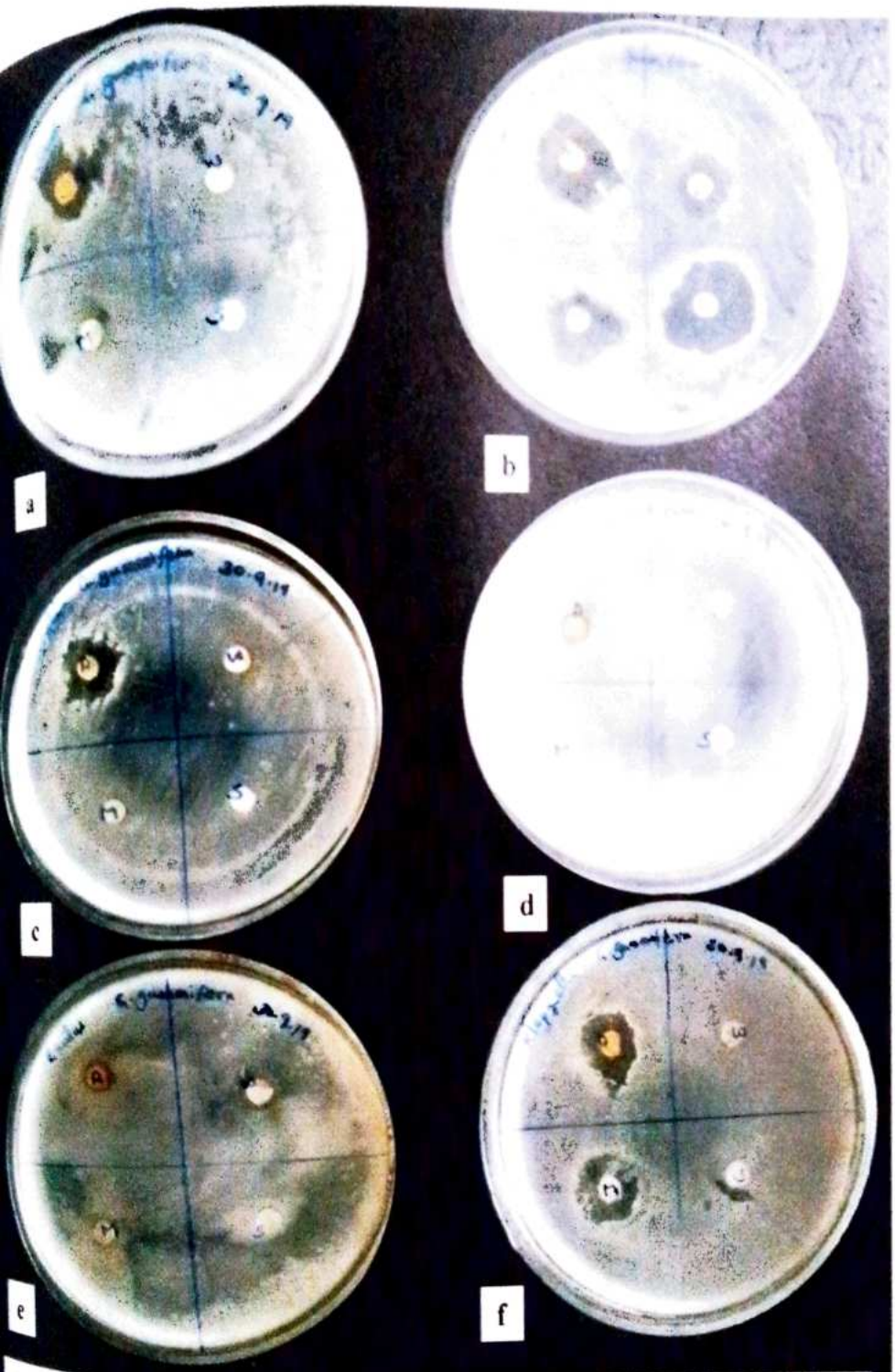
The antibacterial capacity of the experimental plant leaves were screened according to their zone of inhibition against selective clinical pathogens and the results (zone inhibition) were compared with standard streptomycin. The results showed that, all the extracts are active against all the selected microorganisms used in present investigation. Among the different solvent extracts used, acetone showed more inhibition followed by methanol and water extracts (Plate 3; Figure 2 & Table 4).

More antibacterial activity was identified in *E. coli* culture placed with disc dipped in methanolic leaf extract. This indicates *E. coli* are highly susceptible to compounds present in the methanolic extract. Highly intensified colour complexes reported in methanolic extract for quinones, terpenoids, tannin and cardiac glycoside are evidence for possible cause of incubation of *E. coli*. The extraction of the antibacterial compounds depends on the polarity of the compounds. The variation in the antibacterial activity of the various solvents is due to the nature of the polarity of the solvents. Methanol is having higher polarity and thus they tend to dissolve different compounds from the plant materials dipped in them. Reports also revealed that ethanol and methanol are commonly used for extraction of antibacterial compounds (Karaman *et al*, 2003, Wei *et al*, 2008). To confirm the presence of these compounds and better elicitation for the study was extended using high performing instrument FTIR.

FTIR analysis

Fourier Transform Infrared (FTIR) Spectroscopy is commonly referred to as FTIR Spectroscopy method is used to identify organic, polymeric and in some

Plate 3: Antibacterial Activity of *Gardenia gummifera*.



A. *Proteus vulgaris*;B. *Escherichia coli*; C. *Serratia marcescens*; D. *Streptococcus sp.*;E. *Bacillus substilis*; F. *Klebsiella pneumonia*. [Solvents- Acetone, Methanol, Water]

Table 4. Antibacterial Activity of *Gardenia gummifera*

Bacterial strains	Zone of inhibition (in mm)			
	Acetone	Methanol	Water	Streptomycin
Bacillus substilis	9	8	13	7
Escherichia coli	22	23	14	23
Klebsiella pneumonia	15	16	6	10
Proteus vulgaris	13	7	6	26
Serratia marcescens	18	8	6	6
Streptococcus sp.	8	7	6	22

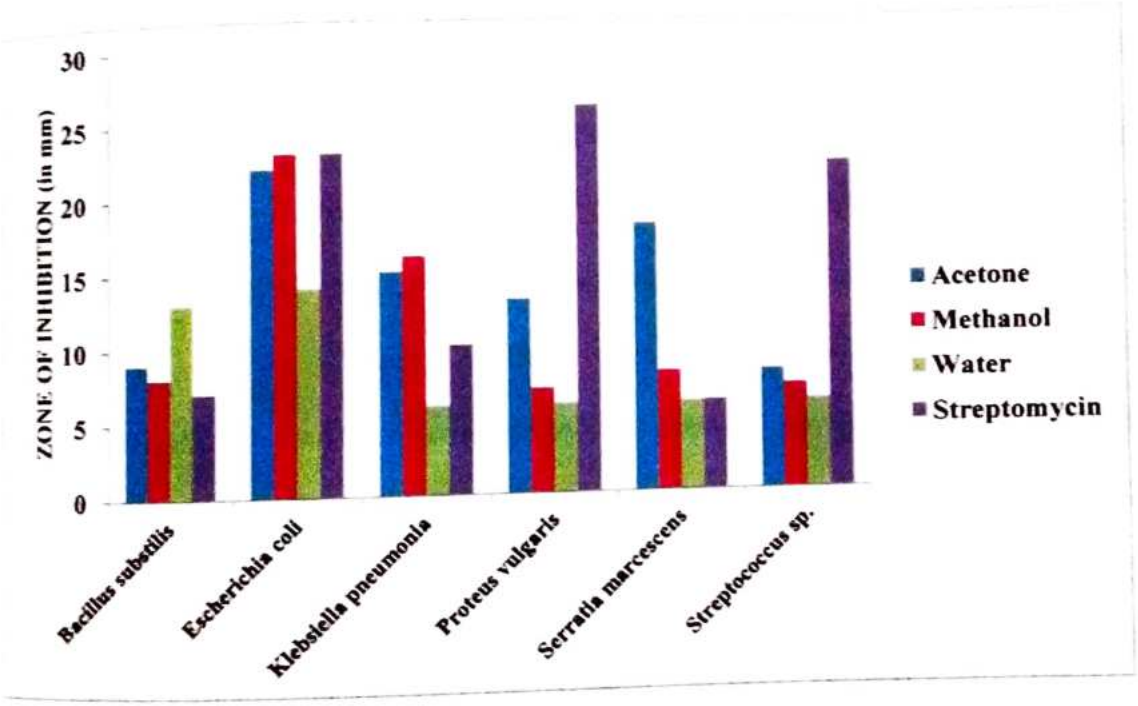


Figure 2. Antibacterial Activity of *Gardenia gummifera*

cases, inorganic materials. The FTIR test relies on infrared light to scan samples and observe bond properties.

Fourier Transform Infrared spectroscopy is an incredibly versatile materials analysis technique, helping identify organic and some inorganic materials. The use of FTIR spectroscopic techniques for the nondestructive analysis of biological specimens is a rapidly expanding research area, with much focus on its utility in cytological and histological diagnosis through the generation of spectral images (Bellisola. and Sorio. 2012; Diem *et al.*, 2004). Molecular bonds with an electric dipole moment that can change by atomic displacement owing to natural vibrations are IR active. These vibrational modes are quantitatively measurable by IR spectroscopy, providing a unique, label-free tool for studying molecular composition and dynamics without perturbing the sample (Griffiths *et al.*, 2007).

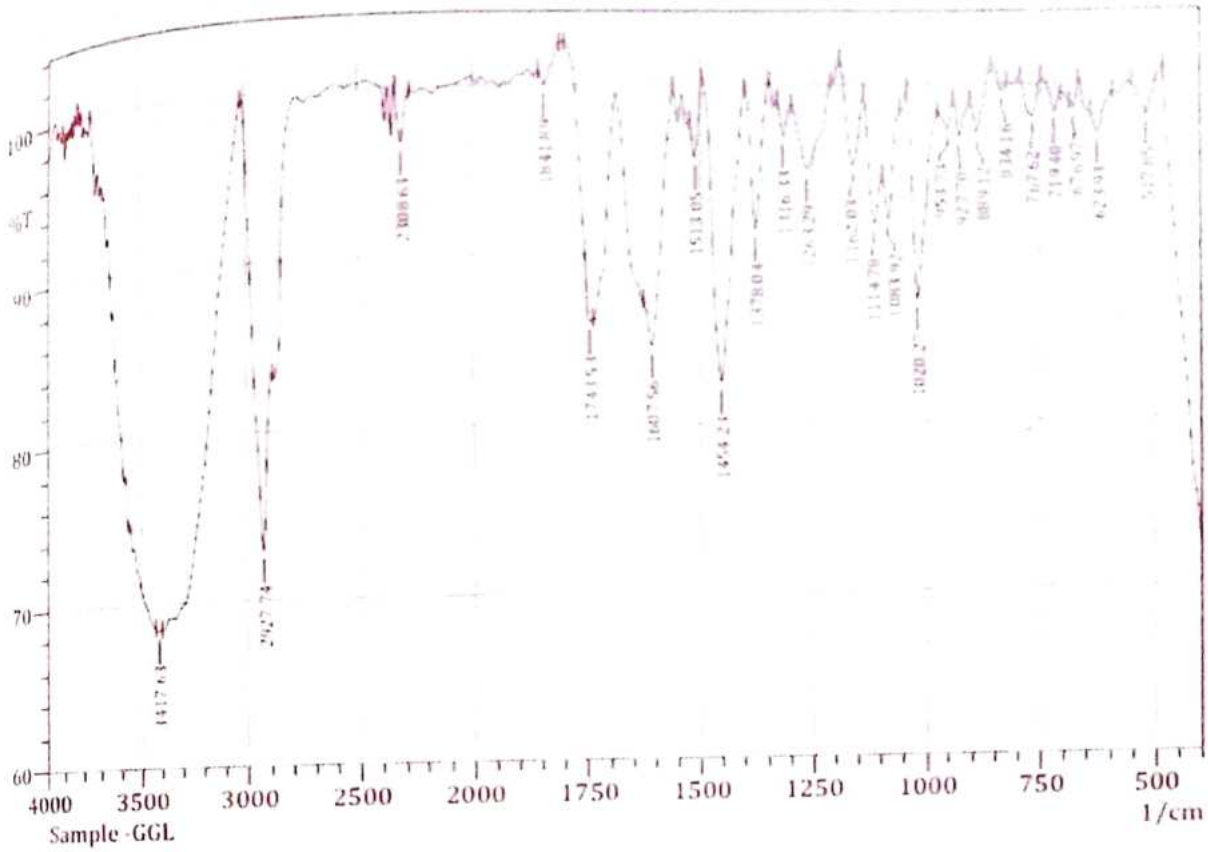
In present study the spectral data shows, presence of C-Br, C-C-C, =C-H, C-Cl, C-F, C-N, C=C, C=O, C-H, OH, N-O, C-CI and N-H. These bonding are responsible for the presence of alkyl halids, alkaline, amine, nitro compounds, aromatic compounds, carbonyl groups, allene, acid, alkane and alcoholic compounds in the leaves of *Gardenia gummifera* (Table 4 & Figure 3). All the above bondings are important functional groups of phytochemical constituents such as alkaloids, phenolic compounds and glycosides. To confirm the presence of these compounds and better elicitation the study was extended using high performing instruments such as HPTLC and GC-MS.

HPTLC profile of leaf extract

Table 5. FTIR Analysis of *Gardenia gummifera*

S.NO	Peak value	Stretching	Interpretation
1	517.85	C-Br	Alkyl Halide
2	623.93	C-CI	Alkyl Halide
3	676.97	=C-H	Alkalene
4	719.4	C-H	Alkalene
5	767.62	C-CI	Alkyl Halide
6	834.16	=C-H	Alkalene
7	889.12	=C-H	Alkalene
8	927.7	=C-H	Alkalene
9	953.73	=C-H	Alkalene
10	1020.27	C-F	Alkyl Halide
11	1083.92	N-H	Amine
12	1114.78	N-H	Amine
13	1162.03	N-H	Amine
14	1263.29	C-N	Amine
15	1316.33	N-O	Nitro
16	1378.04	N-O	Nitro
17	1454.23	C=C	Aromatic
18	1513.05	C=C	Aromatic
19	1607.56	C=O	Carbonyl
20	1743.53	C=O	Carbonyl
21	1841.89	C-C-C	Allene
22	2308.63	OH	Acid
23	2872.77	C-H	Alkane
24	3417.63	OH	Alcohol

Figure 3. FTIR Analysis of *Gardenia gummifera*



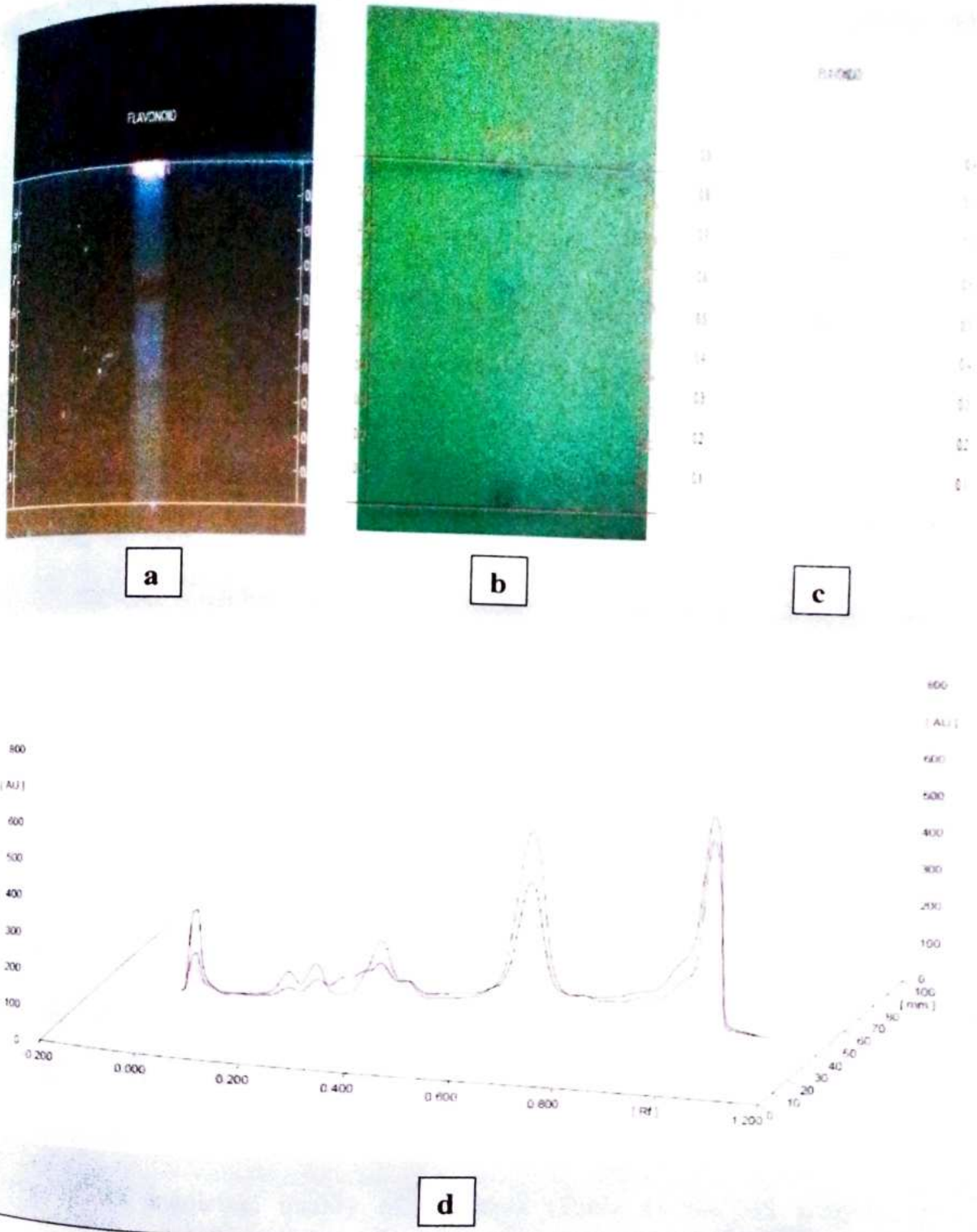
High performance thin layer chromatography (HPTLC) is a valuable tool for reliable identification. In HPTLC profile, each and every metabolite has played specific role and function in harmony with other metabolites within the organization framework of the cells in the defense mechanism of the plants. Chromatographic fingerprinting of phytoconstituents can be used for the assessment of quality consistency and stability of herbal extracts or products by visible observation and comparison of the standardized fingerprint pattern. (Mariswamy *et al.* 2012) According to WHO, it has emphasized the need to ensure the quality of medicinal plant products by using modern controlled techniques and applying suitable standards (Hariprasad and Ramakrishnan *et al.,* 2012). Flavonoids belong to a group of natural substances with variable phenolic structures and are found in fruit, vegetables, grains, bark, roots, stems, flowers, tea, and wine .These natural products were known for their beneficial effects on health. Middleton,(EJ *et al.,*1998). The TLC procedure was optimized with a view to separate the compounds and to identify the phytoccontituent in the extract. Initially, Acidic acid: ethyl acetate: formic acid: methanol in varying ratios was tried along with several combinations of other solvents. The developing system consists of ethyl acetate, water, acidic acid, formic acid acid (8:10:5:5v/v/v/v) gave a sharp and well-defined band with Rf 39.68 for quercetin. The peak display of 100µl of standard (Figure 2) showed the presence of the bioactive compound flavonoid.

HPTLC analysis of methanol extract of *G .gardenia* leaf was carried out along with the standard flavonoid, quercetin and ethyl acetate,water, acidic acid.

Table 6. HPTLC Analysis of *Gardenia gummifera*

Peak	Start Rf	Start Height	Max RF	Max Height	Height %	End Rf	End Height	Area	Area %	Assigned substance
1	-0.05	0.3	-0.02	219.2	15.13	0.03	0.8	3676.4	5.95	FLAVONOID
2	0.09	1.8	0.16	31.8	2.20	0.18	21.8	937.1	1.52	UNKNOWN
3	0.18	24.0	0.21	58.2	4.02	0.23	47.1	1489.9	2.41	UNKNOWN
4	0.23	45.5	0.33	113.7	7.85	0.36	67.3	6764.1	10.94	UNKNOWN
5	0.37	69.3	0.38	72.5	5.01	0.42	43.5	2171.4	3.51	UNKNOWN
6	0.49	52.8	0.61	365.4	25.23	0.70	73.6	18877.1	30.54	UNKNOWN
7	0.78	74.6	0.97	587.5	40.56	1.07	0.6	27903.1	45.14	FLAVONOID
8	0.04	0.2	-0.02	100.5	5.92	0.04	4.1	1798.4	2.79	UNKNOWN
9	0.08	6.4	0.16	71.9	4.23	0.18	42.2	2016.2	3.14	UNKNOWN
10	0.18	43.7	0.20	99.4	5.85	0.25	22.7	2668.1	4.15	FLAVONOID
11	0.25	25.2	0.33	178.9	10.53	0.37	69.8	6901.4	10.65	FLAVONOID
12	0.35	72.3	0.38	73.1	4.31	0.42	31.9	1760.3	2.74	FLAVONOID
13	0.47	38.3	0.61	504.3	29.69	0.70	64.8	25465.1	39.68	FLAVONOID
14	0.70	65.8	0.72	71.8	4.23	0.75	53.6	2321.7	3.62	FLAVONOID
15	0.79	60.4	0.83	76.3	4.49	0.85	70.6	2684.7	4.18	UNKNOWN
16	0.85	71.7	0.97	522.3	30.75	1.03	6.7	18646.3	29.05	UNKNOWN

Figure 4: HPTLC (Chromatogram)- Flavonoid profile



HPTLC (Chromatogram). Flavonoid Profile of *Gardenia gummifera*

a. UV light 396 nm; b. UV light 254 nm; c. White T-image

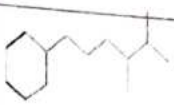
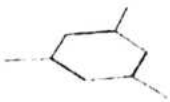
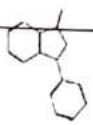
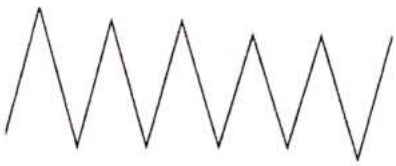
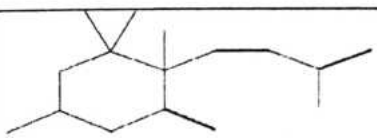
d. Image shows all tracks

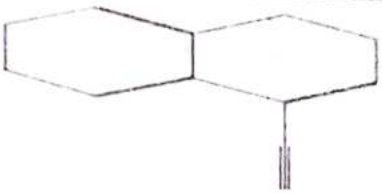
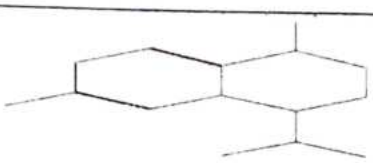
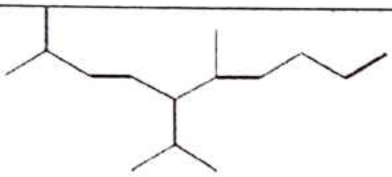
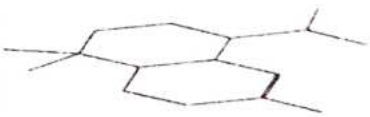
Folic acid (8:10:5:5) as the mobile phase (Table 6 & Figure 4).The identity of the quercetin bands in sample chromatograms was confirmed by the chromatogram obtained from the sample with that obtained from the reference standard solution.(Table 6). The identity of the bands of quercetin in the methanol extract was also confirmed by comparing the UV-Vis absorption spectra with those of standards using a CAMAG TLC scanner 3 (Figure 4). The 3-D spectrum of all tracks scanned at 254nm is shown (Figure 4 b). So many peaks in the spectrum have been detected but identification through peaks is not clear and is not proven too. For better identification of compound and to study the structural configuration investigation was tread to GC-MS analysis.

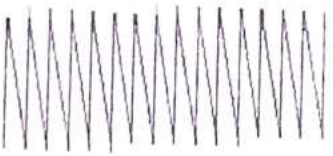
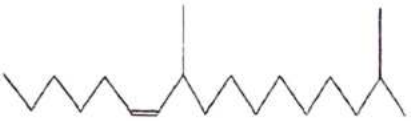
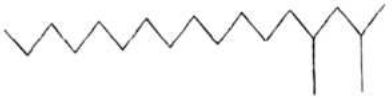
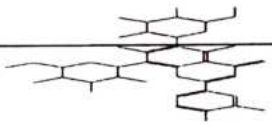
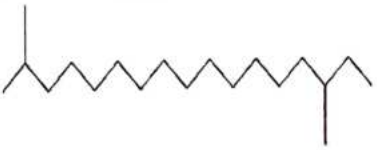
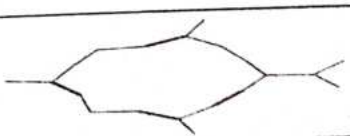
GC-MS analysis

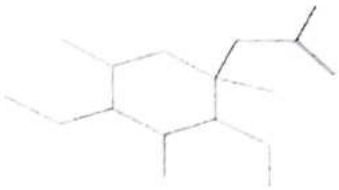
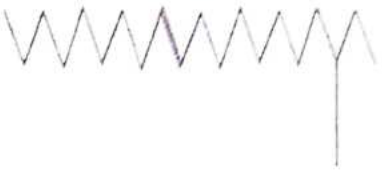
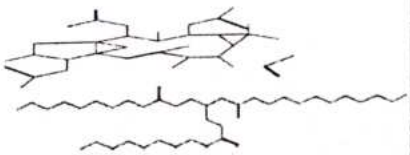
GC-MS is the best techniques to identify the constituents of volatile matter, long chain, branched chain Carbonyl, alcohols acids, alcohol, ester of isopropyl alcohol etc.. The active phytocompounds with their Retention time (RT), Molecular formula, Molecular weight (MW) and peak area and structure identified are presented in Table :7. Twenty seven compounds are identified in leaf powder extract of o *G.gummifera* with the retention time ranging from 0.00 to 37.40 minutes. The compounds with maximum peak area are Benzene, 1,3,5-trimethyl-(CAS) (5.22), N-(4-Hydroxyphenyl) 9-Octadecenoic acid (Z)-, methyl ester (25.62) (Table 7 & Figure 5). From the GC-MS analysis of *G. gummifera* leaves the presence of twenty seven compounds (phytochemical constituents) were revealed the medicinal quality of the plant (Table 8). GC-MS analysis of phytoconstituents in plants gives a clear picture of the pharmaceutical value of that

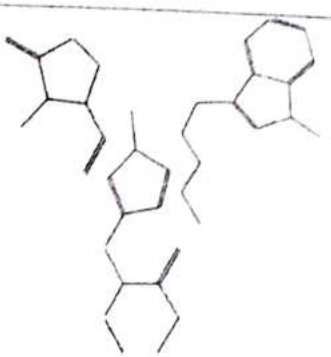
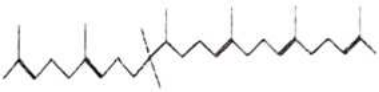
Table 7. Phytocomponents Identified in Leaf Extract of *Gardenia gummifera*

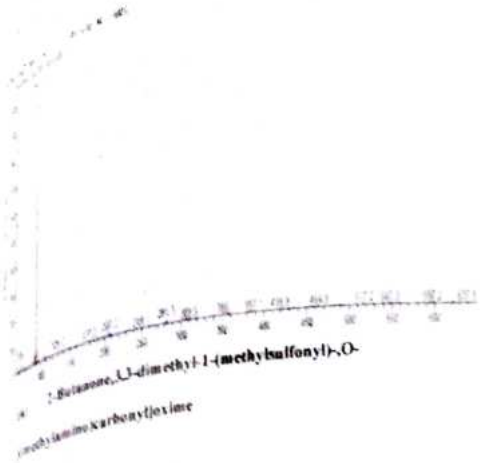
No.	RT	Name,Formula,Molecular weight of the phytocompounds	Peak area	Structure
1	3.53	2-Butanone, 3,3-dimethyl-1-(methylsulfonyl)-, **Formula**: C9H18N2O4S **Mw**:250	4698328.65	
2	4.77	L-Serine, O(phenylmethyl)- **Formula**: C10H13NO3 **Mw:190**	797340.93	
3	5.22	Benzene, 1,3,5-trimethyl- (CAS) **Formula:** C9H12 **Mw:** 120	1041914.10	
4	6.38	(3R)-3-Phenyl-2,3-dihydro-1H-isoindol-1-one **Formula:** C14H11NO **Mw:209**	315030.49	
5	8.22	Dodecane **Formula:** C12H2 **Mw:170**	1614913.58	
6	9.24	Memantine **Formula:** C12H21N **Mw:179**	885325.14	

9.77	QUERCETIN 7,3',4'-TRIMETHOXY **Formula:** C18H16O **Mw:** 344	397119.47	
10.44	4-Cyano-2H-1-benzothiopyran **Formula:** C10H7N **Mw:173**	2259790.64	
11.15	à-Copaene **Formula:** C15H2 **Mw:204**	3309091.59	
13.15	á-Cedrene **Formula:** C15H2 **Mw:204**	372016.10	
13.58	Torreyol **Formula:** C15H26O **Mw:222**	947952.40	
14.11	ë-Cadinene **Formula:** C15H2 **Mw:204**	1141110.11	

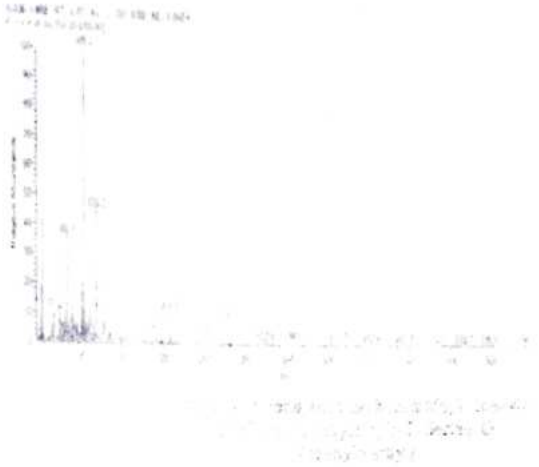
13	15.53	Dotriacontane **Formula:** C32H6 **Mw:450**	847140.04	
14	19.77	7-Methyl-Z-tetradecen-1-ol acetate **Formula:** C17H32O2 **Mw:268**	444111.98	
15	20.28	Isopropyl myristate **Formula:** C17H34O **Mw:270**	4394836.71	
16	20.58	PHYTOL ISOMER **Formula: C20H40** **Mw:296**	433932.32	
17	21.22	Lucenin 2 **Formula:** C27H30O16 **Mw:610**	287424.11	
18	22.30	Pentadecanoic acid, 14-methyl-, methyl ester **Formula:** C17H34O2 **Mw:270**	403371.31	
19	23.23	Cembrene **Formula:** C20H32	1964275.38	

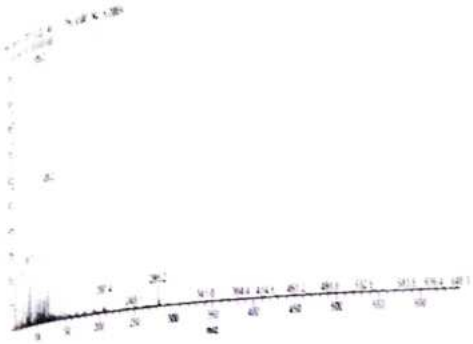
	Mw:270		
23.62	à-L-Mannopyranoside, methyl 6-deoxy-2,4-di-O-methyl-, acetate **Formula:** C11H20O6 **Mw:248**	384274.86	
25.62	9-Octadecenoic acid (Z)-, methyl ester **Formula:** C19H36O2 **Mw:296**	1315099.32	
26.94	3-Hydroxypropanoic acid, 3(2,2,6trimethylbicyclo[4.1.0]hept-1-yl)-, ethyl ester **Formula:** C15H26O3 **Mw:254**	331926.91	
31.50	9-Octadecenamide **Formula:** C18H35NO **Mw:281**	4182810.69	
32.71	9-Desoxo-9-x-acetoxy-3,8,12-tri-O-acetylingol **Formula:** C28H40O10 **Mw:536**	359227.88	
35.07	2-LAURO-1,3-DIDECOIN **Formula:** C35H66O6 **Mw:582**	14966813.29	

36.07	L-Tryptophanamide, 1-methyl-5-oxo-L-prolyl-N,1-dimethyl-L-histidyl-N,N,N à,1-tetramethyl- **Formula:** C29H39N7O4 **Mw:549**	8724401.87	
37.40	N-(4-Hydroxyphenyl)retinamide **Formula:** C26H33NO2 **Mw:391**	3394271.31	

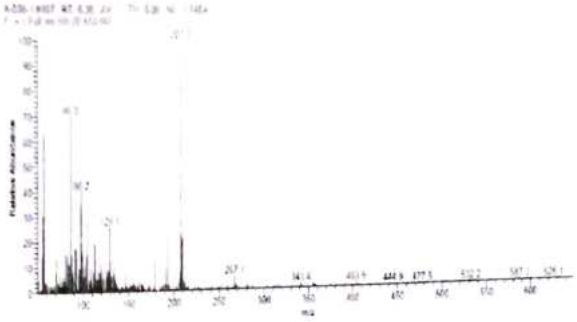


2-Butanone, 3,3-dimethyl-1-(methylsulfonyl)-,O-[methylamino)carbonyl]oxime

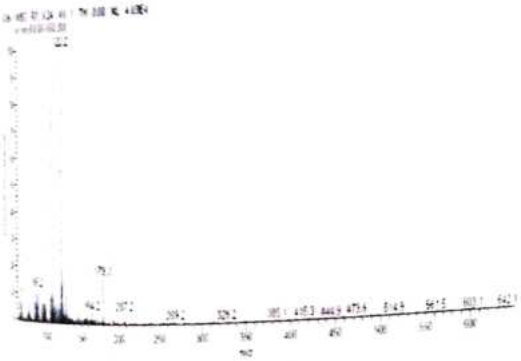




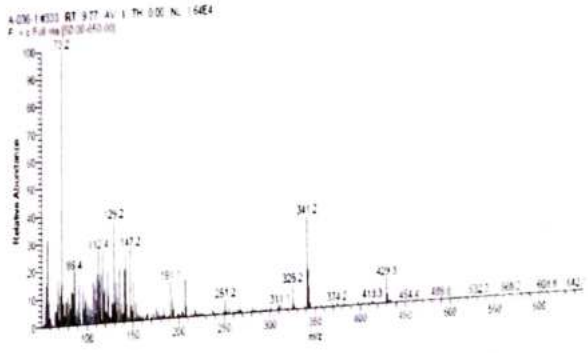
(c) Benzene, 1,3,5-trimethyl



(d) 5H-dibanro[b,f]azepine-5-carboxamide N-oxide



(e) (2s,4r)-4-hydroxy-2-benzylprolin-halbhydrate



(f) 11á-(1-Ethenyl)-21-chloro-(17à,20Z)-19-norpregna-1,3,20-tetraene-3,17á-diol

Figure 5: Mass spectrum of some phytocomponents identified from leaf extract of *Gardenia gummifera*

Table 8. Biological Activity of Phytocomponents Identified in Leaf extract of *Gardenia gummifera*

S.NO	NAME OF THE COMPOUND	Nature of Compound	Activity
1	2-Butanone, 3,3-dimethyl-1-(methylsulfonyl)-, O-[(methylamino)carbonyl]oxime	Carbonyl	Antifibrinolytic, antialcoholic, Antipsoriatic, Antioxidant, HypocholesterolemicNematicide, Antirickettsial, Antileprosy, Pesticide, Flavor, Lubricant, Antiandrogenic, Hemolytic 5-Alpha reductase Inhibitor,Antihistaminic, AnticoronaryInsectifugeAntieczemic Anticancer, Anti-inflammatory.
2	L-Serine, O-(phenylmethyl)-	Amino acid	Treatment for dementia, Treatment for Hypertenstion, Anticancer, Treatment for infertility, diabetes, Obesity. Treatment for HIV, Improve the symtoms of kidney, Blood pressure, Heart attact reciver, Preeclampsia, Antiasthma, Antiinflammation.Convert of the simple sugar glucose into energy, Aerobic activity, Pyruvate, Treating Epstein Barr virus, Insulin depended Diabetes, Prevent night time hypoglycemia.
3	Benzene, 1,3,5-trimethyl	Aromatic methyl hydrocarbon	Anti-inflammatory, Antineoplastic, Antiurolithic, Antiobesity, Antiprotozoal (Toxoplasma, Rheumatoid arthritis treatment, Autoimmune disorders treatment, Transplant rejection treatment, pancreatic disorder treatment, Alzheimer's disease treatm
4	(3R)-3-Phenyl-2,3-dihydro-1H-isoindol-1-one	Phenyl	Antiasthmatic, Anticoagulant, antiallergic, Anti-inflammatory, Antineoplastic, Antiurolithic, Antiobesity, Antiprotozol a (Toxoplasma), Antihypertensive, Anticataract, Fibrinolytic, Sickle-cell anemia treatment, Antidiarorheal, Antiarthritic, Antianemic, Antihypertensive, Antihyperlipo proteinemic, Immunostimulant, Microtubbule formation stimulant, HIV-1 integrase.
5	Dodecane	Alkane hydrocarbon	Antineoplastic, Antineurotoxic, Antiviral, Antibiotic, Antiprotozol, Antihemorrhagic, Antiseptic, Antithrombocytopenic, Antituberculosis, Antiinflammatory, Antineoplastic, Antimycobacterial, Antiperistatic, Antiallergic, Antifungal
6	Memantine	Amantadine derivative	Antidiabetes, It is used to treat moderate to severe Alzheimer's disease. It acts on the glutamatergic system by blocking NMDA receptors.
7	QUERCETIN 7,3',4'-TRIMETHOXY	Flavonoid	Antioestrogenic ,antiproliferative , Anticancer, *anti*-inflammatory, inhibitory activity, anti-tumor cell activity, Antipruritic, Antipruritic, Antihypercholesterolemic, Antiprotozoal,

			Antineurohenic, Antiinflammatory, Antiviral, Antiseborrheic.
8	4-Cyano-2H-1-benzothiopyran	Heterocyclic pyran	Anticoagulant, antiallergic, Anti-inflammatory, Antineoplastic, Antiurolithic, Antiobesity, Antiprotozoal (Toxoplasma), Antihypertensive, Anticataract, Fibrinolytic, Sickle-cell anemia treatment, Antidiarorheal, Antiarthritic, Antianemic, Antihypertensive, Antihyperlipo proteinemic, Immunostimulant, Microtubbule formation.
9	à-Copaene	Resin-exocyclic-methylene	Essential oils, antioxidant, *Antimicrobial activity, Used by skin care product.*
10	a-Cedrene	Cyclohexanone ring	*Antimicrobial activity,* Antioxidant, Antitumer
11	Torreyol	Alcohol	Antimicrobial, Amyotrophoc lateral sclerosis treatment, Atherosclerosis treatment, Hair growth stimulant, HIV-1 reverse transcriptase inhibitor, Antidiarorheal, Antiarthritic, Antianemic, Antihypertensive, Antihyperlipo proteinemic, Immunostimulant.
12	ε-Cadinene	Ketone	Anti-estrogenic, Anti-inflammatory, Antidiarorheal, Antiarthritic, Antianemic, Antihypertensive, Antihyperlipo proteinemic, Immunostimulant.
13	Dotriacontane	Alkanes	Anticardioc, Antipruritic, Antipruritic, Antihypercholesterolemic, Antiprotozoal, Antineurohenic, Antiinflammatory, Antiviral, Antiseborrheic.
14	7-Methyl-Z-tetradecen-1-ol acetate	Acetate	*Antimicrobial activity.*
15	Isopropyl myristate	Ester of isopropyl alcohol	Moisturizing Cream, Age Minimizer Dual Foundation, Emollient, thickening agent, or lubricant in beauty products. Myristic Acid (a naturally-occurring fatty acid), cosmetic and pharmaceutical ingredient.
16	PHYTOL ISOMER	Chlorophyll	Antioxidants, Antibacterial, Antiseptic, Antispirochetal, Anticataract, Antidiarrheal, Antileprosy, Antihyper lipoproteinemic, Antirickettsial, Anticarcinogenic, Antiviral.
17	Lucenin 2	Amino acid	Treatment for dementia, Treatment for Hypertenstion, Anticancer, Treatment for infertility, diabetes, Obesity. Treatment for HIV, Improve the symtoms of kidney, Blood pressure, Heart attact reciver, Preeclampsia, Antiasthma, Antiinflammation.Convert of the simple sugar glucose into energy, Aerobic activity, Pyruvate, Treating Epstein Barr virus, Insulin depended Diabetes, Prevent night time hypoglycemia.
18	Pentadecanoic acid, 14-methyl-, methyl ester	Fatty acid methyl ester	Anticarcinogenic, Antimalarials, Antineoplastics, Treatment of cancer, Antipruritic,

		Antihypercholesteolemic, Antiprotozoal, Antineurohenic, Antiinflammatory, Antiviral, Antiseborrheic, Menopausal disorders treatment, Transplant rejection treatment, Gyneco logical disorders treatment, Alzheimers's disease treatment, Anticholinergic agent, Therapeutic agent.
Cembrene	Diterpene	Antineoplastic, TPS activity, Antyinhibitory, anti-neoplastic agent, anti-microtubule, Anti cancer, Antibacterial, Antiseptic, Antispirochetal, Anticataract, Antidiarrheal, Antileprosy, Antihyper lipoproteinemic, Antirickettsial, Anticarcinogenic, Antiviral.
a-L-Mannopyranoside, methyl 6-deoxy-2,4-di-O-methyl-, acetate	Methyl acetate	*Antimicrobial activity.*
9-Octadecenoic acid (Z)-, methyl ester	Fatty acid methyl ester	Treatment of oral diseases, antimicrobial activity, Anti-inflammatory, Antineoplastic, Antiurolithic, Antiobesity, Antiprotozoal, Antifibrinolytic, antialcoholic, Antipsoriatic, Antioxidant.
3-Hydroxypropanoic acid, 3-(2,2,6-trimethylbicyclo[4.1.0]hept-1-yl)-, ethyl ester	Ester	Flavor, Perfumery, Antioxidant.
9-Octadecenamide	Amide	Convert of the simple sugar glucose into energy, Aerobic activity, Pyruvate, Treating Epstein Barr virus, Insulin depended Diabetes, Prevent night time hypoglycemia.
9-Desoxo-9-x-acetoxy-3,8,12-tri-O-acetylingol	Alcohol	Antiprozoal (Amceba), Antiperistabic, Antibacterial, Antinociceptive, Antineurotoxic, Antianemic, Antiamyloidgenic, Antimutagenic, Antialcoholic, Antimyopathies, Antituberculosic, Antimycobacterial, Antiviral, Antimetastatic, Astrinhent, Antileprosy, Anticataract, Fibrinolytic, Cytoprotectant.
2-LAURO-1,3-DIDECOIN	Diazole	Antifungal drugs, Antibiotics, Sedative midazolam, Antipruritic, Antihypercholesteolemic, Antiprotozoal, Antineurohenic, Antiinflammatory, Antiviral, Antiseborrheic.
L-Tryptophanamide, 1-methyl-5-oxo-L-prolyl-N,1-dimethyl-L-histidyl-N,N,N à,1-tetramethyl-	Amide	Antimutagenic, Antiviral, Antileprosy, Antiprotozol, antimycobacterial, Antiseptic, Antithrombocytopenic, Antituberculosis, Antiinflammatory, Antineoplastic, Menopausal disorders, Chemopreventive, Venom exonuclease inhibitor, Immunosuppressant
N-(4-Hydroxyphenyl)retinamide	Anilide	Antianalgetic, Antibacterial, Antibiotic, treatment of chemotherapeutic approach to cancer.

plant. Thus, this type of GC-MS analysis is the first step towards understanding the nature of medicinal properties in this medicinal plant and this type of study will be helpful for further detailed study.

Dodecanoic acid, 1-(hydroxymethyl)-1, 2-ethanediyl ester is a fatty acid ester with the molecular formula $C_{35}H_{68}O_5$ which is used asemulsifiers for cream, milky lotion and hair conditioner. Tetrahydrofuran-2-carboxylic acid, dibenzofuran-3-ylamide is a volatile organic compound is a component of chemotherapeutic agents. Lauric anhydride is a fatty acid with the molecular formula $C_{24}H_{46}O_3$ which is used to increase high density lipoprotein, investigate molar mass of unknown substance, treatment of wool in presence of cresol and in w afer form as a carrier in the study of drug release (Chaudhary H, 2012). Further investigation is needed to identify the pharmacological importance and phytochemistry of *G. gardenia.*

SUMMARY AND CONCLUSION

SUMMARY AND CONCLUSION

Nature has been the source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources mainly based on their use in traditional medicine. Over 50% of all modern clinical drugs are the natural product origin. According to WHO, medicinal plants would be best source to obtain a variety of drugs. Therefore, plants should be thoroughly investigated therapeutically. Based on that, the present work has been planned to study and evaluate the medicinal potentiality of *G. gummifera*, a shrub distributed in the Maruthuval Malai of Westerns Ghats. The leaves of the G,gummifera were collected at the top of the mountain of Maruthuval Malai. Then those plants were washed before drying. The dried leaves were powdered and stored in the air tight container for future analysis.

The preliminary phytochemical analysis of *G. gummifera* leaves revealed the presence of several compounds includes flavanoids, tannins, phenolic compounds, terpinoids, saponins and glycosides. The results were interpreted by using the intensity of the colour. But this qualitative analysis is not suitable to quantify the amount of phytoconstituents. So my investigation go ahead to identify the amount of components. The quantitative analysis of leaves of G. gummifera divulged the amount of phenols, flavonoids and tannins. All the whole, the leaf exhibited more phenolics (8.242±0.38) than other components like flavonoids (3.49±0.59) and tannins (1.047±0.07). The functional groups (O-H, N-H, C-H, C=O, C-O, C-N, C=C, S=O, C=N and N=C stretching) reported through FTIR analysis includes aldehydes, alkenes, amines amides, alcohols, phenols, aromatics,

carboxylic acids and anhydride, esters and lactones, ethers . All these functional groups are molecule belong to secondary plant metabolites as per researcher's explanations. The prefunctional groups of carboxylic acids, anhydridessence of characteristic alcohols, phenols, amines, amides, esters, ethers, sulphur derivatives, glycosides, and carbohydrate could be responsible for the various medicinal properties of *G. gummifera*, that was confirmed through antimicrobial assay conducted in the present investigation. Further studies required with this herb to identify the unknown functional groups, isolate and characterize the compounds and elucidate the structure of the bioactive compounds which are responsible for the antimicrobial activity was done using high performing instruments such as HPTLC, GC-MS.

HPTLC analysis was carried out by using methonolic leaf extracts of *G .gardenia* along with the standard flavonoid, quercetin and ethyl acetate ,water, acidic acid, Folic acid (8:10:5:5) as the mobile phase .The identity of the quercetin bands in sample chromatograms was confirmed by the chromatogram obtained from the sample with that obtained from the reference standard solution. So many peaks in the spectrum have been detected but identification through peaks is not clear and is not proven too. For better identification of compound and study the structural configuration further study was tread to GC-MS analysis.

GC-MS is the best techniques to identify the constituents of volatile matter, long chain, branched chain Carbonyl, alcohols acids, Alcohol, Ester of isopropyl alcohol etc.. Twenty seven compounds were identified in leaf powder extract of *G.gummifera* with the retention time ranging from 0.00 to 37.40 minutes. The

compounds with maximum peak area are Benzene, 1,3,5-trimethyl- (CAS) (5.22), N-(4-Hydroxyphenyl) 9-Octadecenoic acid (Z)-, methyl ester (25.62). From the GC-MS analysis of *G. gummifera* leaves the presence of twenty seven phytochemical constituents revealed this is the candidate species to face the future challenges in the field of medicine. The research recommend that conservation of this endangered plant is absolutely necessary before evades from the nature

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PHYTOCHEMICAL ANALYSIS , ANTIBACTERIAL ACTIVITIES OF SELECTED SPECIES OF MALVACEAE

A dissertation submitted to

ST.MARY'S COLLEGE (Autonomous), Thoothukudi

Affiliated to

MANONMANIAM SUNDARANAR UNIVERSITY, Thirunelveli

in partial fulfillment of the requirements for the Degree of

MASTER OF SCIENCE IN BOTANY

By

P.NITHIYA
Reg.No.18APBO07

DEPARTMENT OF BOTANY

ST. MARY'S COLLEGE (Autonomous)

(Re- accredited with 'A^{+}' Grade by NAAC)

THOOTHUKUDI -628001

2019-2020

CERTIFICATE

This is to certify that this dissertation entitled, **"Phytochemical Analysis , Antibacterial Activities of Selected Species of Malvaceae"** submitted by **P.NITHIYA Reg.No. 18APBO07** to ST.MARY'S COLLEGE (Autonomous), THOOTHUKUDI in partial fulfillment for the award of the degree of **"Master of Science in Botany"** is done by her under my supervision. It is further certified that this dissertation or any part of this has not been submitted elsewhere for any other degree.

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DECLARATION

I do here by declare that this dissertation entitled **"Phytochemical Analysis , Antibacterial Activities of Selected Species of Malvaceae"** Submitted by me in partial fulfilment for the award of the degree of **'Master of Science in Botany'**, in the result of my original and independent work carried out under the guidance of **Dr. A. Jacintha Tamil Malar M.Sc., M.Phil., Ph.D.** Assistant Professor, Department of Botany, St. Mary's College (Autonomous), Thoothukudi and it has not been submitted elsewhere for the award of any other degree.

Station: Thoothukudi

P. Nithiya
(P.NITHIYA)

Date:

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I am really grateful to **Dr. Rev. Sr. A.S.J. Lucia Rose M.sc., PGDCA., M.Phil., Ph.D.,** Principal, St. Mary's college (Autonomous), Thoothukudi for genuine words of encouragement and support during my study.

I am immensely grateful to **Dr. M. Glory M.sc., M.Phil., Ph.D**., Head of the Department of Botany, St. Mary's college (Autonomous), Thoothukudi for her intellectual inspiration and constant support throughout the course.

I express my sincere thanks to all Staff members and laboratory Assistants, Department of Botany.

Last but not least I thank my family for their lovable care, encouragement and constant help during the course of study.

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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. 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. 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 secor. 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 *Abutilon indicum*. and *Thespesia populnea* 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.

Abutilon indicum belongs to the family Malvaceae; it is a hairy under-shrub with golden yellow flowers, and distributed in all parts of tropical and sub tropical region of India. The plant is commonly called as Thutti. This plant parts are very much used in Siddha medicines. All parts of this plant have been recognized to have medicinal properties; this plant is used to treat anthelmintic, anti-inflammatory, urinary and uterine discharges, piles and lumbago (Porchezhian *et al*., 2000). *A. indicum* leaves are used in the treatment of toothache, anti-fertility and liver disorders (Anyensu *et al*., 1978). Root and bark are used as aphrodisiac, antidiabetic, nervine tonic, and diuretic (Lakshmayya *et al*., 2003). A chemical compound, β-sitosterol, which has been identified as the active ingredient in many medicinal plants, is present in *A. indicum* and a petroleum ether extract provided larvicidal properties against the mosquito larvae *Culex quinquefasciatus*.

Thespesia populnea belongs to the family Malvaceae is a large tree found in the tropical regions and coastal region forests of India. Various parts of *T. populnea* are found to possess useful medicinal properties, such as antibacterial, antioxidant, purgative, antifertility, anti-inflammatory and hepatoprotective activity The bark, leaves, flower and fruits are positive in skin infection such as ringworm, eczema, scabies, psoriasis, and guinea worm in folk medicine The leaves and bark of this tree are still used to mix with oil for the treatment of fracture wounds and as an anti-inflammatory wrapping applied to ulcers and boils.

Plant source is still mostly unexplored and merely a small percentage of them has been subjected to phytochemical investigations, and the fractions submitted to pharmacological screening is very low. The present investigation is under taken to

evaluate the phytochemical constituents using FT-IR , and antibacterial activity of leaf extract *Abutilon indicum* and *Thespesia populnea.*

In view of these fact, the present investigation is under taken

- To elucidate the macroscopic characteristic of *Abutilon indicum* and *Thespesia populnea*
- To carry out preliminary phytochemical screening to establish the phytoconstituents present.
- To analyse the functional groups of the selected plants by FTIR
- To evaluate the Antimicrobial activity of *Abutilon indicum* and *Thespesia populnea*

REVIEW OF LITERATURE

Medicinal plants are the nature's gift for human beings to make disease free healthy life. It plays a vital role to preserve our health. India is one of the most medico-culturally diverse countries in the world where the medicinal plant sector is a part of time-honored tradition that is a respected even today. Here, the main traditional systems of medicine include Ayurveda, Unani and Siddha (Kotnis, *et al.,* 2004). With the emerging worldwide interest in adopting and studying traditional systems and exploiting their potential based on different health care systems, the evaluation of the rich heritage of traditional medicine is essential (Gupta, 2010).

The ethnomedicinal usage of *Abutilon indicum* 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 tuberculosis, ulcers, bleeding disorders, and worms (Gairola *et al.,* 2013). Juice of fresh leaves are used as anti-helminthic, anti-vomitting gastric disorders and ulcers (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 (*T. populnea* Linn. that is also known as "portia tree") 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, swellings, chronic bowel complaints and nervous system disease and acts as analgesic, anti-inflammatory, hypoglycemic activities etc. (Sharma,2013). Various Ayurvedic preparation of *T. populnea* 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, *et al.* (1999) reported the phytochemical analysis of *A. indicum.* The plant 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.

All parts of *T. populnea* are used medicinally (Lander and Morrison, 1962). Leaves are used as a remedy for piles and as demulcent tonic. A decoction of *T. populnea*

is used in bronchitis, catarrhal bilious diarrhoea, gonorrhoea, inflammation of the bladder and fever (Ali *et al.,* 2009). The flowers and leaves are used as a local application to boils and ulcers (Mhasker *et al.,* 2000). Seeds are used in treatment of cold, cough and bronchial infection, inflammation of the urinary tract, gonorrhea, diarrhoea, and ulcers. Seeds are also used as diuretic and demulcent (Vaghasiya and Chanda, 2007). The seeds cakes are used for dairy cattle and fertilizer (Gutkin, 1950).

A. indicum traditionally, the plant is used in inflammation, piles, gonorrhea treatment and as an immune stimulant. Root and bark are used as aphrodisiac, anti diabetic, nervine tonic, and diuretic. Seeds are used in urinary disorders. The seeds are used as a laxative in piles and in the treatment of cough. According to the Chinese in Hong Kong, the seeds are employed as an emollient and demulcent. The bark and the root are used as a diuretic, anthelmintic, pulmonary sedative and in fever. The juice from its leaves has been used to formulate into an ointment for quick ulcer healing. Its extract is also used in relieving thirst; in treating bronchitis, diarrhea, gonorrhea, and inflammation of the bladder; and in reducing fever. In addition, it has good medicinal value and useful to treat diseases like cleaning wounds and ulcers, treating vaginal infections, diabetes, and hemorrhoids; and can also be used as an enema (Kashmiri *et al*., 1992).

The root of the *A. indicum* has a diuretic property and can be taken for the relief of hematuria. It is also effective in the treatment of leprosy. The seeds from this plant are considered to be aphrodisiac and can be used as a laxative for those having hemorrhoids and in the treatment of coughs, puerperal disease, urinary disorders, chronic dysentery, and fever (Thongsiri *et al*., 2001).

Kirtikar *et al*. (1992) reported the ethnomedicinal uses of the plant *T. populnea* (Linn.) Soland. The leaves and fruits are used for treatment of inflammation. The leaves, roots, fruits, bark are used to treat various skin infections such as ring worms, warts,

psoriasis, scabies, sprains, bruises and cutaneous diseases. Fruits and bark are used in treatment of urethritis & gonorrhea. Fruits are used in migraine headache. Bark is used in haemorrhoids and chronic dysentry. In ayurveda, root part suggested to remove 'vatta' and 'pitta' and also used as a tonic, aphrodisiac and treatment of burning of body and heart diseases. Flowers are used in itching.

Phytochemical

Plants produce a great number of secondary metabolites, many of them with antibacterial and antifungal activity. Well-known examples of these compounds include flavonoids, phenols and phenolic glycosides, unsaturated lactones, sulphur compounds, saponins, cyanogenic glycosides and glucosinolates (Gomez *et al.,*1990).

Filho *et al.* (1996) isolated several compounds from *Abutilon indicum,* such as alkaloids, tannins, flavonoids, lignans, phenols and terpenes. Plant prominently contains mucilage, tannins, aspargines, gallicacid (Kashmiri *et al.,* 2009), sesquiterpines, alkaloids, leucoanthocyanins, flavonoids, sterols, triterpenoids, saponins and cardiac glycosides (Khare, 2007). Plant is also reported to contain fructose, galactose, glucose n- alkaline mixture, analkanol fraction, beta - sitosterol, vanillic acid, P- coumaric acid, Phydroxybenzoic acid, caffeic acid, fumaric acid, P- beta-D-glycosyloxybenzoic acid, leucine, histidine, threonine, serine, glutamic acid, aspartic acid, galacturonic acid, the plant contains 2 (new) sesquiterpene lactones (Sharma and Ahmad, 1989).

Saxena *et al.* (2010) studied phytochemicals and antimicrobial activity of stem bark extracts of *T. populnea* and revealed that *T. populnea* extracts exhibited antimicrobial activities at a concentration of 20 mg/ml. The phytochemical analysis of the petroleum ether, benzene and chloroform extract of stem bark of *T. populnea* revealed the presence of phytosterols whereas methanolic and aqueous extract showed the presence of

tannins, flavonoids, carbohydrate, alantolactone and isoalantolactone (Kashmiri *et al.,* 2009). The investigation on the chemical constituents of the whole plant of *Abutilon indicum* has resulted in the isolation of two new compounds, abutilin A and (R)-N-(r-methoxycarbonyl-2'- phenylethyl)-4-hydroxybenzamide (Kuo *et al,* 2008). A study showed that *Abutilon indicum* contained Luteolin, Luteolin 7-0- beta- glucopyranoside (Matlawska and Sikorska, 2002). Gossypetin-8 and 7 glucosides and cynidin - 3 – rutinoside is also-isolated by Subramanian and Nair, (1972).

Saravanakumar *et al.,* (2009) evaluated antibacterial activity of *T. populnea* flower extracts and reported that methanolic extract contains flavonoids, alkaloids, tannins and anthroquinone and glycosides. Antibacterial results showed that 7.2% of methanolic extract were active in the lowest tested concentration of 62.5 µg/ml, 5% active in the concentration of 250 µg/ml, 75.7% active in the concentration of 500 µg/ml and 92.8% active at the concentration of 1000 µg/ml in a dose dependent manner.

Seven flavonoid compounds including quercetin and its glycosides have been isolated from the flowers of *A. indicum* by Irena and Maria, (2002). Viswanatha *et al.,* (2008) investigated the phytochemical constituents and antimicrobial activity of alcoholic (ALTP) and aqueous (AQTP) extracts of *T. populnea* stem bark by cup plate technique. Preliminary phytochemical investigation showed presence of alkaloids, carbohydrate, glycosides, saponins, proteins, flavonoids, tannins and phenolic compounds in alcoholic as well as aqueous extract. AQTP extract showed significant antimicrobial activity against tested bacterial organism compared to ALTP extract. *A. indicum* leaves contain quercetin 72% than flowers (Rajalakshmi and Senthil, 2009). The leaves contain 11.5% asparagines, a diuretic principle (Kashmiri *et al,* 2009).

Huang *et al.,* (2003) reported that the aerial parts of *T. populnea* contain alkaloids, flavonoids, phenols, coumarins, tannins, terpenoids and lignans.

Phytochemical screening of the ethanolic extract of whole plant *A. indicum* Linn was shown the presence of alkaloids, carbohydrates, phenols, sterols, terpenes and flavonoids (Durai Muthumani *et al.,*2005).

Anandjiwala *et al.,* (2007) evaluated antioxidant activity of stem bark of *T. populnea.* The preliminary phytochemical investigation indicates the presence of high amount of phenolics, tannins and flavonoids. Methanolic extract showed very good DPPH radical and superoxide scavenging activity in dose dependent manner.

A.indicum has been explored phytochemically by various researchers and found to possess number of chemical constituents. The whole plant contains mucilaginous substances, asparagines, saponins, flavonoids, alkaloids, hexoses, n-alkanes and mixtures alkanols as the main compounds. Some important constituents reported in the plant are β-sitosterol, vanillic, *p*-coumaric, caffeic and fumaric acids, abutilon A, (R)-N-(1'-methoxycarbonyl1-2' phenylethyl)-4-hydroxybenzamide, galacturonic, *p*-β-Dglycosyloxybenzoic and amino acids. The plant contains of an essential oil consisting of α-pinene, caryophyllene, caryophyllene oxide eudesmol, farnesol, boreniol, geraniol, geranyl acetate and α-cineole along with a number of other minor constituents (Kuo *et al.,* 2008).

Mutiniside, new phenolic glycoside, and the flavonoidal glycoside cephacoside have been isolated from the n-butanol soluble fraction. Seven known compounds namely, lupeol, beta-sitosterol, stigmasterol, methyl-4- hydroxybenzoate, taraxacin, ursolic acid, and beta-sitosterol-3-O-beta-Dglucopyranoside, have been isolated from the EtOAc soluble fraction of *T. populnea* (Ali *et al.,* 2009).

Anandjiwala *et al.,* (2007) observed the preliminary anti-implantation activity of petroleum ether and ethyl acetate and alcoholic extracts of *T. populnea* seeds. The tests were undertaken in female albino rats. This study divulged the anti-implantation activity

at the dose of 110 mg/kg body weight while ethyl acetate extract was effective at the same dose. The alcoholic extract showed no such significant action.

The compounds such as lignans, flavonoids, hydrolysable tannins, polyphenols, triterpenes, sterols and alkaloids isolated from *A. indicum* showed a wide spectrum of pharmacological activities including antiviral, antibacterial, antiplasmodial, anti-inflammatory, antimalarial, antimicrobial, anticancer, antidiabetic, hypolipidemic, antioxidant, hepatoprotective, nephroprotective and diurectic properties. (Patel *et al.,* 2011).

The phytochemical screening of plants of *T. populnea* carried out by Ahirrao, (2011) and confirmed the presence of alkaloids, flavonoids, tannins, steroids, terpenoids, sterols and carbohydrate. .

The methanolic extract of the leaves of *A. indicum* were subjected to phytochemical analysis by standard qualitative analysis and the *in vitro* antioxidant activity was evaluated by determination of total antioxidant capacity, 1.1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity, Antioxidant activity, Nitric oxide potential. The analyses revealed that the methanol extract of *Co* was able to efficiently scavenge the free radicals in a dose dependant manner (Tamizhazhagan *et al*., 2017). Saranya *et al*., (2017) studied the amount of phenolics (gallic acid equivalents 3.89-8.55 mg/g), flavonoids (quercetin equivalents 9.47-37.66 mg/g) and tannins (tannic acid equivalents 10.47-13.58 mg/g).

Prabakaran *et al.,* (2014) investigated the phytochemical and functional group screening of *T. populnea* and *A. indicum* by FTIR spectroscopic analysis. The FTIR analysis of methanol leaf extracts of *T. populnea* and *A. indicum* confirmed the presence of amides, amino acids, proteins, lipids, carbonyl group, nitro compounds, sulpur

compounds, nitrosamine, monofluorinated compounds, sulphinic acid group, thiol group, bromo compounds, iodo compounds, lactams and alkanes which showed major peaks.

Karthika.K *et al.,* (2014) studied the quantitative phytochemical analysis of *A. indicum* species exhibited the presence of alkaloids, total phenolics, total flavonoids, tannins, saponins and ascorbic acid in considerable quantity.

Muruganantham *et al*. (2009) carried out the FTIR and EDS spectral analysis of plant parts like leaf, stem, and root of the medicinal plants, *A. indicum* and *T. populnea* and reported the presence of characteristic functional group of carboxylic acids, amines, amides, sulphur derivatives, polysaccharides, nitrates, chlorates and carbohydrate that are responsible for various medicinal properties of both herbal plants.

The FTIR analysis of methanolic and aqueous leaf extracts of *A. indicum* revealed the presence of protein, oil, fats, phenolic compounds, flavonoids, saponins, tannins and carbohydrate as major functional groups (Gaurav kumar *et al.* 2010)

Ragavendran *et al.* (2011) screened the functional group of carboxylic acids, amines, amides, sulphur derivatives, polysaccharides, organic hydrocarbons, halogens that are responsible for various medicinal properties of *T.populnea*

Thangarajan Starlin *et al*. (2012), while analyzing the edhanolic extracts of *A. indicum* by FTIR, revealed functional group components of amino acids, amides, amines, carboxylic acid, carbonyl compounds, organic hydrocarbons and halogens.

Pednekar and Raman (2013) analyzed the methanolic leaf extract of *T.populnea* by FTIR and reported that the transition metal carbonyl compounds and aliphatic fluoro compounds were present in the extract.

Antibacterial

The increasing prevalence of multidrug resistant strains of bacteria and the recent appearance of strains with reduced susceptibility to antibiotics raises the specter of untreatable bacterial infections and adds urgency to the search for new infection- fighting strategies (Sieradski *et al*., 1999). 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)

Jigna Parekh *et al*.,(2005) tested the potential antibacterial activity of *T. populnea* (Linn.) against 5 medically important bacterial strains, namely *Bacillus subtilis* ATCC6633, *Staphylococcus epidermidis* ATCC12228, *Pseudomonas pseudoalcaligenes* ATCC17440, *Proteus vulgaris* NCTC8313 and *Salmonella typhimurium* ATCC23564. The antibacterial activity of aqueous and methanol extracts was determined by agar disc diffusion and agar well diffusion method. The methanol extracts were more active than the aqueous extracts. The plant extracts were more active against Gram – positive bacteria than against Gram – negative bacteria the most susceptible bacteria were *B. subtilis* followed by *S. epidermidis,* while the most resistant bacteria were *P. vulgaris,* followed by S. typhimurium

Sudhakar *et al*., (2006) compared the antimicrobial activity dry fruits of *T. populnea*, aerial parts of *Asystasia gangeticum* and flowers of *Caesalpinia pulcherrima*, against *Escherichia coli* (enteropathogen), *Proteus vulgaris, Pseudomonas aeruginosa, Staphylococcus aureus, Candida albicans, Aspergillus nigerand* and *Rizopous oligosporus*. Ethanolic extracts of all plants exhibited significant antimicrobial activity against *Escherichia coli , Proteus vulgaris, Pseudomonas aeruginosa* and *Staphylococcus aureus . caesalpinia pulcherrima* only exhibited significant anti fungal activity against *Candida albicans, Aspergillus nigerand, Rhizopuso ligosporus.*

A. indicum Schum and thonn is an another important medicinal plant species due to its antiviral properties and useful against hepatitis infection (Raphael *et al*., 2002). Zorinsahalan *et al.,* (2007) screened two species of plants, *Andrographis paniculata* and *A.indicum* for antibacterial activity against three Gram positive (*Staphylococcus aureus* , *Bacillus subtilis, Staphylococcus epidermidis*) and three gram negatives (*Escherichia coli* ,*Klebsiella pneumonia* and *Pseudomonas aeruginosa*) bacteria. The leaves from both plants were extracted by methanol extraction. Minimum inhibitory concentration (MIC) value of *Andrographis paniculata* ethanol extract for both gram positive and gram negative bacteria ranges between 1.56mg / ml to 12.5mg / ml and for *Abutilon indicum* 3.13mg / ml to 12.5 mg / ml Gram negative bacteria showed higher MIC value than gram positive bacteria due to presence of cell wall.

Shanmugaraju *et al.,* (2007) isolated and reported a antibacterial activity compound of *T. populnea*. Ethanol and aqueous extracts of *T. populnea* tested against *E.coli*, *Klebsiella pneumonia, Proteus species, Pseudomonas aeruginosa, Salmonella typhi, Staphylococcus aureus,* and *Streptococcus species.* For *Klebsiella pneumonia, Streptococcus species, Pseudomonas aeruginosa,* and *Staphylococcus aureus,* ethanol extract alone exhibited antibacterial activity. A maximum antibacterial activity was

achieved by aqueous extract on *Protease* and *Salmonella typi.* Both ethanol and aqueous extracts inhibited growth of *E.coli.* thus, ethanol extract posses more antibacterial activity than aqueous extract. Gas chromatography analysis of ethanol extract of plant showed the presence of ethanol extract of plant showed the presence of citronellal, a compound responsible for antibacterial activity of *T. populnea*

EI- Mahmood Muhammad Abubakar, (2009) discussed the antibacterial activity of three different extracts of *A. indicum.* Methanol, hexane and distilled water were employed against *Escherichia coli, Klebsiella pneumonia, Shigella dysentriae, Salmonella typhi* and *Proteus mirabilis,* cause enteric infections in humans. Phytochemical screening revealed the presence of tannins, saponins, phenolics, flavonoids, cardiac glycosides anthroquinones and alkaloids. Growth of bacteria especially *Escherichia coli* and *Salmonella typhi* more susceptible to plant material.

Aniel kumar *et al.,* (2010) studied the phytochemicals and antimicrobial activity of stem bark extracts of *T. populnea.* The different solvent extracts of stem and bark of *T. populnea* exhibited antimicrobial activities at a concentration of 20 mg/ml. The phytochemical analysis of the petroleum ether, benzene and chloroform extract of stem bark of *T. populnea* revealed the presence of phytosterols whereas methanolic and aqueous extract showed the presence of tannins, flavonoids, carbohydrates and phenols.

Prabahar *et al*., (2009) carried out the antibacterial activity of the plant *A. indicum* to determine the antibacterial activity of different extracts of the leaves on gram possitive 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* .

Ibrahim *et al.,* (2012) reported the synergistic activity of methanolic extract of *T. populnea* flowers with oxytetracyclines. They reported the MIC of methanolic extract in combination with oxytetracycline using 12 different Gram positive and Gram negative bacteria.. The highest synergism rate was attained against *Shigella boydii.*

The antibacterial activity of *A. indicum* was determined by agar well diffusion method. It was found that 50mg / ml of methanolic extract of the plant able to inhibit the growth of nosocomial infection causing bacteria when compared to other solvent extracts. From this it was concluded that the solvent methanol able to leach out antimicrobial principle very effectively from the plant than the other solvents. The phytochemicals present in the *A. indicum* was tested and it conferred that the possible antibacterial principle resided in tannins and alkaloids (Murugan and Saranraj, 2011).

Shanmuga priya perumal *et al.,* (2012) carried out cytotoxic and antibacterial activity of isolated eight new sesquiterpenoids (populene A-H) from dichloromethane extracts of the wood and dark heartwood of *T. populnea* together with 11 known compounds. The cytotoxic activity of isolated compounds was evaluated against four cancer cell lines: MCF-7, HeLa, HT-29 and KB. The known compounds Mansonone E and (+)-gossypol showed significant activities

The antibacterial activity of aqueous, ethanol and acetone extracts of *Abutilon indicum* was determined against UTI isolates i.e *Proteus mirabilis, Escherichia coli, Proteus vulgaris, Klebsiella pneumoniae, Enterbacter cloacae, Providencia pseudomallei. Pseudomonas aeruginosa* and *Klebsiella oxytoca* by disc diffusion method. Acetone and ethanol extracts provided more consistent antimicrobial activity as compared to aqueous extract. The acetone extract showed maximum activity against all the isolates tested except *E. coli, P. pseudomonas* and *K oxytoca* and ethanolic extract significantly

inhibited growth of *P. aeruginosa* and *E. cloacae.* Aqueous extract was found to be inactive against any of the isolates (Sharma *et al,* 2009). Similarly Parekh *et al,* observed that aqueous extract was not effective against *K pneumoniae, E.coli* and *P. pseudoalkaligens* (Parekh and Chanda, 2007).

Gowtham *et al.,* (2018) investigated the leaf extracts of *T. populnea* and *Abutilon indicum* with different solvent like aqueous, methanol, and diethyl. Those extracts were treated against the bacteria like *Bacillus* cereus, *E. coli*, *K.pnemoniae*, Pseudomonas aeuroginosa and and fungi such as *Aspergillus flavus, A.niger. A.terreus, Penicillium* sp. and *Fsarium solani* were performed respectively. However the antimicrobial properties of *Thespesia populnea* and *A.indicum* leaf with methanolic and diethyl ether extract of maximum zone inhibition and excellent performance when compared to other solvent of aqueous extract.

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 *et al*.,(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.*

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.*

MATERIALS AND METHODS

Collection and identification of plant materials

The fresh plant materials of *Abutilon indicum* and *Thespesia populnea* are collected from the Botany garden of St.Mary's College and Thoothukudi in the month of July 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.

Morphological studies:

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

Preparation of the plant extracts :

5 grams of *Abutilon indicum and Thespesia populnea* leaf samples are extracted separately with aqueous, methanol, ethanol, petroleum ether and chloroform by maceration (24 hrs for each solvent) with constant shaking, the homogenates are filtered through Whatman No 2 filter paper and the extracts are stored at 4°c the extract thus obtained are used for various analyses.

Preliminary phytochemical screening of different extracts (Harbrone,1998)

The qualitative phytochemical test for alkaloids, flavonoids, tannins, sterols, saponins, quinines, phenols, terpenoids, coumarins, betacyanins, anthraquinone, carbohydrates, glycosides, proteins are carried out in the concentrated extracts using the standard procedures to identify the constituents in the leaf extracts of *Abutilon indicum*

and *Thespesia populnea*. The chemical test for various phytoconstituents in the extracts are carried out as described below.

Test for tannins (Ciulei,1994)

1 ml of extract, 2 ml of 5% Fecl3 is added. A dark green or a blue-black indicates the presence of taninns.

Test for saponin: (Kokate, 1999)

Foam test

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

Test for flavonoids: (Savithramma *et al*., 2011)

2 ml of extract,1ml of 2N sodium hydroxide added. The yellow colour is presence of flavonoids.

Test for alkaloid: (Clarke, 1970)

Wagner's test:

A fraction of extract is low amount, wagner's regant(1.27g of iodine and 2g of pottassium iodide in 100ml of water) and formation of reddish brown colour precipitate,confrims the presence of alkaloid.

Test for glycosides: (Ramakrishnan *et al*., 1994)

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

Test for phenol:

To 1 ml of the extract,2ml of distilled water is added,followed by few drops of 10% aqueous ferric chloride. Appearance of the blue or green colour indicates the presence of phenols.

Test for steroids:

2 ml of acetic anhydride was added to 2 ml extract of each sample followed by careful addition of 2 ml H_2SO_4. The colour changed from violet to blue or green indicate the presence of steroids.

Test for carbohydrate (Ramakrishnan *et al*., 1994)

Fehling's test

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

Test for protein

Ninhydrin test

About 0.5 ml of extract is taken and 2 drops of freshly prepared 0.2% ninhydrin reagent is added and heated. The appearance of pink or purple colour indicates the presence of proteins, peptides or amino acids.

Test for coumarins

To 1 ml of plant extract, 1 ml of 10% NaOH is added, formation of yellow colour indicates the presence of coumarins.

Test for terpenoids

To 0.5 ml of the plant extract, 2 ml of chloroform along with concentrated sulphuric acid is added . formation of red brown colour at the interface indicates the presence of terpenoids.

Test for betacyanin (Selvaraj *et al.,* 2014)

To 2 ml of the plant extract, 1 ml of 2 N sodium hydroxide is added and heated for 5 min at 100°c formation of yellow colour indicates the presence of betacyanin.

Test for quinines (Egwaikhide and Gimba,2007)

A small amount of extract is treated with concentrated Hcl and formation of yellow colour precipitate indicates the presence of quinines

Test for anthraquinone (Kokate *et.al.,*1995)

About 0.5 ml of the extract is taken into a dry test tube and 5 ml of chloroform is added and shaken for 5 minutes. The extract is filtered and the filtrate was shaken with equal volume of 10% ammonia solution a pink violet or red colour in the lower layer indicates the presence of anthraquinone.

Quantitative test:

Flavonoid (Zhinshen *et al.,*1999)

100mg of plant material is homogenized with 10ml of distilled water and filtered through a muslin cloth.0.5 ml of the extract is added with 2.5 ml distilled water and mixed. After 6 minutes 0.15 ml NaNo2, is added and again after 6min 0.3 ml of 10% AICI3 is added. After 5 minutes 1 ml of 1M NaOH and 0.5 ml of water are added

following through mixing of the solution the absorbance against blank are recorded at 510nm. Quercetin is used as standared and the results are expressed as my qucertin equivalents (QE)1 g fresh weight.

Tannin (Julkunen-Tritto,1985)

100 mg of the sample is homogenized with 10 ml of distilled water and filtered through a muslin cloth. 0.1 ml aliquot of aqueous extract is mixed with 3 ml of 4%vanillin (prepared with methanol) and 1.5 ml of concentrated HCl. The solution is shaken vigorously and left to stand at room temperature for 20 minutes in darkness. Absorbance against blank is red at 500 nm using UV visible spectrophotometer. Results are expressed as mg catechin equivalent (CE)/g dry weight.

Phenol (Duan *et al.*,2006)

100 mg of sample was homogenized with 10 ml of distilled water and filtered through a muslin cloth 1 ml of the filtrate was added to 1.5 ml of deionized water and o.5 ml of 50% folin ciocalteau reagent and the contents were mixed thoroughly. After 1 min, 1 ml of 20% sodium carbonate solution was added mixed the control contained all the reagents except the sample. After 30 minutes of incubation at 37°C, the absorbance was measured at 750 nm. Total phenolics were calculated as gallic acid equivalent (GAE) Per gram fresh weight.

FT-IR analysis (Kareru *et al*., 2008)

10 mg of the dried extract powder is encapsulated in 100 mg of KBr pellet, in order to prepare translucent sample disc. The powered sample of each plant specimen is loaded in FTIR spectroscope (Shimadzu, iraffinity 1 Japan), with a scan range from 400 to 4000 cm-1 with a resolution of 4 cm-1.

Growth and maintenance of test microorganism for antimicrobial studies:

Bacterial cultures of *Bacillus subtilis* and *Escherichia coli,* are obtained from the Department of Microbiology, St.Mary's College (Autonomous) Thoothukudi. The bacteria are maintained on nutrient broth (NB) at 37^{o}C

Description of bacteria used for testing Antibacterial activity:

1.*Escherichia coli*

Family : Enterobacteriaceae

E.coli is a Gram negative, rod shaped bacterium that is commonly found in lower intesting of warm – blood organisms, can cause serious food poisoning in humans.

2. *Bacillus subtilis*

Family: Bacillaceae

Bacillus subtilis is a Gram positive, aerobic and motile bacterium. It is human pathogens. *Bacillus subtilis* can contaminate food and cause food poisoning in rare case.

Broth medium

Nutrient broth Himedia MOOI

Nutrient broth 1.3 gm

Distilled water 100 ml

2-3 ml of sterilized broth medium is taken in the sterilized culture tube. The inoculating loop is flamed and after a few minutes a loop full bacterial colony is transferred to the broth medium. This microbe culture is 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 are dissolved and sterilized.

Antibacterial activity:

Antibacterial studies are carried out by agar diffusion method, against the organisms *Bacillus subtilis* and *Escherichia coli.* Nutrient agar medium is prepared and poured into petri plates and inoculated with 1 ml of the test organism. The type of organism, medium, date and extract used are marked on the plates. When the agar gets solidified the disc loaded with each sample are placed on the surface of the medium. For positive control, streptomycin disc (100 µg / ml) is used, where as for negative control, respective solvents are loaded on sterile discs. All the assays are carried out in duplicates. Plates are incubated at 37^{o}c for 24 hours at room temperature. After 24 hours the diameter of the zone of inhibition are measured in mm and tabulated.

RESULTS AND DISCUSSION

In the present investication , leaf of *Abutilon indicum* and *Thespesia populnea* are subjected to macroscopic analysis, preliminary qualitative phytochemical analysis, quantitative analysis, FTIR analysis, and antibacterial activity.

Systematic position:

In Benthem Hooker's system of Classification, the systematic position of *Thespesia populnea* is as follows.

***Thespesia populnea* (Linn.)**

Kingdom : Plantae

Class : Dicotyledons

Order : Malvales

Family : Malvaceae

Genus : *Thespesia*

Species : *populnea*

Macroscopic Characters:

Habit : A medium-sized tree with a spreading crown of branches. (Plate-1).

Leaves : Smooth, somewhat similar to those of the Pipal tree, broadly ovate, cordate, acuminate entire, 5- nerved, often with a glandular pore in one or more of the intercostal spaces below. Stipules triangular, deciduous.

Inflorescence : A large Axillary solitary flower

Flowers : Flower large, at first yellow with a purple spot on the inner side near the base, at maturity the flowers turn pinkish red, the spot becoming almost black. Calyx copular,

truncate. Corolla large, yellow with purple base. Staminal tube toothed at the apex. Ovary half-5 celled, style club-shaped at the apex, 5-furrowed.

Fruit : Capsules, globose, covered with minute peltate scales, surrounded at the base by persistent calyx.

Flowering and Fruiting Time : Almost throughout the year, particularly at the beginning of the cold season.

Vernacular names: Tamil : Cheelnathi; Poovarasam

Distribution

A common strand plant extends from the shores of West Bengal to Peninsular India and the Andmans. It is also grown as a road side tree in tropical regions. Probably it originated in India, but is a common plant of coastal strands across old World tropics. It has naturalised in Florida and West Indies; it is also cultivated occasionally in Central and South America and has probably naturalised there.

Systematic position:

In Benthem Hooker's system of Classification, the systematic position of *Abutilon indicum* (Linn.) is as follows.

***Abutilon indicum* (Linn.)**

Kingdom : Plantae

Class : Dicotyledons

Order : Malvales

Family : Malvaceae

Genus : *Abutilon*

Species : *indicum*

Macroscopic Characters:

Habit : An erect shrub, hairy. (Plate-2).

Stem : Herbaceous, aerial, erect, cylindrical, branched, solid, pubescent and green.

Leaves : Cauline and ramal, alternate, suborbicular, broadly ovate, acuminate, velvety on both sides, simple, stipulate, petiolate, deltoid, serrate, slightly hairy, multicostate, reticulate, divergent type.

Inflorescence : . A large Axillary solitary flower

Flowers : Bright-yellow, opening in the evening, bracteates, pedicellate, complete, actinomorphic, hermaphrodite, pentamerous, hypogynous and cyclic. Epicalyx absent. Calyx of 5 sepals, gamosepalous, free at the tips, valvate, persistent, green. Corollayellow, about 3 cm in diameter, made up of 5 petals, polypetalous, slightly connate at the base and adnate to staminal tube, twisted. Androecium consists of indefinite stamens, monadelphous forming a tube around the style, the tube being united with the petals (epipetalous). In the upper part of the staminal tube are borne

monothecous and extrorse anthers. Gynoecium multicarpellary, syncarpous, carpels with a distinct acute point, hairy, shining, ovary superior, multilocular, with one ovule in each locule, placentation axile, style long and stigmas as many as carpels.

Fruits : Multiridged Capsule (carserule)

Seeds : Reniform, blackish-brown, minutely pitted and stellate-hairy.

Flowering and Fruiting time : Throughout the year.

Vernacular names *:* Tamil -Tutti, Paniara, Hutti

Distribution

The plant is found in India, Sri Lanka, topical regions of America an Malesia. It is found as a weed in sub-Himalayan tracts, hills up to 1200 m and in hotter parts of India.

Preliminary Phytochemical screening

The presence of different phytochemical constituents in aqueous, methanol, ethanol, petroleum ether and chloroform extract of leaf of *Abutilon indicum and Thespesia populnea* are evaluated qualitatively and presented in (table 1 and 2). The phytocomponents such as alkaloids, anthraquinones, catechins, xanthoproteins, steroids, tannins carbohydrates, coumarins, flavonoids, phenols and quinines are present in both ethanol and methanolic leaf extract of *A. indicum and T. populnea*

In the current study methanolic extracts of leaves of *T. populnea* contains flavonoids, tannins, phenol, terpenoids, sterol, betacyanin, saponin, carbohydrate, protein, and glycoside. Badoni *et a*l. (2016) reported that the methanolic extract of *T. bellirica* contains alkaloids, tannins, coumarins, flavonoids, phenols and carbohydrate.

The Petroleum ether extract of leaves of *T. populnea* contains of alkaloids, tannins, phenol, terpenoids, sterol, quinones, betacyanin, coumarins, protein, glycoside. Osabor *et al.,* (2015) revealed the presence of cardiac glycosides, saponins, flavonoids, polyphenols, and reducing sugars in the petroleum ether extracts of Zingiber officinale.

The ethanol extract of *A, indicum consist of* flavonoids, tannins, phenol, saponin, anthraquinone and glycoside. The preliminary phytochemical screening of *Mirabilis jalapa* extract showed the presence of bioactive components like alkaloids, flavonoids, phenols, glycosides, tannins, saponins, and lignin (Kumar *et al.,* 2010).

Alkaloids commonly are concentrated in particular organs such as the leaves, bark or roots. Alkaloids play an important role in the defence systems against pathogens and animals. The applications of alkaloids are not limited to biological control of herbivores but also have pharmacological, veterinary and medical importance. Alkaloids belonging to beta-carboline group possess antimicrobial, anti-HIV and antiparasitic

activities (Bouayad *et el.,* 2011). Alkaloids are present in a wide range of plant families and have a variety of biological effects (Roberts and Wink, 1998).

Terpenes also act as diuretics and helps in relieving gastrointestinal spasms. Terpenes are added to creams and ointments to relieve pain and itching terpenes also possess antimicrobial properties thus, helps to fight microorganisms resistant to antibiotics such as yeast and other fungi. Terpenes like menthol when consumed as a tea aids to reduce flatulence and indigestion (Santos *et al.,* 2014). The coumarin acts anticoagulants, which block multiple steps in the coagulation cascade. Coumarins can be used treat the side effects caused by radiotherapy (Agarwal, 2000).

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

Plant sterols are secondary metabolites occurring in plants in small quantities with the highest concentrations in vegetable oils. They reduce total and LDL cholesterol levels in plasma by inhibiting its absorption from the small intestine. Hence , they lower the atherosclerotic risk and offer protection against cardiovascular diseases (Berdiel *et al.,* 2000). Phytosterols may attenuate the inflammatory activity of immune cells, including macrophages and neutrophils (Awad *et al.,* 2004).

Total phenol, flavonoid and tannin

The total phenolic, flavonoid, and tannin content of leaf methanolic extract of *Thespesia populnea* and *Abutilon indicum* are presented in (Table 3-5)

The phenol content of the aqueous extract of *T. populnea* was found to be (1.14 mg QE/g) which is higher than *A. indicum.* Natural phenolic compounds play an important role in cancer prevention and treatment. Various bioactivities of phenolic compounds are responsible for their chemopreventive properties (e.g, antioxidant, anticacinegetic, antimutagenic and anti inflammatory effects).

The flavonoind contens of leaves of *A. indicum and T. populnea* shown in (Fig .1) is 0.29 mg QE/g in *A. indicum* which is more than that of *T. populnea* (0.13mg QE/g). The presence of flavanoid in the leaf of *A. indicum* could accurent for its use as an antiinflammatry agent (Ekwueme *et al* .2011) and for treatment of diarrhoea (Scheie *et al*,2005) fever reducing, pain relieving and anticancer activities.

The tannin content of the aqueous extract of *T. populnea* to be 7.11mg QE/ g which is more than the tannin content of *A. indicum* (5.72mg QE/g) . Tannins are known to possess immunoshinulating activities.(Kumar and Subramanyan 2013) .

FT-IR analysis

FTIR analysis is proved to be a reliable and sensitive method as it provided a unique fingerprint for the biomolecules. It has been used as a requisite method to identify the various function groups responsible for medicinal properties in the herbal drug (Devika *et al.,* 2013). Most researchers applied FTIR spectrum as a tool for discriminating closely associated species (Liu *et al.,* 2006). As the chemical functional groups are responsible for the absorption of the radiation at different frequencies, the frequencies are helpful for the identification of the chemical make up of the samples (Chakraborty, 2016).

The FT-IR spectroscopic analysis of the present study reveals the different characteristic peak values with functional groups of phytocomponents in leaf ethanolic extract of *A. indicum* and *T. populnea* (Fig.4 and 5). FT-IR analysis of leaf extract of *A. indicum* confirms the presence of alcohol, urethanes, alkane, ester, carbonyl, guanidine, aromatic, nitro group, acid, acetals, ether, sulphinic acid, alkyl halide, and halogen compound corresponding to the major peak values at 3609.53, 3285.51, 2926.78, 2855.42, 2310.56, 1744.49, 1649.99, 1553.55, 1514.98, 1455.19, 1373.22, 1333.68, 1241.11, 1165.89, 1036.67, 895.87, 669.25, 537.14, 431.06 cm^{-1} (Table 6). The FT-IR analysis results of ethanolic extract of *T. Populnea* leaf reveals the presence of aromatic amines, alcohol group, urethanes, alkane, ester, carbonyl, guanidine, aromatic, nitro group, acid, acetals, ether, sulphinic acid, aliphatic chloroformate, primary amines, and alkyl halide are the functional groups found in the leaf extract of *T. Populnea* which shows major peaks at 3908.48, 3786.97, 3723.32, 3593.14, 3281.65, 2925.81, 2853.49, 2307.67, 1744.49, 1607.56, 1514.02, 1449.41, 1395.40, 1372.26, 1318.25, 1287.40, 1244.97, 1160.10, 1112.85, 1039.56, 1007.74, 830.30, 768.58, 678.90, 649.00, 581.50, and 513.99 cm^{-1} (Table 7).

Antibacterial activity:

Antimicrobial properties of medicinal plants are being increasing 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 methanolicextracts of *T.populnea* and *A. indicum* against *E.coli* and *B.subtilis.*

Life threatening disease and high rate of mortality occur in animal and human population due to bacterial infection. Many bacteria both Gram positive and Gram negative contaminate food, water, air, soil, ect, and cause biological / microbial pollution. *B. subtilis* is responsible for causing food borne gastroenteritis, *E. coli* , cause diseases like mastitis, abortion and upper .respiratory complication (Jawetz *et al.,*1995)

The ethanol , methanol , chloroform , petroleum ether, and water extracts of two selected plants are tested against pathogenic microbes *E. coli* and *B. subtilis* . The results of antibacterial activity of different plant extracts are shown in (Fig.6 & 7). The antibacterial activity of these plant extract range from 7 mm to 12 mm against *B. subtilis.* The antibacterial properties exhibited by the extracts may be associated with the presence of the secondary metabolites through different mechanism.

The methanolic extract of *T. populnea* showed highest antibacterial activity against both the tested organism (plate 4) .These activities may be due to the phytochemical such as tannin, saponin and glycosides which could serve as the lead to the isolation of haemotherapatic agent. This is an indication that the extract posses substances that can inhibit the growth of some microorganism.

Meanwhile *A. indicum* showed low antibacterial activity against *E. coli* and *B. subtilis* which range from 6 mm to 19 mm and 9mm to 12 mm respectively. This low activity might be due to negligible amount of active principles that the present in the plant (plate 3) The methanolic extract of *A. indicum showed high antibacterial activity against the E. coli* 19 mm followed by the ethanol, 16 mm chloroform, 12 mm petroleum ether, 9 mm and water extract 8 mm. *A. indicum showed lower inhibition against B. subtilis* .

Devi *et al.,* (2009) reviewed that methanol extraction yielded higher antimicrobial activity than other extract. The variation of antibacterial activity among different crude extracts of this investigation might be due to distribution of varied antimicrobial similarly, Owoseni *et al.,* (2010) reviewed that different extracts of plants show different antimicrobial activities on an organism. The present study showed that Gram negative bacteria were more sensitive to the tested methanolic and Ethanol extracts as compare to the Gram positive bacteria.

Some of the extracts has a good potential for therapeutic uses against some pathogens. It appears that extracts with high antimicrobial activity against Gram- negative bacteria do not necessarily have 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 1 : ***Thespesia populnea***

Plate 2 : *Abutilon indicum*

Table 1: Phytochemical screening of different extracts of *Abutilon indicum*

S.NO	Phytochemical	Solvents				
		Aqueous	**Ethanol**	**Methanol**	**Chloroform**	**Petroleum Ether**
1.	Alkaloids	+	–	–	+	+
2.	Flavonoids	+	+	+	+	–
3.	Tannins	–	+	+	+	+
4.	Phenol	–	+	+	+	+
5.	Terpenoids	+	–	+	+	+
6.	Sterol	+	+	+	+	+
7.	Quinones	+	+	+	–	–
8.	Betacyanin	+	–	+	+	+
9.	Coumarins	+	+	+	+	+
10.	Saponin	–	+	+	+	–
11.	Anthraquinone	+	+	+	+	+
12.	Carbohydrate	+	–	–	+	+
13.	Protein	+	+	+	–	–
14.	Glycoside	+	+	+	+	+

Table 2: Phytochemical screening of different extracts of *Thespesia populnea*

S.NO	Phytochemical	Solvents				
		Aqueous	**Ethanol**	**Methanol**	**Chloroform**	**Petroleum Ether**
1.	Alkaloids	+	+	–	–	+
2.	Flavonoids	+	+	+	+	+
3.	Tannins	+	+	+	+	–
4.	Phenol	+	+	+	+	–
5.	Terpenoids	+	+	+	+	+
6.	Sterol	+	+	+	+	+
7.	Quinones	+	+	+	+	+
8.	Betacyanin	–	+	+	–	+
9.	Coumarins	+	–	+	+	+
10.	Saponin	+	+	+	+	+
11.	Anthraquinone	–	+	–	+	+
12.	Carbohydrate	+	+	+	+	–
13.	Protein	+	+	+	+	+
14.	Glycoside	+	+	+	+	+

Table 3: Amount of flavonoids present in the leaves of *Abutilon indicum and Thespesia populnea*

Samples	Amount of flavonoids (mg(QE)/g)	
	Abutilon indicum	***Thespesia populnea***
Leaf	0.293±0.011	0.136±0.036

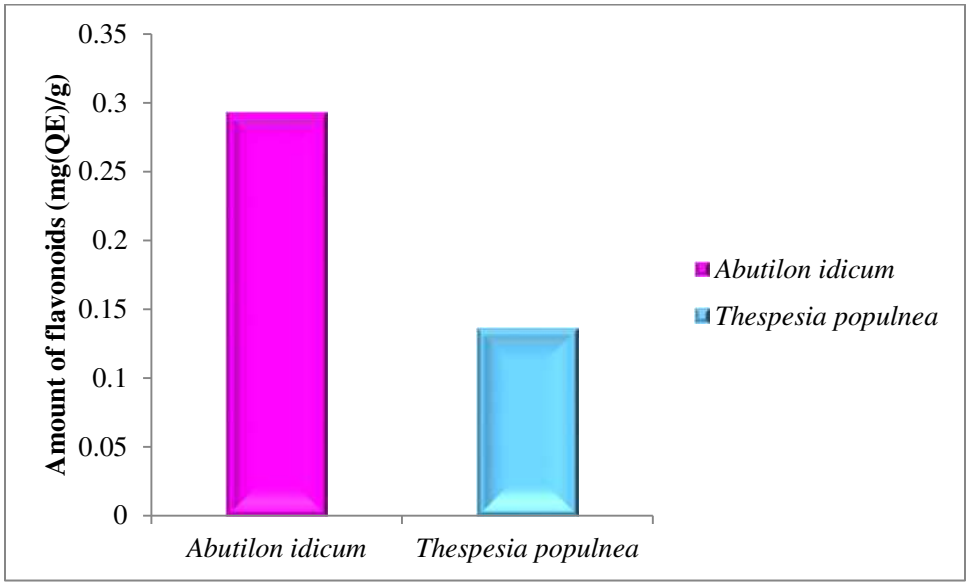


Figure 1. Amount of flavonoids present in the leaves of *Abutilon indicum and Thespesia populnea*

Table 4 : Amount of Phenol present in the leaves of *Abutilon indicum and Thespesia populnea*

Samples	Amount of Phenol (mg(QE)/g)	
	Abutilon indicum	***Thespesia populnea***
Leaf	0.499±0.169	1.143±0.057

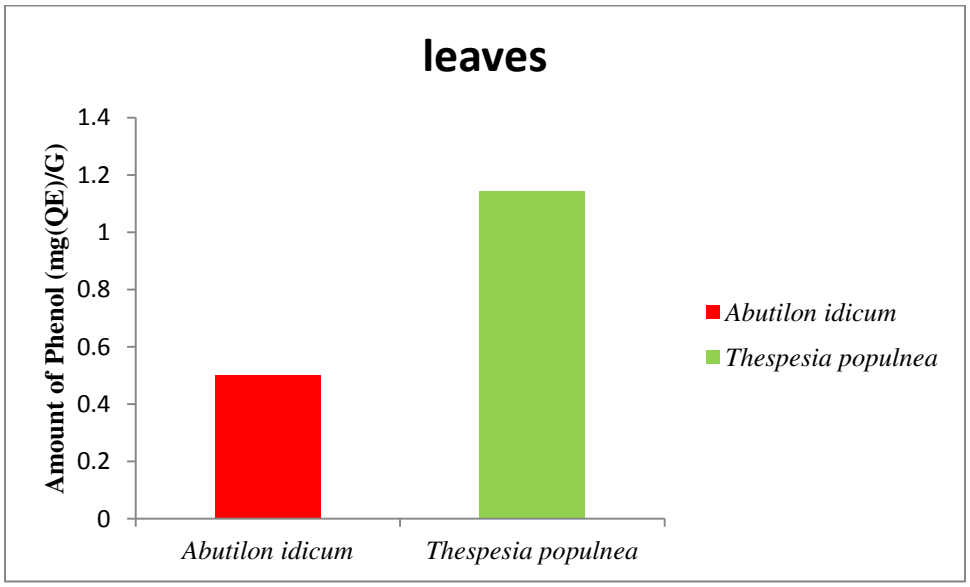


Figure 2. Amount of Phenol present in the leaves of *Abutilon indicum and Thespesia populnea*

Table 5 : Amount of Tannins present in the leaves of *Abutilon indicum and Thespesia populnea*

Samples	Amount of Tannins (mg(QE)/g)	
	Abutilon indicum	***Thespesia populnea***
Leaf	5.722±4.271	7.113±0.461

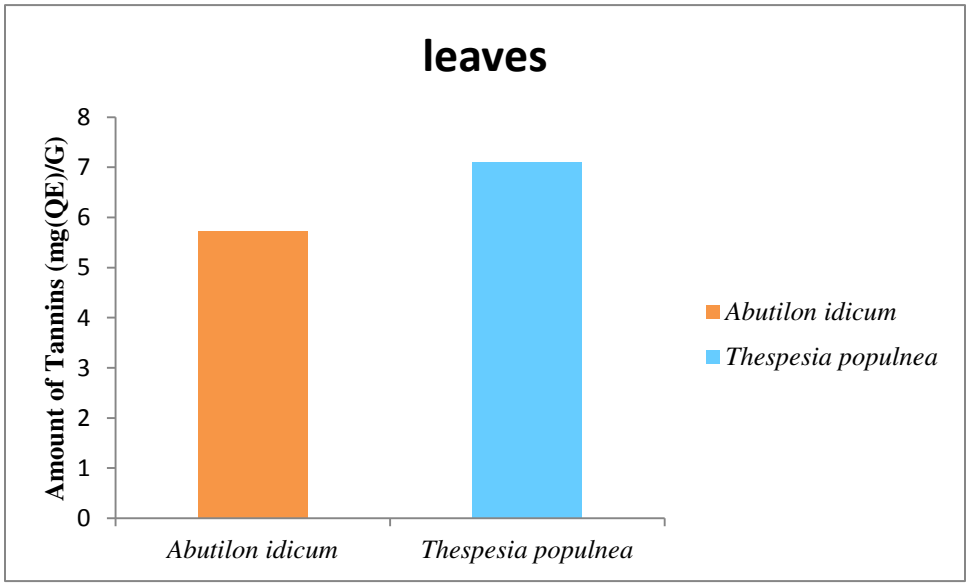


Figure 3. Amount of Tannins present in the leaves of *Abutilon indicum and Thespesia populnea*

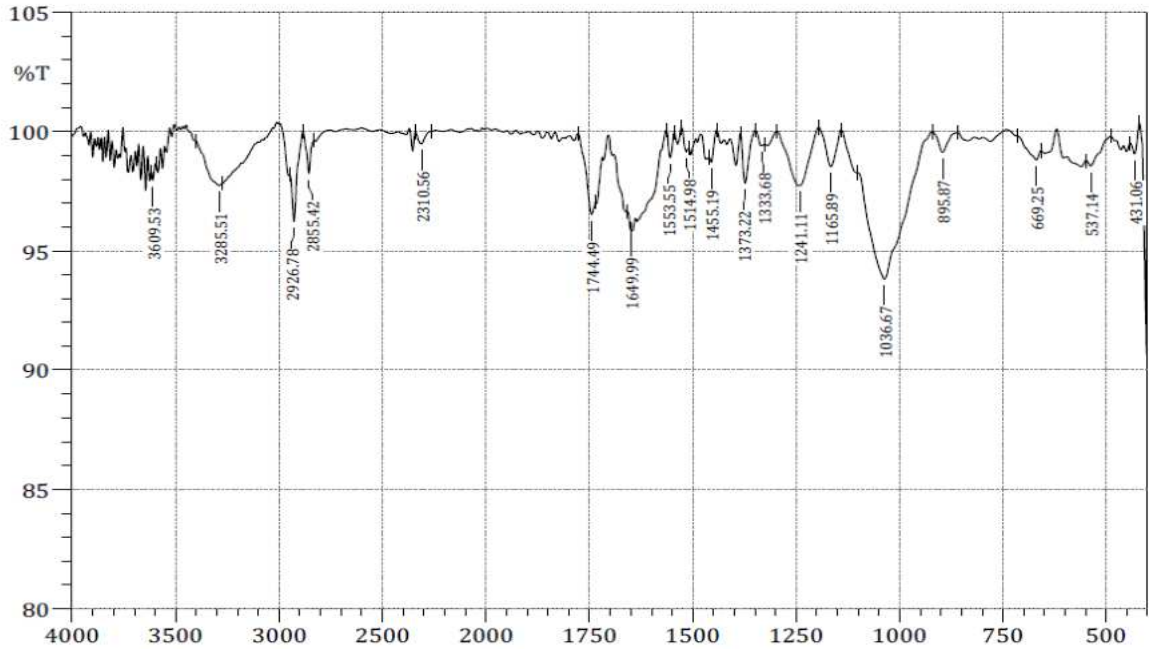


Fig 4 : FT-IR spectrum of ethanol extract of leaf of *Abutilon indicum*

Table 6 : FT-IR Peak values and functional groups of ethanol extract of *Abutilon indicum* leaf

PEAK VALUE	BOND	FUNCTIONAL GROUP
3609.53	STRONG, ALCOHOL GROUP	O-H
3285.51	MEDIUM, URETHANES	N-H
2926.78	STRONG, ALKANE	C-H
2855.42	MEDIUM, ESTER	C-H
2310.56	MEDIUM	O-H
1744.49	STRONG, CARBONYL	C=O
1649.99	STRONG, GUANIDINE	C=N
1553.55	MEDIUM, AROMATIC	C=C
1514.98	MEDIUM, AROMATIC	C=C
1455.19	STRONG, NITRO GROUP	N=O
1373.22	STRONG, NITRO GROUP	N=O
1333.68	STRONG, NITRO GROUP	N=O
1241.11	STRONG, ACID	C-O
1165.89	STRONG, ACETALS	C-H
1036.67	STRONG, ACETALS	C-H
895.87	STRONG, ETHER	C-O
669.25	WEAK, SULPHINIC ACID	O-H
537.14	STRONG, ALKYL HALIDE	C-BR
431.06	HALOGEN COMPOUND (IODO COMPOUND)	C-I

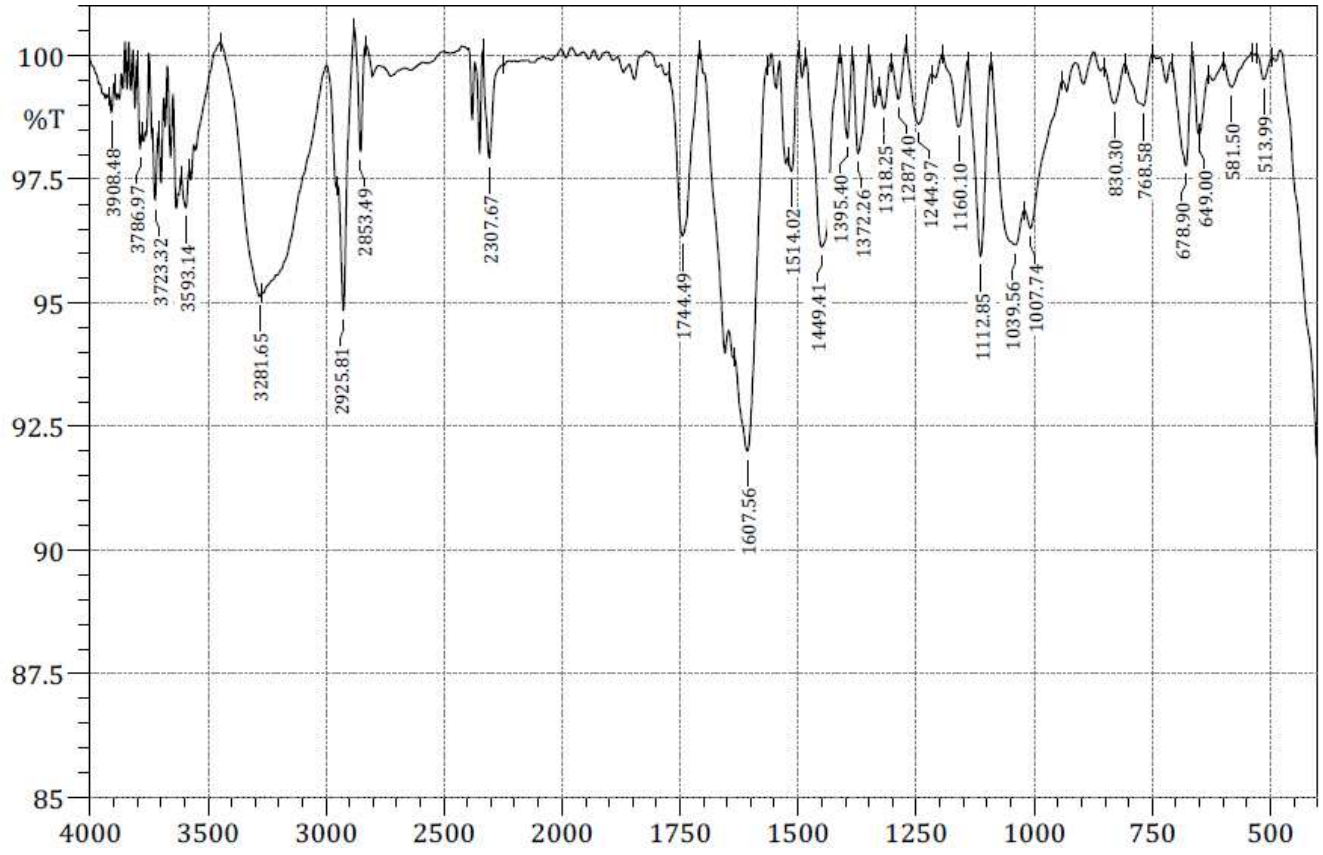
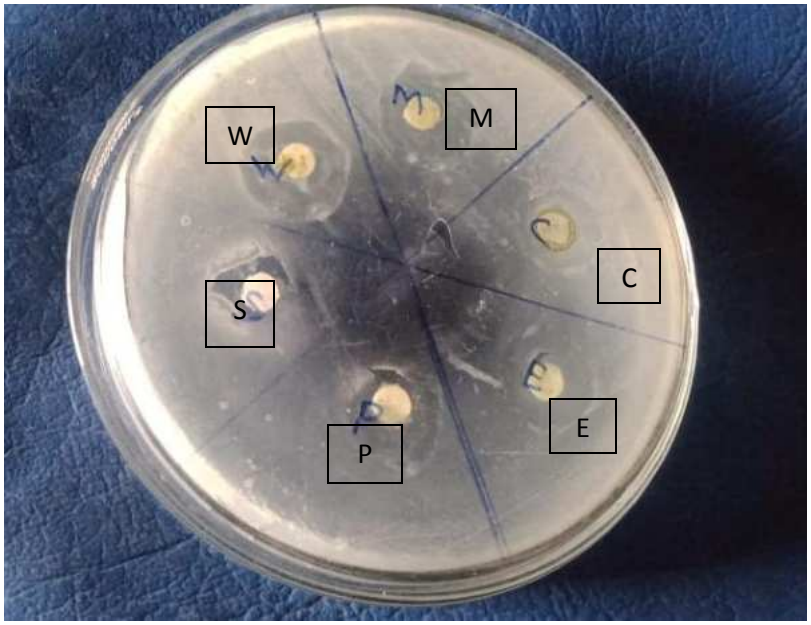


Fig 5 : FT-IR spectrum of ethanol extract of leaf of *Thespesia populnea*

Table 7 : FT-IR Peak values and functional groups of ethanol extract of *Thespesia populnea* leaf

PEAK VALUE	BOND	FUNCTIONAL GROUP
3908.48	MEDIUM, AROMATIC AMINES	N-H
3786.97	STRONG, ALCOHOL GROUP	O-H
3723.32	STRONG, ALCOHOL GROUP	O-H
3593.14	STRONG, ALCOHOL GROUP	O-H
3281.65	MEDIUM, URETHANES	N-H
2925.81	STRONG, ALKANE	C-H
2853.49	MEDIUM, ESTER	C-H
2307.67	MEDIUM	O-H
1744.49	STRONG, CARBONYL	C=O
1607.56	STRONG, GUANIDINE	C=N
1514.02	MEDIUM, AROMATIC	C=C
1449.41	STRONG, NITRO GROUP	N=O
1395.40	STRONG, NITRO GROUP	N=O
1372.26	STRONG, NITRO GROUP	N=O
1318.25	STRONG, NITRO GROUP	N=O
1287.40	STRONG, ACID	C-O
1244.97	STRONG, ACID	C-O
1160.10	STRONG, ACETALS	C-H
1112.85	STRONG, ACETALS	C-H
1039.56	STRONG, ETHER	C-O
1007.74	STRONG, ETHER	C-O
830.30	WEAK, SULPHINIC ACID	O-H
768.58	STRONG, ALIPHATIC CHLOROFORMATE	C-O-H
678.90	STRONG, PRIMARY AMINES	N-H
649.00	STRONG, PRIMARY AMINES	N-H
581.50	STRONG, ALKYL HALIDE	C-BR
513.99	STRONG, ALKYL HALIDE	C-BR

Plate 3: Antibacterial activity of *Abutilon indicum* against *B. subtilis*



Antibacterial activity of *Abutilon indicum* against *E.coli*

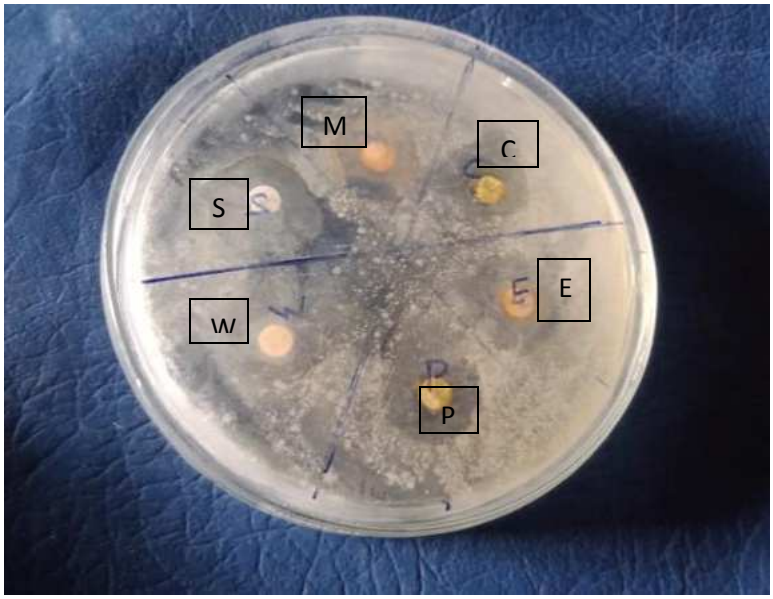
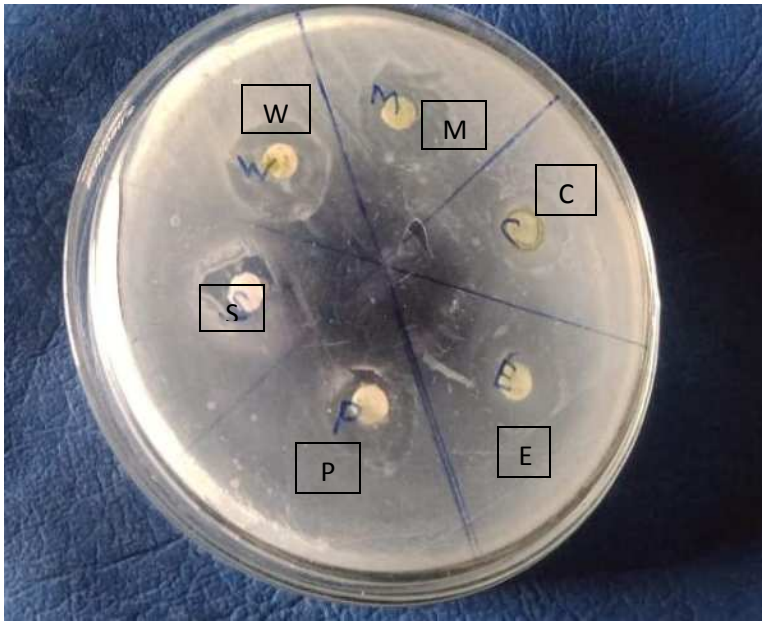
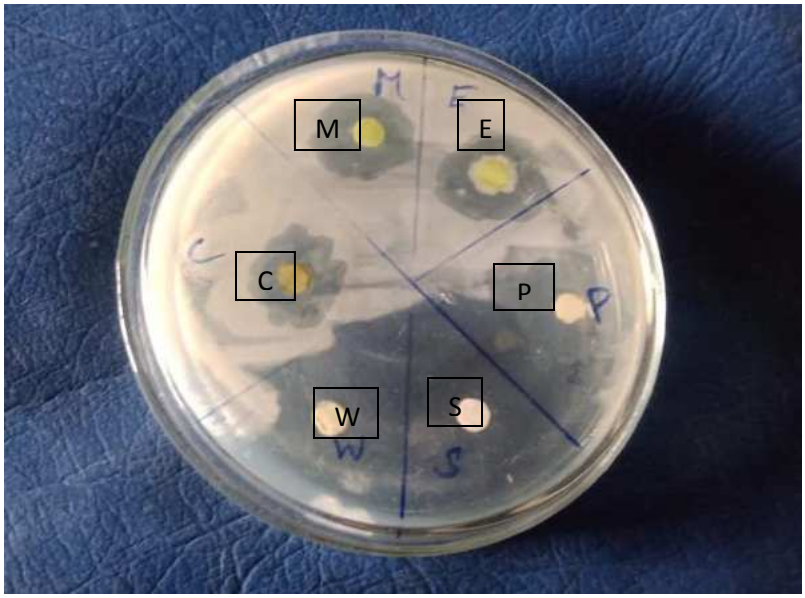


Plate 4: Antibacterial activity of *Thespesia populnea* against *B. subtilis*



Antibacterial activity of *Thespesia populnea* against *E.coli*



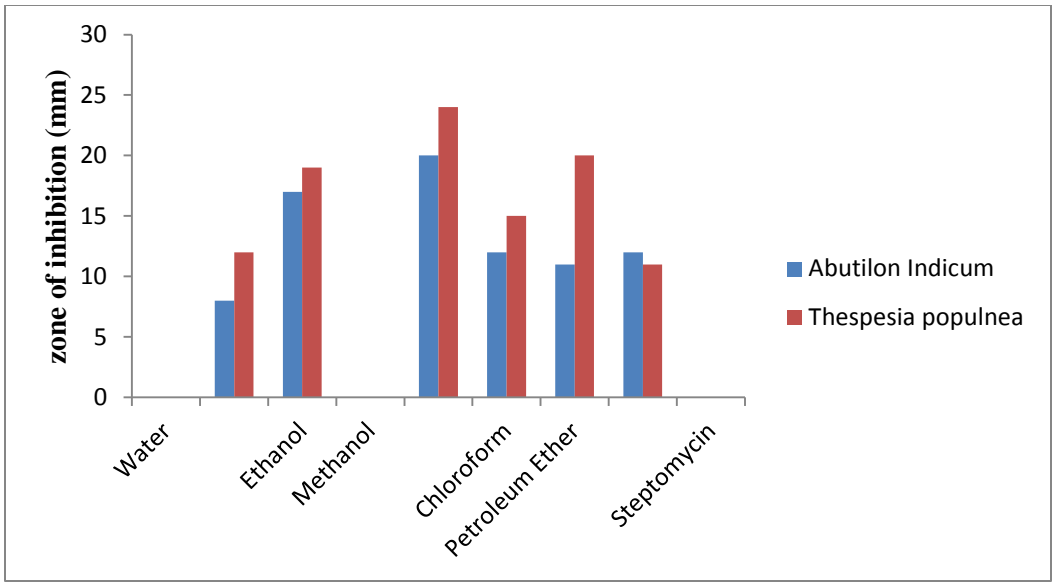


Fig.6: Antibacterial activity of *Abutilon indicum* and *Thespesia populnea* against *B. subtilis*

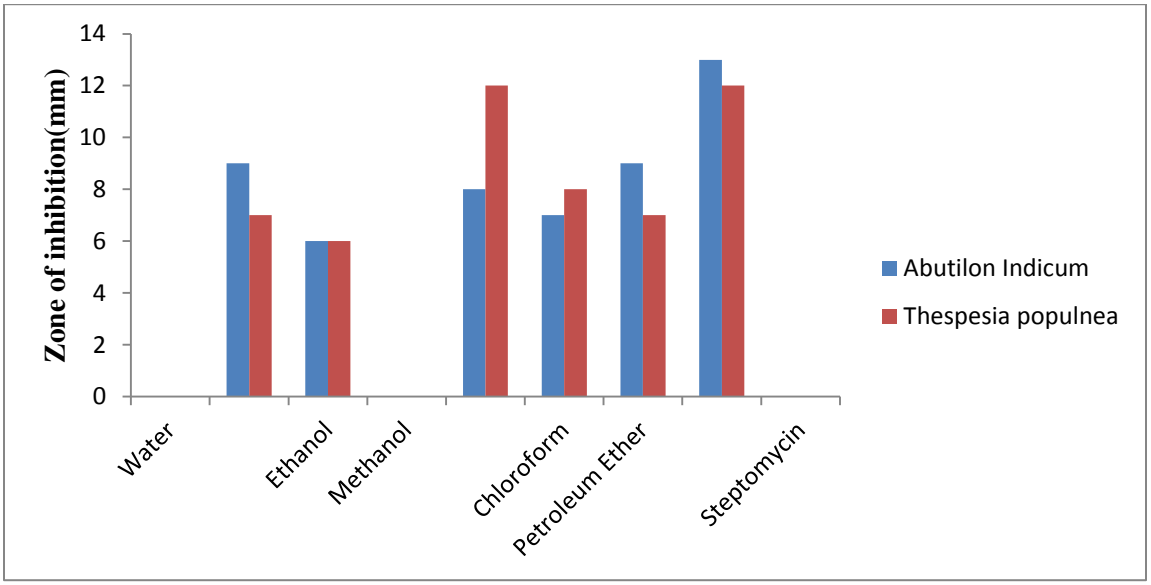


Fig. 7 :Antibacterial activity of *Abutilon indicum* and *Thespesia populnea* against *E.coli*

SUMMARY AND CONCLUSION

The thesis entitled "Phytochemical analysis, antibacterial activities of selected species of Malvaceae" deals with a systematic evaluation of macroscopic characters, phytochemical,and antibacterial activity of leaves of *Abutilon indicum and Thespesia populnea* belongs to family Malvaceae.

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

- Macroscopical characters of *Abutilon indicum and Thespesia populnea*
- preliminary phytochemical screening to establish the phytoconstituents present
- functional groups of the selected plants by FTIR
- Antibacterial potential of *Abutilon indicum and Thespesia populnea* against *Escherichia coli* and *Bacillus subtilis*

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

Basic phytochemical screening was performed using suitable reagent to detect the presence of secondary metabolites, in the methanol, ethanol, petroleum ether and chloroform leaf extracts of selected plants. Antioxidants are quantitatively estimated using standard procedure. The antioxidants were high in the *Thespesia populnea* than *Abutilon indicum.* The result of the antimicrobial screening on the methanolic extract of *Thespesia populnea* showed high inhibitory activity towards *E.coli.*

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

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