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COMPARATIVE STUDY OF IN-VITRO ANTI-MICROBIAL ACTIVITY OF *SESBANIA GRANIFLORA* LEAVES EXTRACT BY USING DIFFERENT SOLVENTS

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ABSTRACT

To compare the antimicrobial activity of *Sesbania graniflora* leaves extracts by using different solvents (Aqueous, Ethanol & Petroleum ether) .The essential part of *Sesbania graniflora* sample was extracted by macerated for 7days, the extraction was efficiently done and the extract was collected to perform the anti-microbial activity by measuring the zone of inhibition of the bacterial culture. In this study, we concluded that the Sesbania graniflora leaves show more anti-microbial activity in Gram positive bacteria when compared to the Gram negative bacteria.The ethanolic extract shows more anti-microbial activity than aqueous and petroleum ether extracts.

Keywords: *Sesbania Graniflora Leaves*, Maceration, Zone Of Inhibition, Phytochemical Screening, Antibacterial Activity.

I. INTRODUCTION

A medicinal plant is any plant which, in one or more of its part contains substance that can be used for therapeutic purpose or which are the precursors for the synthesis of useful drugs.[5] The Sesbania graniflora is a most commonly used plant which is also popularly called as “Agati”. The Sesbania graniflora is a short living plant, soft-wooded, loosely branching with a rather open crown; it can grow 8-15 meters tall. The tree bears a big white flower that is hertialy used in Bengali cuisine. White flower variety of Sesbania graniflora found to be non-toxic, the purple flower is highly toxic. The white flowers and leaves are enriched with vitamins and minerals. The leaves, flowers and young pods are edible, and Sesbania flowers are commonly used as a vegetable in Southern Asia, especially in Thailand, Vietnam and Cambodia**.**

Several activities has been studied on the plant Sesbania graniflora like treating the small pox and eruptive fevers. The barks, leaves, gums and flowers are considered as medicinally active. The juice from the flowers is used to treat the head ache, head congestion or stuffy nose. As a stuff, the juice is supported to clear the nasal sinus.

The present study examines the phytochemical constituents and antibacterial activity of leaves of Sesbania grandiflora using different solvents such as water, ethanol and petroleum ether. Antibacterial activities of leaves of Sesbania grandiflora was analyzed through well diffusion technique. The anti-miocrobial activity was compared between different extracts from solvents such as water, ethanol and petroleum ether. The bacteria used in the culture was (*Escherichia coli, Salmonella spp, Shigella, Vibrio*) from the rain water as gram (–ve) bacxteria[3] and (*Streptococcus salivariu, Streptococcus porphymonas ginivalis, Staphylococus, mutans and Lactobacillus*) from the Saliva as gram (+ve) bacteria for comparision[2]. In the study also analyse the potential anti-microbial activity between the water, ethanol and petroleum ether extract of Sesbania graniflora.

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Fig 1: *Sesbania graniflora* Plant

II. MATERIALS AND METHOD

Sample Collection and Preparation:

The Sesbania graniflora leaves used in this study is collected from Kaveripattinam at Krishnagiri; the collected sample was cleaned, washed and shaded dried for 15 days. Then the dried leaves was coarsly grinded and kept in store room for further analytical and antimicrobial study.

Preparation of plant material:

The collected plant leaves were washed thoroughly 2-3 times with running water and with distilled water. The leaves were air-dried under shade. The leaves were crushed to make possible coarse powder with the help of mortar and pestle and stored for further analysis.

Extraction of sample material:

The essential part of *Sesbania graniflora* sample was extracted by maceration method by using conical flask as mentioned by Experimental pharmacognosy in Nirali Prakasahan:[4] About Each 200g of sample mixed separately with 500ml of various solvents such as water, ethanol, petroleum ether was taken in conical flask and then extraction run for 7 days with the occasional shaking, the process was ready to collect extraction sample. The extracted sample was kept in room temperature and stored in conical flask closed with aluminium foil. Aqueous, ethanolic, petroleum ether extracts stored as sample A, B and C separately.

The crude extracts were subjected to phytochemical screening for the presence or absence of alkaloids, saponin, glycosides, carbohydrate, tannins, reducing sugar, steroid and flavonoids.

Phytochemical Screening of the Plant Extracts:

The *Sesbania graniflora* sample extracts were screened for alkaloids, saponin, glycosides, carbohydrate, tannins, reducing sugar, steroid and flavonoid. The portion of the dry extract was subjected to the Phytochemical screening using the method adopted by Trease, Evans and Harbourne.

Chemical tests for *Sesbania graniflora* leaves:

Test for alkaloid (Mayer's reagent tests)

The identification of alkaloids was carried out using the Mayer's test. A portion of the plant extract was mixed with 5ml of sulphuric acid in 50% ethanol. 1ml of Mayer's reagent was added drop by drop. The formation of a greenish color or cream precipitate indicated the presence of alkaloids.

Test for Flavonoids:

The identification of Flavonoids was carried out using the sodium hydroxide test. 5ml of plant extract was mixed with few magnesium chips and 2 drops of concentrated hydrochloric acid were added and warmed. The presence of a pink/red color indicated the presence of flavonoids

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Test for Reducing Sugars:

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

Test for saponin's

Saponins were identified via the frothing test. 3ml of the plant extract was added to 10ml distilled water and shaken vigorously for 30 seconds. Froth formation indicates the presence of saponins.

Test for 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) Molisch test:

Filtrate was treated with 1 drop of Molisch reagent and add 2ml of con.HCl was added from the side of test tube. The test tube was observed for formation of violet ring at the junction of two solutions indicated that presence of carbohydrates.

b) Iodine test:

2ml of iodine solution added in plant extract gives development of Dark blue color. Simultaneously, presence of phenols and tannins were tested. 2 ml of 2% Of FeCl3 solution were added in the plant extracts. Dark green color was developed for phenolic compounds and black color for presence of tannins.

c) Fehling test:

Equal volume of Fehling's reagent A and B mixed together and 2ml of Mixture was added to plant extracts followed gentle heat, the mixture turned Brick red color.

d) Benedict test:

2ml of Benedict's solution was added to crude plant extracts followed. Gentle boiling gives reddish brown precipitate.

Test for Glycosides

2ml of chloroform, 2 ml of acetic acid were added to plant extract and allowed to cool, followed by addition of 2ml of concentrated H_2SO_4 changes the violet to blue then green, indicates the presence of steroidal nucleus that is glycone portion of glycoside. In another way, the available cardiac glycosides are tested by addition of 1-2 drops of glacial acetic acid and 2% of $FeCl_3$ solution in crude plant extract followed by 2ml of H_2SO_4, gives brown ring at the interphase indicates the presence of cardiac glycosides.

Test for Tannins

Tannins were identified using the Bromine Water test. 5ml of plant extract was extracted with 20ml of 50% alcohol and then filtered. A few drops of bromine water were added to the resulting filtrate. The formation of a buff/white precipitate indicated the presence of tannins.

Test for Steroid:

Finally the presence of steroids was detected using the Libermann-Burchard test. 2ml of the test plant extract were mixed with 2 drops of chloroform and 2ml of acetic anhydride, along with 1ml of concentrated sulphuric acid added down the side of the tube. The formation of a reddish ring at the contact zone of the two liquids and a greenish color in the separate layer indicates the presence of steroids.

BIOLOGICAL CHARACTERIZATION:

ANTIBACTERIAL ACTIVITY OF PLANT EXTRACTS

The bacterial species used for study anti-microbial activity such as gram positive bacteria (*Streptococcus salivarius , Streptococcus porphymonas ginivalis, Staphylococus, mutans and Lactobacillus*) from Saliva and gram negative bacteria (*Escherichia coli, Salmonella spp, Shigella, Vibrio*) from the rain water[1] . The Agar disc diffusion method was influenced for detect the antibacterial activity of plant extract of water as (sample A) and ethanol extract as (sample B) and petroleum ether extract as (sample C). The disc diffusion method was found to simple, cheap and reproducible practical method. The microorganism were inoculated in the nutrient broth and incubated on a Biological oxygen demand incubator. 25ml of inoculum was poured into the molten Muller Hinton agar media in the petri plate.

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The test compounds such as water, ethanol and petroleum ether extracts of Sesbania graniflora leaves were disc was introduced into the well (spot) and the plates were incubated for 12h. The zones of inhibition were measured in millimeters. Amoxicillin was used as positive reference standards to determine the selectivity of each tested microbial species.

P. Sarasu Packiyalakshmi, et al.., demonastrated the bacterial species used for the test were act against Sample A (water extract), Sample B (Ethanol extract) and Sample C (petroleum ether extract) of *Sesbania graniflora* leaves, The zone of inhibition and Comparative study of antibacterial activity of sample A, sample B and sample C found in plant extracts A ,B and C were detailed discussed in below[1],

III. RESULT AND DISCUSSION

Phytochemical Screening of the Plant Extracts:

In this study, The phytochemicals constituents that present in the different extracts (Aqueous, Etahanol and Petroleum ether) of the *Sesbania graniflora* leaves are mentioned in the below table.

Table 1: Phytochemical screening of leaf extracts

S.NO	Phytochemicals	Aqueous extract	Ethanol extract	Petroleum Ether extract
1	Alkaloids	+	+	+
2	Carbohydrate	+	+	+
3	Saponin	+	+	-
4	Glycosides	-	+	+
5	Flavanoid	+	+	-
6	Steroid	+	-	+
7	Reducing sugar	+	+	-
8	Tannins	-	+	+

Notes: (+) positive & (-) negative

Antibacterial Activity of Plant Extracts:

In this study, Aqueous extract (Samlple A), Ethanolic extract (Sample B) and Petroleumm ether extract (SampleC) of Sesbanioab graniflora leaves were taken for comparatively studied. The gram positive bacteria from the saliva (bacteria (*Streptococcus salivarius , Streptococcus porphymonas ginivalis, Staphylococus, mutans and Lactobacillus*) show more zone of inhibition when compared to the gram negative bacteria from the rain water(*Escherichia coli, Salmonella spp, Shigella, Vibrio*).

Table 2: Zone Of Inhibition Of Plant Extracts (A, B &C) With Standard Drug

Tested Drug	Gram (+) bacteria Zone of inhibition(mm)	Gram (-) bacteria Zone of inhibition(mm)
Amoxicillin (Standard)	18mm	12mm
Water Extract (Sample A)	10.8mm	8mm
Ethanol (Sample B)	13mm	9.5mm
Petroleum ether(Sample C)	8.5mm	7mm

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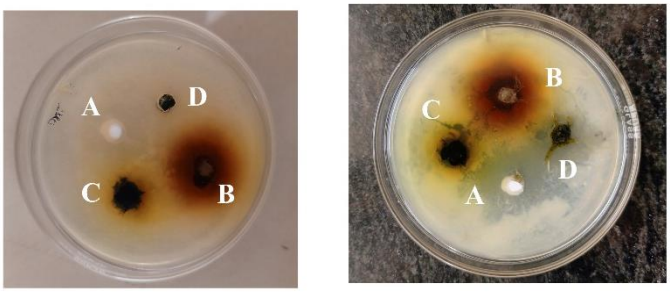

Fig 2: Zone of inhibiton of Test and Standard

IV. CONCLUSION

From the above mentioned results we concluded that the Sesbania graniflora leaves show more anti-microbial activity in Gram positive bacteria when compared to the Gram negative bacteria.

Gram (+ ve) bacteria > Gram (– ve) bacteria.

And the order of activity of the extracts fron the leaves are

Ethanol > Water > petroleum ether

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