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# Invitro Antimicrobial Activity of Leaf extracts from *Sesbania grandiflora*

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**A B S T R A C T**

*Sesbania grandiflora* Linn belonging to family *Leguminosae* is a well-recognized medicinal plant in numerous countries like India, Srilanka and Southeast Asia. The present study examines the phytochemical constituents and antibacterial activity of leaves of *Sesbania grandiflora*. The quantitative examination of numerous phytochemicals was analyzed in different solvent systems. Antibacterial activities of leaves of S*esbania grandiflora* was analyzed through well diffusion technique. Current outcome shows the rich amount of phytochemical constituents present in the leaves. Among the three extracts ethanol showed the maximum activity against *Staphylococci sp* compared to gram negative bacteria. Minimum inhibitory concentration the least value of the range between 320mm to488mm indicated the high activity. Maximum activity recorded against S*taphylococci sp* for ethanol leaf extract was 316mm. This proved that *Sesbania grandiflora* leaves exhibit the highest activity.

## Introduction

Plant materials are main sources to treat serious diseases in the world. So far a systematic study of plants to determine their antimicrobial active compounds is a comparatively new field. *Sesbania grandiflora* is a small tree in the genus *Sesbani*. It is a fast-growing shrub highly distributed in tropic and subtropics. The selected green leafy vegetable *Sesbania grandiflora* has various traditional uses. *Leaves* are used as tonic, diuretic, laxative, antipyretic, chewed to disinfect mouth and throat. Leaves are chewed to purify mouth and throat and are beneficial in stomatalgia (Nadkarni *et al.*, 2009).

*Flower* is used to treat the headache, dimness of vision, Catarrh, and also used to improve appetite, antipyretic. *Bark* is used for bitter tonic for anthelmintic, febrifuge, diarrhea, small pox, Astringent. *Fruits* are used in case of fever, pain, bronchitis, anemia, tumors, colic, jaundice, poisoning (Kirthikar *et al.*, 1998). *Root* issued in Rheumatism, Expectorant, Painful swelling. Warrier *et al.* (1996). *Sesbania grandiflora* is an easily available plant and based on the medicinal properties the present study investigates the phytochemical constituents and antibacterial activity of leaves of *Sesbania grandiflora.*

## Materials and Methods

### Collection of Plant Samples

The fresh leaves of *Sesbania grandiflora* were concurrently collected from open fields of Theni district. 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.

The methanolic extracts of the Fenugreek Leaf extracted previously were screened for phytochemicals.

For phytochemical analysis, the extracts were prepared by taking each dried powder into separate 100 ml conical flask. To this 50ml of each solvent (Aqueous, Methanol, ethanol) was added. The conical flasks were allowed to stand for 24hours and then filtered using what man No.1 filter paper. Thus, the filtrates gained were used as test solutions.

The plant extracts were tested for the presence of bioactive compounds by standard method (Yadha *et al.,* 2011)

### 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) **Fehling's 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.

(b) **Benedict's test**: 2ml of Benedict's solution was added to crude plant extracts followed.

Gentle boiling gives reddish brown precipitate.

(c) **Molisch's test**: 2ml of Molisch's reagent was added in plant extract followed

By addition of H2SO4 which develops the appearance of violet rings in the interphase.

(d) **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

### Alkaloids

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.

### 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.

### 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.

### Saponins

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.

### Terpenoids

The identification of terpenoids was performed using Noller's test. The test plant extract was warmed along with a tin piece and 3drop of thionyl chloride. Terpenoids are present if the solution turned a purplish colour.

### Steroids

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.

### 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 H2SO4 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 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 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 Organism

The following bacterial strains were obtained from the laboratory of Department of Microbiology, Nadar Saraswathi College. 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.

### Well Diffusion Method

### Muller Hinton agar Formula and preparation

Muller Hinton agar medium was employed for well diffusion sensitivity testing. The medium contained per litter, infusion from 300g beef, 17.5g casein hydro lysate, 1.5g starch and 17g agar (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˚cfor 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 extract 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 (Ragahavendra *et al.*, 2010).

## Determination of Minimum Inhibitory Concentration

The MIC of the extracts was determined according to the macro broth dilution technique (NCCLS, 2010).Standardized suspensions of the test organisms existed 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.

## Result and Discussion

The powdered leaves of *Sesbania grandiflora* extracted with different solvent. The plant extract was then performed for identify the numerous phytochemical constituents.

Phytochemical analysis showed the presence of alkaloids, flavonoids, saponins, glycosides and steroids. Maximum antibacterial activity was observed due to presence of secondary metabolites. All the extracts from *Sesbania grandiflora* display antibacterial activity against all tested strains. Test for concentration ranging from 25mg/ml, 50mg/ml, 75mg/ml, and 100mg/ml antibacterial activity tested for well diffusion method. Ethanol extracts showed the maximum zone of inhibition on all organisms especially S*taphylococcus sp* (19.3mm at 75µl).The result was conformed with reports of K. Padmalochana *et al.*, (2014).same result we found in the present investigation.

**Table.1** Pηytochemical Constituents of *Sesbania grandiflora*

| Compound | Methanol | Ethanol | Aqueous |
|---|---|---|---|
| Alkaloids | + | + | + |
| Saponins | + | + | + |
| Flavonoids | + | + | + |
| Tannins | + | + | _ |
| Amino acid | _ | _ | _ |
| Reducing sugar | + | + | + |
| Carbohydrates | - | + | + |
| Glycosides | + | + | _ |
| Phenols | + | + | + |
| Steroids | + | + | + |
| Terpenoids | _ | _ | _ |

**Table.2** Invitro Antimicrobial Activity of *Sesbania grandiflora* Extract Against *Bacillus*

| **Zone of diameter(mm)** | | | | |
|---|---|---|---|---|
| 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.3** Invitro Antimicrobial Activity of *Sesbania grandiflora* Extract Against *Staphylococcus Sp*

| **Zone of diameter(mm)** | | | | |
|---|---|---|---|---|
| S.No | Solvent | Leaf 25 | 50 | 75 |
| 1 | Ethanol | 13 | 14.5 | 19.3 |
| 2 | Methanol | 11.7 | 13 | 16 |
| 3 | Aqueous | 12.6 | 13.7 | 14.5 |
| 4 | Standard | 18 | 16 | 17 |

**Table.4** Invitro Antimicrobial Activity of *Sesbania grandiflora* Extract against *Pseudomonas sp*

| | **Zone of diameter(mm)** | | | |
|---|---|---|---|---|
| S.NO | Solvent | Leaf 25 | 50 | 75 |
| 1 | Ethanol | 15 | 13.5 | 14 |
| 2 | Methanol | 13 | 13.5 | 12.8 |
| 3 | Aqueous | 9.5 | 10 | 13.6 |
| 4 | Standard | 14 | 15 | 16 |

**Table.5** Invitro Antimicrobial Activity of *Sesbania grandiflora* Extract Against *E.coli*

| | **Zone of diameter(mm)** | | | |
|---|---|---|---|---|
| **S.NO** | **Solvent** | **Leaf 25** | **50** | **75** |
| **1** | Ethanol | 10.3 | 12 | 13.8 |
| **2** | Methanol | 11.5 | 12.8 | 14 |
| **3** | Aqueous | 8.5 | 13 | 15 |
| **4** | Standard | 12 | 15 | 17 |

**Table.6** Invitro Antimicrobial Activity of *Sesbania grandiflora* Extract against *Klebsiella sp*

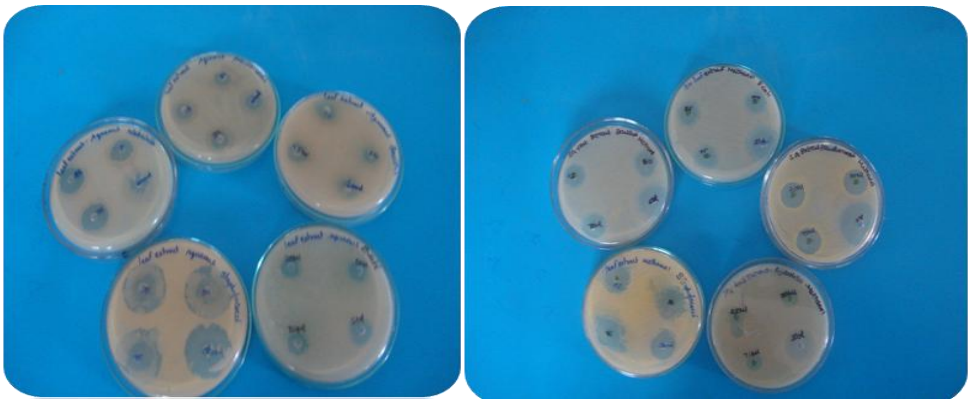
| **Zone of diameter(mm)** | | | | |
|---|---|---|---|---|
| **S.NO** | **Solvent** | **Leaf 25** | **50** | **75** |
| **1** | Ethanol | 6.8 | 8.5 | 13.4 |
| **2** | Methanol | 8.2 | 12 | 14 |
| **3** | Aqueous | 6 | 9.6 | 10.7 |
| **4** | Standard | 11.4 | 12 | 15 |

**Table.7** Mean MIC (mg / ml)

| S.NO | Plants | *E.coli* | *Klebsiella* | *Pseudomonas* | *Bacillus* | *Staphylococcus* |
|---|---|---|---|---|---|---|
| 1 | *Sesbania grandiflora* | 0.164 | 0.285 | 0.237 | 0.199 | 0.316 |

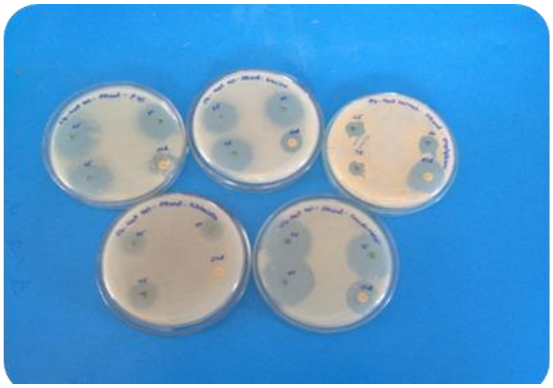
Qualitative Analysis of Phytochemical Analysis

In Vitro Antibacterial Activity of *Sesbaniagrandiflora*



**AQUEOUS EXTRACT** **METHANOL EXTRACT**

Ethanol Extract



MIC was performed against five organism such as *E.coli sp, Klebisiella sp, Pseudomonas sp, Staphylococcus sp, Bacillus sp.* Minimum inhibitory concentration least value the range between the 320mm to488mm indicated the high activity. Maximum activity was recorded for 316mm against *staphylococcus sp* for ethanol leaf extract. This proved that S*esbania grandiflora* leaves exhibit the highest activity and so theS*esbania grandiflora* leaves are potentially used as natural drug.

In conclusion, for this current study, the simple available plant *Sesbania grandiflora* was selected for the phytochemical screening. Phytochemical analysis showed the presence of alkaloids, flavonoids, saponins, glycosides and steroids. Ethanol extracts showed the maximum zone of inhibition on all organisms especially S*taphylococcus sp* (19.3mm at 75µl) due to presence of secondary metabolites. Based on the results, it is conclude that the ethanol extract of *Sesbania grandiflora* leaves potentially act as antimicrobial agent.

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