



Phytochemical study on *Sesbania grandiflora*

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ABSTRACT

Sesbania grandiflora Linn. (Family: Fabaceae) is widespread distributed in West Bengal, Assam, Karnataka and North-Eastern parts. The present study intended with various phytochemical screening and toxicity studies on *Sesbania grandiflora*. The present study was done with the help of Soxhlet extraction, Kirby Bauer well diffusion assay, Minimum Inhibitory concentration assay (MIC) and Colony forming unit (CFU). Preliminary phytochemical evaluation of the aqueous extracts revealed that presence of carbohydrate, proteins, flavonoids, alkaloids, tannins and glycosides. As per the antimicrobial investigations in the present study the CLP (Crude Leaf Powder) of *Sesbania grandiflora* exhibited low level antibacterial activity but the NPL (Nanosized leaf powder) exhibited highest level of antibacterial activity.

Key Words: Screening of phytochemicals, Kirby Bauer well diffusion assay, Minimum Inhibitory concentration assay (MIC), and Colony forming unit (CFU).

INTRODUCTION

The exact origin of *S. grandiflora* is not known but it is considered native to many south east Asian countries until recently, the use of perennial *Sesbania* species has largely been restricted to south and south east Asia.

Sesbania grandiflora[14,21] is a loosely branching tree up to 15m tall. Its leaves are pinnately compound up to 30 cm long with 20 – 50 leaflets in pairs, dimensions 12.4 x 5.15 mm oblong to elliptical in shape. Flowers were large, white yellowish, rose pink or red with a calyx 15.22 mm long. The standard has dimensions up to 10.5 x 6 cm. Pods are long (20 – 60 cm) and thin (6 – 9 mm) with broad sutures containing 15.50 seeds.

It is well adapted to hot, humid environments and does not grow well in the subtropics particularly in areas with temperatures below about 10°C. It is outstanding in its ability to tolerate water logging and is ideally suited to seasonally water logged or flooded environments when flooded, they initiate floating adventitious roots and protect their stems, roots and nodules with spongy, aerenchyma tissue. *S. grandiflora* is adopted to rain fall conditions of 2,000 – 4,000 mm but will grow in areas receiving only 800 mm. Another outstanding feature is its tolerance of both saline and alkaline soil conditions. The trees are grown on rice paddy walls at 1.5 – 2m intervals and forage is harvested in this manner for 3-4 years, yielding up to 2 kg dry matter per harvest per tree.

The tender leaves, green fruit, and flowers are eaten[14,22,20] alone as a vegetable or mixed into curries or salads. Flowers may be dipped in batter and fried in butter. Tender portions serve as cattle fodder. Overeating is said to

cause diarrhea[2]. Ripe pods apparently are not eaten. The flowers and young leaves of *S. grandiflora* are edible and are often used to supplement meals. Tender pods may also be eaten as vegetables. The dried leaves of *S. grandiflora* are used in some countries as a tea which is considered to have antibiotic, anthelmintic, [5, 15, and 16] anti – tumor [5] and contraceptive properties.

The wood is used, like bamboo, in Asian countries for construction. The tree is grown as an ornamental shade tree, and for reforestation. A gum resembling Kino fresh when red, nearly black after exposure, exudes from wounds. This astringent gum is partially soluble in water and in alcohol, and is applied to fishing cord.

Based on the above medicinal properties it is used in the present study for the experimentation of using bioactive compounds obtained from the extracts of plant leaves.

EXPERIMENTAL SECTION

SOXHLET EXTRACTION OF BIOACTIVE COMPOUNDS

Sesbania grandiflora plants were collected from vegetable market. The leaves of the plants were carefully separated and washed in sterile water to remove the soil and other debris. Then the leaves were dried in clean shadow place. After drying the leaves were grounded into powders. The bioactive principles from the plant were extracted by using various solvents like chloroform, Ethanol, petroleum ether and water in soxhlet extraction apparatus. The known amount of powdered leaf materials were packed and placed in the soxhlet apparatus. Then the solvents were added and the apparatus was switched on in order to get the bioactive compounds.

Under each solvent, the extraction was continued for a minimum 8 to 6 hours (until almost no plant residues was left in the recycled solvents). Then the excess solvent was evaporated in an oven at 50⁰C for 2 hours or 40⁰C for 3 hours. After evaporation, the sample was in the form of powder (concentrated form) and this form was stored at 4⁰C until further use.

During assay the bioactive compound was diluted by using double distilled water of standard physiological saline (0.9%, NaCl).

PREPARATION OF LEAF POWDERS

Crude Leaf powder was prepared by powder blender. Nanosized Leaf Powder was prepared by Nano wet ball milling

Phytochemical study on Bioactive compounds

The leaves of plant samples were air-dried and ground into uniform powder. The aqueous extract of each sample was prepared by soaking 100g of dried, powdered sample in 200ml of distilled water for 12 hour. The extracts were filtered using whattman Filter paper No.42 (125mm). Phytochemical tests were carried out on the aqueous extract and on the powdered specimens.

Test for alkaloids:

0.5 gm of *Sesbania grandiflora* leaf extract powder was added to 5ml of 1% aqueous HCl on a steam bath. This was filtered and 1 ml of the filtrate was treated with few drops of Draggendorf's reagent and a second 1 ml portion was treated with Wagner's reagent. The formation of precipitates was an indication of the presence of alkaloids.

Test for Flavonoids:

0.5g of the extract was dissolved in 2ml of dilute NaOH solution. A few drops of concentrated H₂SO₄ were then added. Formation of yellow solution confirmed the presence of flavonoids.

Test for glycosides:

0.5g of leaf extract was stirred with 10 ml of boiling water. This was filtered and 2 ml of the filtrate was hydrolyzed with a few drops of concentrated HCl and the solution was rendered alkaline with a few drops of ammonia solution. 5 drops of this solution was added to 2 ml of Benedict qualitative reagent and boiled. A reddish – brown precipitate showed the presence of glycosides.

Test for tannin:

0.5 g of *Sesbania grandiflora* leaf was stirred with 10 ml of distilled water. This was filtered and a few milliliters of 5% ferric chloride were added to the Filtrate. A green coloration showed the presence of tannin.

Test for anthraquinone:

0.5 g of leaf extract was taken into a dry test tube and 5 ml of chloroform was added and shaken for 5 minutes. The extract was filtered, and the Filtrate was shaken with an equal volume of 100% ammonia solution. A red color in the ammoniacal layer (lower layer) indicated the presence of free anthraquinone.

Test for steroid:

5 g of leaf extract was dissolved in 2 ml of chloroform. Sulfuric acid was carefully added to form a lower layer. A blue color at the interface indicated the presence of steroidal ring.

Test for Phlobatannins:

Aqueous extract of plant sample was boiled with 1% aqueous hydrochloric acid. A red precipitate is formed, which confirmed the presence of Phlobatannins

Test for terpenoids:

5 ml of leaf extract was mixed in 2 ml of chloroform and concentrated H₂SO₄ (3 ml). A reddish brown coloration at the inter face showed the presence of terpenoids.

Test for protein:

5 ml of leaf extract solution was cooled on ice and 1 ml of 10% NaOH, 1 ml of 0.02% α -Naphthol solutions were added. After few minutes 10 drops of alkaline hypo bromide solution was added. The formation of intense red color showed the presence of protein.

Test for carbohydrate:

5 gm of leaf extract was mixed with a few drops of iodine solution appearance of deep blue color indicated the presence of carbohydrates.

TEST WITH MICROORGANISMS:**Bacterial strains:**

- i) Gram-positive: Staphylococcus aureus (ATCC 2267)
- ii) Gram-negative: Pseudomonas aeruginosa (ATCC 25619)

Fungal strains:

- i) Candida albicans (NCTM 3102)
 - ii) Aspergillus niger(NCTM 105)
- were obtained from the department of microbiology, Bharathidasan university, Tiruchirappalli.

ANTIMICROBIAL ASSAYS:

Essential bioactive compounds from *Sesbania grandiflora* have antimicrobial activity against both Gram positive and Gram negative bacteria, yeast and filamentous fungi. The antileptospiral activity of the drug efficacy of the plant *Sesbania grandiflora* based phytonanompounds has been studied by the following methods.

- ❖ Kirby Bauer well diffusion assay.
- ❖ Minimum Inhibitory concentration assay (MIC)
- ❖ Colony forming unit (CFU).

i) Kirby – Bauer well – Diffusion assay.

Petriplates containing 10 to 15 ml of agar media were swabbed with 100 μ l of 48 hours old culture of test microbial strains. The swab is uniformly spreaded on the agar plate using L-rod. The plates were allowed to dry for 10 minutes. On each plate, reference antibiotics also applied in disc form (20mg/ml). The cutting discs Sesbania leaf extract based crude medicine sinked in and nano phytocompounds (20mg/ml) were left to dry in aseptic condition. The plates were incubated for 24 hours at 37⁰C. Following 24 hours or 48 hours incubation the antimicrobial activity

of the plant extract was indicated by clear zone of growth inhibition formed around the well. The plates were examined carefully.

ii) Minimum inhibitory concentration assay (MIC)

Minimum inhibitory concentration values are used to measure the in-vitro activity of *Sesbania grandiflora* based nanomedicine against test microbes. From the prepared plant extracts 0.5, 1.0, 1.5, 2.0, 2.5 and 9 ml was taken. It is added to the series of test tubes containing 10 ml of nutrient broth. About 20 μ l of microbial culture is also added to each and every tube. The tubes were incubated at 37°C. After overnight or 48 hours the turbidity is measured using spectrophotometer in terms of optical density at 540nm.

iii) Colony forming unit (CFU)

This technique is used to determine the number of colonies produced against the *Sesbania grandiflora* based phytonanocompounds.

In this method, the inoculum was taken from the tubes of time course method it is maintained by pour plate method. The plates were incubated at 37°C. The plates were observed for colonies and the total numbers of colonies were counted and it is recorded

RESULTS AND DISCUSSION

Table: 1 Screening of Phytochemicals in *S.grandiflora*

| S. No | Parameter | <i>S.grandiflora</i> |
|-------|---------------|----------------------|
| 1 | Alkaloids | Presence |
| 2 | Flavonoids | Presence |
| 3 | Glycosides | Presence |
| 4 | Tannin | Presence |
| 5 | Anthraquinone | Presence |
| 6 | Steroid | Presence |
| 7 | Phlobatannins | Presence |
| 8 | Terpenoids | Presence |
| 9 | Protein | Presence |
| 10 | Carbohydrate | Presence |

CHARACTERIZATION OF LEAF MATERIALS:

The *Sesbania grandiflora* leaf material prepared by powder blender and Nano-wet ball milling were characterized

CHARACTERIZATION OF SIZE AND SHAPE:

The size and shape of the powdered leaf materials are investigated using Transmission Electron Microscope. Results were shown in table: 2

Table: 2 Size and Shape of Crude and Nano powders using TEM

| S.No | Parameter | Size | Shape |
|------|-----------------------|-------------|---------------------------|
| 1 | Crude Leaf powder | 0.5 μ m | Irregular oval cluster |
| 2 | Nanosized Leaf Powder | 72-78 nm | uniform and monodispersed |

KIRBY-BAUER'S DISC DIFFUSION ASSAY

The antimicrobial activity of the T2 (phytonanocompounds) indicated greater efficiency than T1 (crude phytocompounds). The results explored through Kirby-Bauer's disc diffusion assay are given in table: 3

Table: 3 Antimicrobial activity of *S.grandiflora* through Kirby-Bauer's disc diffusion assay

| S.No | Parameter | Antimicrobial activity (Inhibition %) | |
|------|-------------------------------|---------------------------------------|----------------------------|
| | | Crude Leaf Powder (T1) | Nanosized Leaf Powder (T2) |
| 1 | <i>Staphylococcus aureus</i> | 63 | 76 |
| 2 | <i>Pseudomonas aeruginosa</i> | 66 | 79 |
| 3 | <i>Candida albicans</i> | 68 | 81 |
| 4 | <i>Aspergillus niger</i> | 64 | 89 |

MINIMUM INHIBITORY CONCENTRATION ASSAY (MIC):

The effect of direct addition of *Sesbania grandiflora* crude leaf powder and Nanosized leaf powder to the microbial culture using liquid broth medium was also assayed. After 24 hours of incubation of inoculated medium (medium contains test microorganism such as staphylococcus aureus, pseudomonas aeruginosa, Candida albicans and aspergillus niger) with different concentration of *Sesbania grandiflora* crude leaf powder (CLP) and Nanosized leaf powder (NLP), (0.5, 1.0, 1.5, 2.0 and 2.5mg). The absorbance was taken at 540nm.

The crude leaf powder possess coarse irregular size particle and it cannot intact with microbes. Hence its efficiency is very low compared with Nanosized leaf powder. Nanosized leaf powder easily binds on the surface of the microbial cell membrane and initiates their medicinal effect quickly. The results explored through minimum inhibitory concentration assay are given in table: 4

Table: 4 Minimum Inhibitory Concentration Assay of S.grandiflora

| S.No | Quantity in mg | 0.5 | 1.0 | 1.5 | 2.0 | 2.5 |
|------|------------------------|------|------|------|------|------|
| 1 | Optical Density of CLP | 0.26 | 0.24 | 0.22 | 0.20 | 0.19 |
| 2 | Optical Density of NLP | 0.16 | 0.12 | 0.09 | 0.04 | 0.01 |

COLONY FORMING UNIT (CFU)

The previous results were further confirmed by CFU using petriplate spread method.

Only at lower concentration of CLP and NLP ranging from 2-4mg extract /10ml, colony formation was observed whereas at higher concentration (6-10mg/10ml) hardly any growth of microbes was noticed.

CONCLUSION

Qualitative phytochemicals were screened in *S.grandiflora*, according to the results Alkaloids, Flavonoids, Glycosides, Tannin, Anthraquinone, Steroid, Phlobatannins, Terpenoids, Proteins and Carbohydrates are present. Antimicrobial activity of crude leaf powder and Nanosized leaf powder was determined. According to the results the antimicrobial activity of Nanosized leaf powder was very potent due to its uniform size when compared with Crude leaf powder. It was found that the Nanosized leaf powder had a high ability to kill microbes

Acknowledgements

Authors are thankful to M.S University Tirunelveli, Scott Christian College Nagercoil, N.M.C.College Marthandam, and Bharathidasan University, Tiruchirappalli to carry out the project.

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