Antimicrobial Activity and Phytochemical Characterization of Ethanol and Acetone extracts of *Sesbania grandiflora*

1. SCOPE AND PLAN OF WORK

The aim of the project was to do a comparative study of antimicrobial activity using plant extracts and commercially available antibiotics and phytochemical. The study deals with the comparative analysis of the plant extracts against commercial antibiotics. The present research includes,

1. Selection of plants
2. Collection of plant material
3. Preparation of plant extracts
4. Collection of Test organism
5. Screening of phytochemical constituents
6. Antibacterial activity test using plant extracts
7. Minimum inhibitory concentration (MIC)

In the present study, simply available plant *Sesbania grandiflora* was selected for the phytochemical screening of aqueous, ethanol and acetone extracts and its antimicrobial activity against pathogenic bacteria was assessed.

2. INTRODUCTION

The World Health Organization (WHO) recognizes traditional medicine, particularly plant medicine as an important alternative healthcare delivery system for most of the world's population. In recent years, drug resistance to human pathogenic bacteria has been commonly reported all over the world (**Singh et al., 1992).**

There is a need to develop alternative antimicrobial drugs for the treatment of infectious diseases. One approach is to screen local medicinal plants for possible antimicrobial properties. Plant materials remain an important resource to combat serious diseases in the world. According to (1993), 80% of the world population is dependent on the traditional medicine and a major part of the traditional therapies involves the use of plant extract or their active constituents. Yet a scientific study of plants to determine their antimicrobial active compounds is a comparatively new field. The traditional methods, especially the use of medicinal plants, still play a vital role to cover the basic health needs in the developing countries.

Medicinal plants constitute an effective source of both traditional and modern medicines. Herbal medicine has been shown to have genuine utility. The World Health Organization advocated that countries should encourage traditional medicine with a view to identify and exploit aspects that provide safe and effective remedies for ailments of both microbial and non ‗ microbial origins (WHO 1978).

The search for newer sources of antibiotics is a global challenge pre-occupying research institutions, pharmaceutical companies, and academia, since many infectious agents are becoming resistant to synthetic drugs Latha and Kannabiran *et al.,* (2006). Scientific experiments on the antimicrobial properties of plant constituents were first documented in the late 19th century (Zaika 1975).

It is estimated that today, plant materials have provided the models for 50% western drugs (Robbers *et al*.1996). Many commercially proven drugs used in modern medicine were initially used in crude form in traditional for folk healing practices, or for other purposes that suggested potentially useful biological activity. The primary benefits of using plant derived medicines are that they are relatively safer than synthetic alternatives, offering profound therapeutic benefits and

more affordable treatment. The medicinal plants around the world contain many compounds with antibacterial activity (Marjorie 1999).

Many efforts have been made to discover new antimicrobial compounds from various sources such as microorganisms, animals and plants. Systematic screening of them may result in the discovery of novel effective antimicrobial compounds (Tomoko *et al*. 2002). The use of botanical medicines is generally on the rise in many parts of the world (Bbosa et *al*.2007). The screening of plant extracts and plant products for antimicrobial activity has shown that plants represent a potential source of new anti ‗ infective agents (Amani *et al* .1998: Salvat *et al.* 2001 Costa *et al.*2008). Since prehistoric times, man has used plants for various purposes and he will continue to do so as long as life continues on this planet (Abbiw, 1990).

Man's symbiotic relationship over time with plants has given the world many invaluable benefits. Apart from the raw materials that go to form our variety of foods, the most important plant products are medicines, cosmetic and flavour products, as well as other pharmaceuticals (Sofowora, 1996). The Centre for Research in Plant Medicine has identified one thousand medicinal plants in Ghana and forty (40) of them are used in treatments of thirty-three diseases such as: malaria, jaundice, asthma, diabetes, epilepsy, typhoid fever, hypertension and anemia (Yidana et *al.*, 2002).

Screening of phytochemical constituents:

Various medicinal properties have been attributed to natural herbs. Medicinal plants constitute the main source of new pharmaceuticals and healthcare products (Ivanova et al. 2005). The history of plants being used for medicinal purpose is probably as old as the history of mankind. Extraction and characterization of several active phytocompounds from these green factories have given birth to some high activity profile drugs (Mandal et al. 2007).

A growing body of evidence indicates that secondary plant metabolites play critical roles in human health and may be nutritionally important (Hertog et al. 1993). Phytochemical screening of plants has revealed the presence of numerous chemicals including alkaloids, tannins, flavonoids, steroids, glycosides, saponins etc. Secondary metabolites of plants serve as a defense mechanism against predation by many microorganisms, insects and herbivores (Lutterodt et al. 1999; Marjorie 1999). Flavonoids are a broad class of plant phenolic that are known to possess a well-established protective ability against membrane lipoperoxidative damages (Sen et al. 2005).

Phenolic compounds are one of the largest and most ubiquitous groups of plant metabolites that possess an aromatic ring bearing one or more hydroxyl constituents (Singh et al. 2007). The Phenolic compounds are widely found in the secondary products of medicinal plants, as well as in many edible plants (Hagerman et al. 1998).

Plant based antibacterial have enormous therapeutic potential as they can serve the purpose with lesser side effects that are often associated with synthetic antibacterial. Biomolecules of plant origin appear to be one of the alternatives for the control of these antibiotic resistant human pathogens (Kumaraswamy *et. al*., 2008).

Knowledge of the chemical constituents of plants is desirable, not only for the discovery of therapeutic agents, but also because the medicinal value of plant lies in the chemical substances that produce a definite therapeutic action on the human body. Some of these important bioactive compounds are alkaloids, flavonoids, tannins and phenolic compounds. In addition, the knowledge of the chemical constituents of plants would further be valuable in the discovery of the actual value of folkloric remedies. The phytochemical research based on ethnopharmacological information is generally considered an effective approach in the discovery of new anti – infective agents from higher plants (Chhetri *et al*., 2008)

Botanical Description:

Sesbania grandiflora is a small, loosely branching tree that grows up to 8- 15 m tall and 25 – 30 cm in diameter; stems tomentose, unarmed; roots normally heavily nodulated with large nodules; the tree can develop floating roots. Leaves alternate and compound; pinnate 15 – 30 cm long with 12 – 20 paris of oblong, rounded leaflets, 3 – 4 cm long and about 1 cm wide; Leaves borne only on terminal ends of branches; leaves turn bright yellow before shedding. . Flower clusters hanging at leaf base have 2 – 5 large or giant flowers; pink, red or white, pea like, 5 – 10 cm in length, curved about 3 cm wide before opening. Pods long and narrow, hanging down 30 – 50 cm by 8 mm; septate, wide, flat, with swollen margins and about 15 -40 pale – colored seeds; seed is beanlike, elliptical, red brown, 6-8 in a pod, 3.5 mm. Flowering in sharad ritu and fruition in winter. Fruit and flower are used as vegetable and as pickles (Orwa *et al*., 2009).

Traditional medicinal uses:

The root – bark of the red – flowered variety is useful in vitiated condition of vata and arthralgia. The bark is astringent, cooling, bitter, tonic, anthelmintic and febrifuge the pounded bark is externally applied to cure Scabies. The juice of the bark is good for dyspepsia, diarrhea and gastralgia.

The leaves are acrid, bitter, Sweet, cooling, aperient, tonic and diuretic and contain a non – poisonous saponins like substance. The leaf juice is used is nasal catarrh, nyctalopia and cephalagia. Leaves are chewed to disinfect mouth and throat and are useful in stomatalgia.

The flowers are cooling, bitter, astringent, acrid and antipyretic. The juice of the flowers is applied to the eyes for nyctalopia and is used for intermittent fevers. The fruits are sweet, bitter, laxative and alexiteric and are useful in flatulent – colic, astringent, cooling, bitter, tonic, anthelmintic, febrifuge, cure scabies, dyspepsia, diarrhea and gastralgia, astringent, antipyretic, for nyctalopia, anemia, emaciation and vitiated conditions of tridosa.

PLANT DESCRIPTION:

Taxonomical description:

Kingdom : plantae – plant

Subkingdom : Tracheobionta – Vascular plant

Super division : Spermatophyta – Seed plant

Division : Magnoliophyta – flowering plant

Class : Magnoliopsida – Dicotyledons

Subclass : Rosidae

Order : Fabales

Family : Fabaceae – pea family

Genus : Sesbania Scop – Riverhemp

Species : *Sesbania grandiflora* (Linn)

3. REVIEW OF LITERATURE:

World is endowed with a rich wealth of medicinal plants. Man cannot survive on this earth for long without the plant king because the plant products and their active constituents played an important role. Medicinal plants have been used to cure a number of diseases. Though the recovery is slow, the therapeutic use of medicinal plant is becoming popular because of its inability to cause side effects. Commercially available antibiotics caused many side effects because of its component.

Kanitta *et al*.,(2015) reported that *Sesbania grandiflora* one of the medicinal plants used for antioxidant activities. It contains several kinds of alkaloids, flavonoids, saponins, tannin, triterpenoids, glycosides and phenols. Many researchers have evaluated that these phytochemical substances have major impact on diabetes mellitus.

K. Padmalochana *et al*., (2014) stated that among these three extracts ethanol extracts have shown good antibacterial activity compared with aqueous and acetone extracts. Because of the presence of alkaloids, flavonoids, tannins and steroids, ethanol extract shows high antibacterial activity. So these active compounds can be used in the field of medicine as therapeutic agent.

Malviya reeta *et al*., (2013) reported that these chemical constituents are well known for their potential health benefits and have been reported to possess valuable biological activities such as antibacterial and antifungal, antioxidant, antiurolithiatic, anticonvulsant and antiolytic and hepatoprotective properties. A number of experiments have been carried out on *Sesbania grandiflora* to assure its analgesic and CNS depressant effects, cardioprotective effects and kindly protective effects on mice. Bark juice is given in diarrhea and abdominal colic and also gastric- intestinal disorders and wound healing activity of its flower due to having tannin and other nutritious content.

Dayanaada Bhoumik *et al*., (2014) stated that traditionally *Sesbania grandiflora* is used alone or with other medicinal plants to treat a variety of ailments. Research studies leading to extraction, isolation and biological study of plant constituents have now formed the major field of study. In recent years, ethno medicinal studies received much attention as this brings to light the numerous little known and unknown medicinal virtues especially of *Sesbania grandiflora* revealed as a valuable medicinal plant with several medicinal properties.

K.J.AI- Dawah *et al*., (2014) reported the detection of the bioactive chemical consituents of *Sesban* leaves extract by a qualitative analysis for each ethanol and methanol extract It further reflects a possibility for the development of many more novel chemotherapeutic agents or templates from the plant which in future may serve for the production of improved therapeutic plant based drugs.

Abbs fen reji *et al*., (2013) stated that as per the antimicrobial investigations in the presence study the CLP (Crude Leaf Power) of *Sesbania grandiflora* exhibited low level antibacterial activity but the NPL (Nanosized Leaf Power) exhibited highest level of antibacterial activity. Antimicrobial activity of crude leaf power and nanosized leaf power was determined. According to the results, the antimicrobial activity of nanosized leaf power was very potent due to its uniform size when compared with crude leaf power. It was found that the nanosized leaf power had a high ability to kill microbes.

Aravind *et al*., (2013) described that the benefits of papaya owed due to high content of vitamins A, B and C proteolytic enzymes like papania and chymopapain which have antiviral, antifungal and antibacterial properties. The present article reviews the pharmacological use of *Carica papaya* and side / toxic effects. *Carica papaya* contains an enzyme known as papain which is present in the bark, leaves and fruit. *Carica papaya* is a neutraceutical plant having a wide range of pharmacological activities. The wide range of enzymes, vitamins present in *Carica papaya* makes it a neutraceutical plant.

Vijay yogiraj *et al*., (2015) reported that papaya (*Carica papaya Linn*) is commonly called as paw – paw and it belongs to the family Caricaceae. Papaya is commonly known for its food and nutritional values throughout the world.

N.Nirosha *et al*., (2013) stated that papaya leaf and stem extracts were tested against both gram positive and gram negative bacteria such as *staphylococcus aureus, streptococcus pneumonia, Bacillus cereus, Salmonella typhi, Escherichia coli* and P*seudomonas aeroginosa* by diffusion method. *Carica papaya* may be used for the treatment of gastroenteritis urethritis media, typhoid fever and wound infections.

Bhawana pandey *et al*., (2015) described that the antimicrobial properties of plant have been investigated by a number of studies worldwide and many of them have been used as therapeutic alternatives because of their antimicrobial properties. Plants are the cheaper and safer alternative source of antimicrobials. The study deals with the antibacterial activity of aqueous, ethanol and ethyl ether extract of leaves of *Carica papaya* through agar well diffusion

I.I.Anibijuwon *et al*., (2009) reported that the bioactive compound of leaf and root extracts of *Carica papaya* was extracted, using water and organic solvents, and were investigated for antibacterial activity against some human pathogenic bacteria using the agar diffusion method. The aqueous extracts of the root extracts did not show significant activity, but the organic extracts had significant activity with the methanol extracts demonstrating the highest activity against the test bacteria.

Boshra v *et al*., (2013) stated that it is low in calories and rich in natural vitamins and minerals, like vitamin c, vitamin A, thiamine, iron and fibre. Papaya have been much studied in pharmaceutical and has wide applications in the food industry. The delicious papaya fruit has nutritional values that make it potent as a raw material in the food processing industry beyond mere raw consumption.

Tatyasaheb patil *et al*., (2014) described that many scientific studies been conducted to the same end. Yet there is deficiency and opportunity to enlarge the framework of the research to include research to Parkinsonism and oxidant related damage.

KL Krishna *et al*., (2008) reported that during the last few decades' considerable progress have been achieved regarding the biological activity and now it is considered as valuable nutraceutical fruit plant. Papaya popularly known as food article is the unique source of various types of compounds having diverse structure.

Arumugan *et al*., (2014) stated that the presence of various phytochemical compounds in *cynogon dactylon* and *Carica papaya* was qualitatively and quantitatively determined by various standard of analysis. The suitable extraction method was identified for phytochemical compound extraction in above selected plants. The present study revealed that ethanol is a suitable solvent system which showed the presence of high percentages in the range of 0.01 to 1.46 and 0.02 to 10.0 percentage of phytochemicals compared to other solvent extracts.

Doughari J. H. *et al*., (2007) described that the bioactive compounds of root extracts of *Carica papaya L*. were extracted, using water and organic solvents and were investigated for antibacterial activity against some pathogenic bacteria using the cup plate agar diffusion method. Further pharmacological evaluations, toxicological studies and therapeutic antibacterial from this plant are the further challenges.

S.Aruljothi *et al*., (2014) reported that nowadays, there is an increased sustained interest in the production of plant – based – drugs for the treatment of many diseases. Moreover; people are welcoming traditional medicines to overcome mild/ serious illness. Due to increase in the thrust for the production of plant – based antimicrobials, the present study was performed on *Carica papaya* leaves. The results obviously justified the importance of topical application of papaya leaf extracts to treat the wound infection as a traditional practice.

Jyotsna kiran peter *et al*., (2014) stated that the aqueous as well as the methanolic extract of seeds were effective to inhibit the bacterial pathogens while in case of chloroform extract of *Carica papaya*, leaves did not show any inhibition against the bacteria and the aqueous leaf extract was potent to inhibit them.

Orhue P.O *et al*., (2013) described that aside its nutritional values, there are speculations that *Carica papaya*, also known as paw. Paw, has antibacterial potentials. This study evaluates the antibacterial potentials of different extracts of *C. papaya* parts, in comparison with standard drugs. These suggest that *C. papaya* may be used as an antibiotic and extracts in petroleum ether seems more potent.

K.Kayalvizi *et al*., (2015) reported that leaves of female *Carica papaya.*L were Shaded, powdered and were extracted using different solvents ethanol, methanol, acetone, chloroform, petroleum ether, hexane and ethyl acetate. It is very necessary to introduce new and biologically safe and active drugs eco-friendly in nature and effective as antibacterial agents.

V.N Chidozie *et al*., (2014) stated that these were tested against S*almonella typhi, (s.typhi*) and six other bacteria by agar gel diffusion method and the zones of inhibition recorded. This inferred that anogeissus leiocarpus aqueous leaf extract had a potentiating effect on the antibacterial activities of the other two plant extracts. The combination of these plants has greater antibacterial actions than any single one of the plants appears to have a potentiating effect on the other two plants.

R Sumathi *et al*., (2014) described that the plant materials such as leaves, stem and root of disease free *Carica papaya* were collected from Kaveripakkam, Vellore district, Tamilnadu. The dried powdered plant material is subjected to solvent extraction using the solvents cold water, hot water and ethanol. Antimicrobial assay of plant extract against clinical isolates by AWD assay. Only the leaf extracts showed inhibitory effect against *Candida albicans*, whereas stem and root extracts were infective.

Neethu S. *et al.*, (2016) reported that plants have been one of the important sources of medicines since the beginning of human civilization. There is a growing demand for plant based medicines, health products, pharmaceuticals, food supplements and cosmetics. *Sesbania grandiflora* (L) is a multipurpose tree with edible flowers and is a source of one of the medicinal products. *S. grandiflora* (L) has unique medicinal properties and used as a herbal drug for its antibiotic, anthelmintic, antitumor and contraceptive properties. Therefore future research should be addressed on the application of using *S. grandiflora (L*) leaves as natural remedy and to protect against infectious diseases.

S.Gomathinayagam *et al.*, (2013) stated that the plant extract displays antimicrobial activity and therefore justifies its ethnobotanical uses for the treatment of ophthalmic, coughs, colic and haemorrhoids.

Mohamed abd elgadir *et al*.,(2014) described that extracts from different parts of *Carica papaya* plant have shown protective effects against many diseases such as intestinal worms infection and different types of wounds. As a conclusion *Carica papaya* is one of the most effective sources of natural medicine and widely used in pharmacological applications. It is used to treat several diseases such as tumors, nervous, asthma and wounds.

Pedro Chavez – Quintal *et al*.,(2001) An ethanol extraction was used to obtain bioactive compounds from *Carica papaya* L.cv The extract exhibited the broadest action spectrum. Ethanolic extracts from *Carica papaya* L. cv. Maradol leaves are a potential source of secondary metabolities with antifungal properties.

Tarun Vij et al., (2015) Papaya (*Carica papaya L.*) is a popular and important fruit tree in tropical and subtropical parts of the world. The fruit is consumed worldwide as fresh fruit and vegetable are used as processed product. The fruit is healthy and delicious and the whole plant parts including fruit, root, bark, peel, seeds and pulp are known to have medicinal properties. The many benetifs of papaya are owed due to high content of vitamin A, B and C, proteolytic enzymes like papain and chymopapain which have antiviral, antifungal and antibacterial properties.

Okunola A et al., (2012) The efficacy of treatment with *C. papaya* is dependent on the quantity of the different chemical substances present in the preparation. The quantity of chemical substances varies in the fruit, latex, leaves, and roots and varies with the extraction method, age of the plant part, and the cultivar and sex of the tree. The antibacterial and antifungal ability of both fresh and dried leaves of *C. papaya* against bacteria and fungi of medical importance was carried out.

Biresh Kumar Sarkal et al., (2012) The extraction of fruit of *Sesbania grandiflora* was carried out by using solvent aqueous methanol. The antioxidant activity of plant *Sesbania grandiflora* determined by using different *in vitro* antioxidant assays the extract of *Sesbania grandiflora* was found to have potent antioxidant activity which may be due to the abundance of phenolic and flavonoid contents.

4. MATERIALS AND METHODS

MATERIALS: PLANT SAMPLES, EQUIPMENTS AND REAGENTS

Collection of Plant Samples:

The fresh leaves of *Sesbania grandiflora and Carica papaya* were simultaneously collected from cultivated farms and the open fields of Theni district. Fresh parts of the plants were identified and authenticated prior to phytochemical analysis. The leaves were rinsed thrice with distilled water followed by double distilled water to remove the dust and other contaminants. Then dried at room temperature to remove the moisture.

Qualitative analysis of phytochemical screening:

For phytochemical analysis, the extracts were prepared by suspending 2gms of each dried powder into separate 100 ml conical flasks and 50ml of each solvent (Aqueous, Methanol, Ethanol) was added. The conical flasks were plugged with cotton plugs, labeled and allowed to stand for 1-2 hrs. Then filtered using what man No.1 filter paper. Thus, the filtrates obtained were used as test solutions.

The plant extracts were tested for the presence of bioactive compounds such as alkaloids, cardiac glycosides, amino acids, reducing sugars, flavonoids, phenols, saponins, steroids, tannins, carbohydrates, terpenoids by standard method.

Qualitative analysis of carbohydrates

The presence of carbohydrates in solvent extracts was determined by different methods such as, Fehling's test, Benedict's test, Molisch's test and iodine test.

a) **Fehling's test**: The equal volume of Fehling's reagent A and B were mixed together and 2ml of mixture was added to plant extracts. On gentle heat, the mixture turned brick red color (Fehling, 1984).

(b) **Benedict's test**: 2ml of Benedict's solution was added to crude plant extracts followed gentle boiling gives reddish brown precipitate (Benedict, 1909).

(c) **Molisch's test**: 2ml of Molisch's reagent were added in plant extract followed addition of H_2SO_4 develops the appearance of violet ring in the interphase.

(d) **Iodine test**:Addition of 2ml of iodine solution in plant extract gives development of dark blue. Simultaneously, presence of phenols and tannins were tested: 2 ml of 2% of $FeCl_3$ solution was added in the plant extracts, development of dark green for phenolic compounds and black color for presence of tannins (Segelman*et al.*, 1969).

Qualitative analysis of Flavonoids

2ml of 2% NaOH was added to the plant extract, which gives intense yellow color, which disappeared on standing for few min. Further addition of few drops of 1% aluminum solution added in each filtrate turns reappearance of yellow color indicates the presence of flavonoids (Edeoga*et al.*,2005)

Qualitative analysis of Saponin by Obadoni and Ochuko method

5ml of plant extracts were mixed with equal volume of distilled water and mixed vigorously for 3 to 5min which gives intense stable foam development. In addition, 3ml of olive oil was mixed vigorously, observed the development of emulsion (Edeoga*et al.,* 2005)

Qualitative analysis of Glycoside by Keller- Killani test method

2ml of chloroform, 2 ml of acetic acid were added to plant extract and allowed to cool, followed by addition of 2ml of concentrated H2SO4 changes the violet to blue then green, indicates the presence of steroidal nucleus that is glycone portion of glycoside, (Siddiqui and Ali *et al.,* 1997). In another way, the available cardiac glycosides are tested by the addition of 1-2 drops of glacial acetic acid and 2% of FeCl3 solution in crude plant extract followed by 2ml of H2SO4, gives brown ring at the interphase indicates the presence of cardiac glycosides.

Qualitative analysis of Steroids:

Plant extracts mixed with 2ml of chloroform and Conc. H2SO4 were added gently, which leads to the development of red color in the lower chloroform layer indicating the presence of steroid, and was further confirmed with addition of acetic acid which develops the greenish color formation.

Qualitative analysis of Terpenoids by Salkowski test method

Presence of terpenoids in plant extract was determined according to Salkowski method (Singh *et al*., 2004). 5 ml (1 mg/ml) of fraction was combined with few drops of chloroform and 3 ml of concentrated H2SO4. Change of reddish brown color revealed the presence of terpenoids (Siddiqui and Ali, 1991).

Qualitative analysis of Alkaloids by Phenonthroline method

Alkaloids in plant extract were detected according to Phenonthroline method (Singh *et al*., 2004). Plant extract was added with 8 ml of HCl (1%), warmed and filtered. 2ml of each filtrate was titrated separately by means of (a) potassium mercuric iodide (Mayer's reagent) and (b) potassium bismuth (Dragendroff's reagent). Turbidity of precipitation indicated the presence of alkaloid.

Qualitative analysis of Anthocyanins

The presence of Anthocyanins were tested by the addition of 2ml of HCl and ammonia with 2ml of aqueous plant extract, gives the development of pink red turn to violet color (Kokate*et al.*, 2007).

Qualitative analysis of Coumarins

5ml of the moistened solvent plant extract was taken in a test tube. The mouth of the tube was covered with filter paper treated with 1N NaOH solution. Test tube was placed for few minutes in boiling water and then the filter paper was removed and examined under the UV light for yellow fluorescence indicated the presence of coumarins (Maique *et al.*, 2003).

Qualitative analysis of Phenols and Tannins

Plant extract was mixed with 2ml of 2% solution of FeCl3. A blue-green or black coloration indicated the presence of phenols and tannins.

Qualitative analysis of reducing sugars:

1ml of the extract was added with 2ml of fehling´s reagent and 3ml of water. It was then boiled for 2minutes.

IN VITRO ANTIBACTERIAL STUDIES:

Test organisms:

The following bacterial strains were obtained from the laboratory of Department of Microbiology, Nadar Saraswathi College Theni. Gram negative bacterial strain *Escherichia coli, Pseudomonas sp, Klebsiella sp* and gram positive strain *Staphylococcus sp, Bacillus sp* were used for the present study. They were maintained at 4˚c on the slants of nutrient agar medium for further use.

Preparation of inoculums:

The nutrient broth cultures of the organisms grown at 37^{0}C for approximately 3 to 4 hrs were used as inoculum.

Well diffusion method:

Muller Hinton agar Formula &preparation:

Muller Hinton agar medium was employed for well diffusion sensitivity testing. The medium contained per litter, infusion from 300g beef, casein hydro lysate, 17.5 g starch 1.5g and agar 17g (Monica, 1985). The medium was prepared by dissolving the dehydrated mixture of ingredients in distilled water. After boiling, pH was adjusted to7.4 and sterilized by autoclaving at 121^{0}c for 15minutues. The medium was poured into the petriplates

Experimental procedure:

The three solvent extracts were screened against a number of selected pathogenic bacteria by agar well diffusion. In this method, 10ml aliquots of nutrient broth were inoculated at 37˚C for 24 hours. Sterile cotton swabs were dipped in the bacterial suspension and evenly streaked over the entire surface of the agar plate to obtain uniform inoculums. Six wells per plate were made with the reverse side of a sterilized micropipette. 25ml, 50ml, 75ml, 100ml of different extracts were then poured into the respective wells using a micropipette. Distilled water was used as negative control. All plates were incubated for 24hours at 37˚C. The antibacterial activity was determined by measuring the diameter of the zone of inhibition to the nearest (mm) that observed from the clear zone surrounding the well (Raghavendra et al., 2010).

Determination of Minimum inhibitory concentration:

The MIC of the extracts were determined according to the macro broth dilution technique (Baron and Finegold, 1990). Standardized suspensions of the test organisms were inoculated into a series of sterile tubes of nutrient broth containing two-fold dilution of leaf extracts and incubated at 37$_0$C for 24h. MICs were read as the least concentration that inhibited the growth of the test organisms

5. RESULT AND DISCUSSION

The powdered leaves of *Sesbania grandiflora* and *Carica papaya* were extracted with different solvent. The plant extract was then performed for the phytochemical characteristics to identify various phytochemical constituents

Table1: PHYTOCHEMICAL CONSTITUENTS OF *SESBANIA GRANDIFLORA:*

Compound	Methanol	Ethanol	Aqueous
Alkaloids	+	+	+
Saponins	+	+	+
Flavonoids	+	+	+
Tannins	+	+	_
Amino acids	_	_	_
Reducing sugar	+	+	+
carbohydrates	+	+	+
Glycosides	+	+	-
Phenols	+	+	+
Steroids	+	+	+
Terpenoids	-	-	-

Table [1] shows the phytochemical analysis of aqueous leaf extract of *S. grandiflora.* It revealed the presence of alkaloids, phenols, saponins, flavonoids, carbohydrates, reducing sugar, steroids and absence of tannins, amino acid, glycosides, terpenoids. The ethanol leaf extract confirmed the presence of alkaloid, phenols, saponins, tannins, flavonoids, carbohydrates, reducing sugar, glycosides, steroids and absence of amino acid, terpenoids. The methanol leaf extract revealed the presence of alkaloid, phenols, saponins, tannins, flavonoids, reducing sugar, glycosides, steroids and absence of amino acid, carbohydrates and terpenoids.

Table2: Invitro antimicrobial activity of *Sesbania grandiflora* extract against *Bacillus sp.*

S.No.	Solvent	Leaf 25	50	75
1	Ethanol	10	12	14
2	Methanol	8	10	14
3	Aqueous	11	13	14
4	Standard	16	18	12

Table [2] represents the antibacterial activity of leaf extract against isolated five pathogens. The antibacterial activity of the aqueous and ethanol extract of *Sesbania grandiflora* by well diffusion method showed the highest inhibitory zone of 14mm and methanol extract showed lowest inhibitory zone of 8mm against inhibitory of was measured in millimeter highest activity of ethanolic extracts against *Bacillus sp.* in all parts of the plant.

Table 3:

Invitro antimicrobial activity of *Sesbania grandiflora extract* against *staphylococcus sp.*

S.No.	Solvent	Leaf 25	50	75
1	Ethanol	13	14.5	19.3
2	Methanol	11.7	13	16
3	Aqueous	13	13.7	14.5
4	Standard	18	16	17

Table [3] represents the antibacterial activity of leaf extract against isolated five pathogens. The antibacterial activity of aqueous and different organic solvent (ethanol and methanol) extract of *Sesbania grandiflora* was assessed by well diffusion method and was measured in millimeter. The ethanol extract showed the highest inhibitory zone of 19.3mm against *Staphylococcus sp*. and methanol extract showed lowest inhibitory zone of 11.7mm against Staphylococcus *sp*.

Table 4:

Invitro antimicrobial activity of *Sesbania grandiflora extract* against *Pseudomonas sp.*

S.No.	Solvent	Leaf 25	50	75
1	Ethanol	7.8	9	16.5
2	Methanol	8	12.8	15
3	Aqueous	9	11	10
4	Standard	14.4	15	16

Table [4] represents the antibacterial activity of leaf extract against isolated five pathogens. The antibacterial activity of aqueous, organic solvent (ethanol and methanol) extract of *Sesbania grandiflora* was assessed by well diffusion method and was measured in millimeter. The ethanol extract showed the highest inhibitory zone of 16.5mm against *Pseudomonas sp*. and methanol extract showed lowest inhibitory zone of 8mm against *Pseudomonas sp*.

Table 5: Invitro antimicrobial activity of *Sesbania grandiflora* extract against *E.coli sp*

S.No.	Solvent	Leaf 25	50	75
1	Ethanol	13	15	17.5
2	Methanol	11.5	12.8	14
3	Aqueous	12.5	13	15
4	Standard	12	15	15.5

Table [5] represents the antibacterial activity of leaf extract against isolated five pathogens. The antibacterial activity of aqueous, organic solvent (ethanol and methanol) extract of *Sesbania grandiflora* was assessed by well diffusion method and was measured in millimeter. The ethanol extract showed the highest inhibitory zone of 17.5mm against E.coli *sp*. and methanol extract showed lowest inhibitory zone of 11.5mm against *E.coli sp*.

Table 6: Invitro antimicrobial activity of *Sesbania grandiflora* extract against *Klebsiella sp*

S.No.	Solvent	Leaf 25	50	75
1	Ethanol	8	8.5	13.4
2	Methanol	7.2	12	13
3	Aqueous	9	9.6	10.7
4	Standard	11.4	12	12.8

Table [6] represents the antibacterial activity of leaf extract against isolated five pathogens. The antibacterial activity of aqueous, organic solvent (ethanol and methanol) extract of *Sesbania grandiflora* was assessed by well diffusion method and was measured in millimeter. The ethanol extract showed the highest inhibitory zone of 13.4mm against Klebsiella sp. and methanol extract showed lowest inhibitory zone of 7.2mm against Klebsiella sp.

Table 7: Mean MIC (mg / ml)

S.No.	Plant	*E.coli*	*Bacillus*	*Pseudomonas*	*Klebsiella*	*Staphylococcus*
1	***Sesbania grandiflora***	0.460	0.453	0.426	0.412	0.386

Table [7] MIC was performed against five organism such as *E.coli sp, Klebisiella sp, Pseudomonas sp, Staphylococcus sp, Bacillus sp.* Minimum inhibitory concentration ranged between 0.350mm to 0.480mm indicated the high activity against *staphylococcus sp*. Maximum activity was recorded as 316mm by the ethanol leaf extract against *staphylococcus sp*. This proved that *Sesbania grandiflora* leaves exhibit the highest activity and so the *Sesbania grandiflora* leaves are potentially used as natural drug.

6. PHOTOSECTION

Fig: 1 antibacterial activity aqueous extract of *Sesbania grandiflora* against pathogen

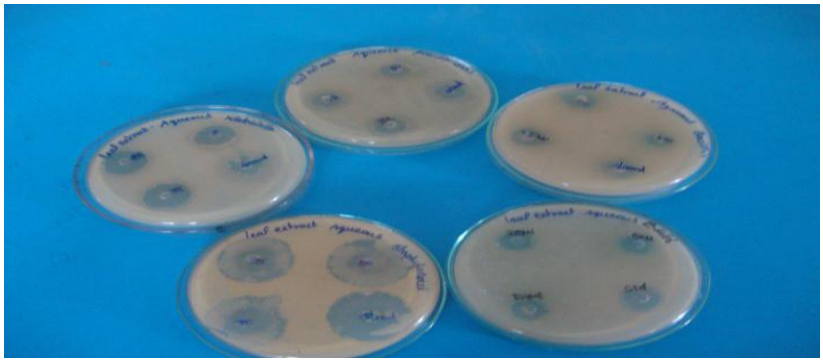


Fig: 2 antibacterial activity methanol extract of *Sesbania grandiflora* against pathogen

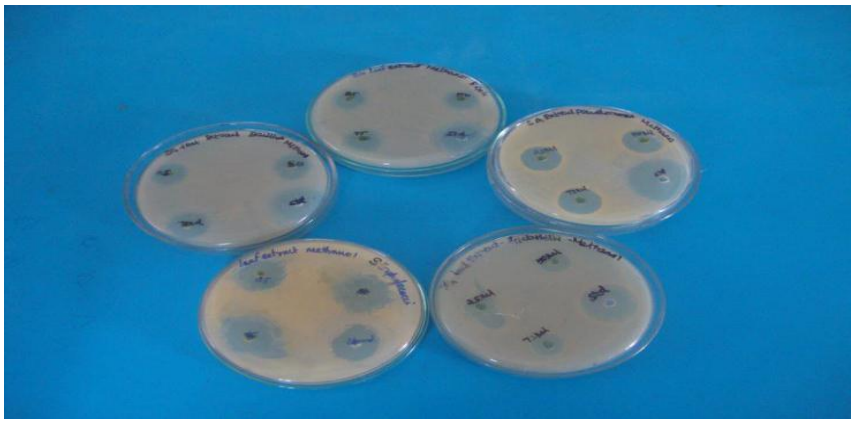
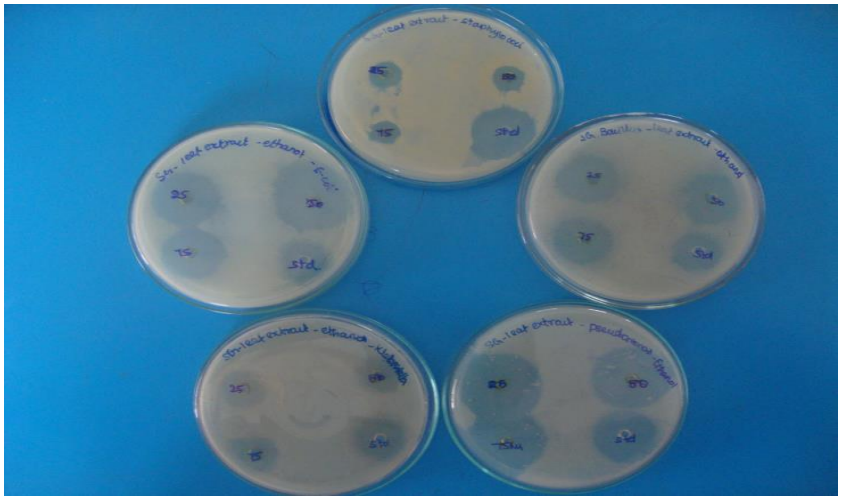


Fig: 3 Antibacterial activity of the ethanol extract of S. *grandiflora* against pathogens



7. APPENDIX

LIST OF ABBREVIATIONS

- μL - Microliter
- $CHCl_3$ - Chloroform
- *E. coli* - *Escherichia coli*
- $FeCl_3$ - Ferric Chloride
- S grandiflora - *Sesbania grandiflora*
- C.papaya - Carica papaya
- g - Gram
- H_2SO_4 - Sulfuric Acid
- HCl - Hydrochloric Acid
- $HgCl_2$ - Mercuric Chloride
- HNO_3 - Nitric Acid
- Kg - Kilogram
- KI - Potassium Iodide
- m - Meter
- mm - Millimeter
- mMol - Milimole
- N – Normality
- NaCl - Sodium Chloride
- NaOH - Sodium Hydroxide
- nm - Nanometer
- Ppt. - Precipitate
- ROS - Reactive Oxygen Species
- Rpm- - Rotation per minute
- Sol - Solution
- *Spp.* - Species
- UV - Ultraviolet

Reagents used for phytochemical screening

I. Mayer's Reagent: 1.358 g of HgCl2 was dissolved in 60 ml of water and it was mixed with a solution of 5 g of KI in 10 ml of water.

2. Wagner's Reagent: 16.6 g of potassium iodide was dissolved in 100 ml of distilled Water and few crystals of iodine were added to the solution and stirred properly.

3. Benedict's Reagent: Solution I: 50 g of crystalline sodium carbonate 50 g of crystalline sodium citrate And 31.25 g of potassium thiocyanate were dissolved in 200 ml hot distilled water. Solution II: 4.5 g of CuSO4 dissolved in 25 ml water to prepare copper sulphate solution. Solution III: 5% solution of potassium ferrocyanate was prepared. Finally Benedict's reagent was prepared by mixing 200 ml of solution I, 25 ml of Solution II and 5 g of solution III and the final volume was adjusted to 250 ml with Water.

4. Fehling's Reagent:

Solution I: 31.66 g of CuSO4 was dissolved in sufficient amount of water to produce 500 ml solution. Solution II : 176 g of sodium potassium tartar ate and 77 g of sodium hydroxide was dissolved in sufficient amount of water to produce 500 ml solution. Finally equal volume of solution I and II were mixed to prepare Fehling's solution.

5. Concentrated sulphuric acid solution: 36 N concentrated sulfuric acid solutions was used.

6. Dilute sulphuric acid solution: Concentrated sulfuric acid was diluted 10 times with Water to produce dilutes sulfuric acid solution.

7. Ferric chloride solution: 15 g of ferric chloride hexahydrate was dissolved in 100 ml of distilled water.

8. Ammonia solution: 25% of ammonia solution was used.

9. Sodium hydroxide solution: 20 g of NaOH was dissolved in 100 ml of distilled water.

10. Lead acetate solution: 10 g of lead acetate was dissolved in 100 ml of Carbon dioxide free water.

11. Concentrated nitric acid solution: Nitric acid solution (69 – 72%) was used.

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CONCLUSION & SUMMARY

In the present investigation the preliminary screening was done for the two plants. The commercial antibiotics were used to perform the antibacterial activity. These plants are mentioned in the traditional systems of medicine. They are most importantly used in the treatment of infective gastro intestinal diseases especially in India due to the major causes of infant and adult mortality. All the plant extracts used were found to possess antibacterial activity which are screened using well diffusion method and antibiotic sensitivity test to know the activity of selected pathogens.

In conclusion, it can be stated that the leaf extract of *Sesbania grandiflora* possessed rich phytochemical constituents and antibacterial activity which may be potentially responsible for its free radical scavenging capacity. Thus may be recommended as an anti-stressor. Medicinal plants were the potent source of human health due to the presence of active phytochemical compounds that are responsible for its various pharmacological activities.

On the basis of the results obtained, the present work concluded that the leaves of *S. grandiflora* are rich in phytochemical constituents even though the phytochemical screening of the leaf extracts of samples had shown variation in their phytochemical constituents with the presence and or absence of some components. Most components were present in aqueous, ethanol and methanol leaf extracts. The presence of various secondary metabolites such as glycosides, alkaloids, saponins, phenols and flavonoids were believed to exhibit the antibiotic properties and confirmed their antimicrobial efficacy against selected pathogens.

The antibacterial activity of *Sesbania grandiflora* against both gram positive and gram negative bacteria indicated that the plants are the potential source for production of drugs with a broad spectrum of antibacterial activity. Methanol leaf extracts showed better antibacterial activity against *Staphylococcus sp.* while ethanol extracts were found to be better on *E. coli*. From the results of this study it is concluded that antibacterial extracts of *Sesbania grandiflora* may be helpful in treatment of many infectious enteric diseases, as the extracts of the major part of *Sesbania grandiflora* were effective in controlling the growth of entero pathogenic bacteria.

Aqueous extract of *Sesbania grandiflora* showed maximum active against *Bacillus* sp, *Staphylococcus* sp, *E.coli* sp, *Klebsiella* sp. Ethanol extracts obtained from *Sesbania grandiflora* were highly active against all the pathogenic organisms. Methanol extracts from *Sesbania grandiflora* showed maximum active against *Staphylococcus* sp, *E.coli* sp, *and Klebsiella* sp.