Research Article

Phytochemical Analysis and Antimicrobial Activities of *Sesbania Grandiflora* (L) Leaf Extracts

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ABSTRACT

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, anti-tumor and contraceptive properties. The present study intends to provide an overview of the chemical constituents present in the crude leaf extracts of *S. grandiflora* (L) with special emphasis on their pharmacological actions. Qualitative phytochemical screening was carried out using the crude leaf extracts in four different solvents such as water, acetone, ethanol and methanol. Preliminary phytochemical analysis revealed the presence of eleven compounds such as carbohydrates, tannins, steroids, terpenoids, alkaloids, flavanoids, cardiac glycosides, oils, saponins, coumarins, gum and mucilage. A comparative antimicrobial activity of dried leaf extracts of *S. grandiflora* (L) were evaluated against two gram negative bacterial pathogens namely *Escherichia coli* and *Pseudomonas aeroginosa* and two clinical fungal pathogens namely *Candida albicans* and *Aspergillus niger* by agar cup plate assay method. The leaf extracts of *S. grandiflora* (L) was found to have high anti fungal activity than antibacterial activity. The results suggest that the leaves are a rich source of valuable primary and secondary metabolites exhibiting the antimicrobial activity.

Keywords: *Sesbania grandiflora* (L), Antimicrobial activity, Phytochemical Analysis, Leaf extracts, Agar cup method.

INTRODUCTION

Since ancient times, people have been exploring the nature particularly plants in search of new drugs. This has resulted in the use of large number of medicinal plants with curative properties to treat various diseases[1]. Nearly 80% of the world's population relies on traditional medicines for primary health care, most of which involve the use of plant extracts[2]. In India, almost 95% of the prescriptions were plant based in the traditional systems of Unani, Ayurveda, Homeopathy and siddha[3]. The study of plants continues principally for the discovery of novel secondary metabolites. Plants have the ability to synthesize a wide variety of chemical compounds that are used to perform important biological functions and to defend against attack from bacteria, fungus and other pathogens. The great Sanskrit writings such as the Rig Veda and Atharva Veda are some of the earliest available documents detailing the medical knowledge that formed the basis of some plants that are used for medicinal purposes. Chemical compounds in plants mediate their effect on human body for healing purposes. The presence of bioactive compounds indicates the medicinal value of plants. Antioxidant and antimicrobial properties of various extracts from many plants have recently been of great interest both in research and food industry, because of their possible use as natural additives to replace synthetic antioxidants and antimicrobials with natural ones.[4] Phytochemicals are the antibiotic properties of plants and have been reported to possess antibacterial, antifungal and anti-inflammatory activities.[5] Thus medicinal plants play an important role in the development of newer drugs due to their effectiveness, less side effects and relatively low cost when compared to synthetic drugs.[6,7]

Plant secondary metabolites can be divided into three chemically distinct groups such as Terpenes, Phenolics and nitrogen containing compounds.[8] Another defensive response of plants towards infection is the synthesis of hydrolytic enzymes that attack the cell wall of pathogens. An assortment of glucanases, chitinases and other hydrolases are influenced by fungal invasion. Perhaps the best studied response of plants to bacterial and fungal invasion is the synthesis of phytoalexins. Plant products have been part of phytomedicines since time immemorial. These can be derived from any part of the plant like bark, leaves, flowers, seeds etc.[9] Knowledge of the chemical constituents of plant is desirable since such information will be of great value for the synthesis of complex chemical substances properties were carried out in the leaf extracts of *S. grandiflora* (L).

Sesbania grandiflora also known as agate or hummingbird tree, is a small tree about 10 m high with unique medicinal properties as all parts of the plant serves as a natural anti-oxidant.[10,11] The tree thrives under full exposure to sunshine and is extremely frost sensitive. Agathi is native to tropical Asia and is widespread in India, Malaysia, Indonesia and Philippines. It is commonly found in disturbed and agricultural environments including along roadsides, on dikes between rice paddies and in backyard vegetable gardens. The flowers, young leaves

and tender pods of the white flowered Agati are edible and are sold in local ethnic markets[12]. All parts of *S. grandiflora* are used as medicine in Southeastern Asia and India including preparations derived from the roots, bark, gum, leaves, flowers and fruits.[10]

Powdered roots of *S. grandiflora* var. coccinea are mixed in water and applied externally as a poultice or rub to rheumatic swellings. The bark is considered as an astringent and is used for the treatment of smallpox, in Philippines for the treatment of ulcers in the mouth and alimentary canal, in Java for the treatment of thrush and infantile disorders of the stomach and in Cambodia the pounded bark is applied to scabies. The juice of the leaves is considered anthelmintic and tonic and is used to treat worms, biliousness, fever, gout, itchiness and leprosy[13]. Malayans apply crushed leaves to sprains and bruises. In Ayurvedic medicine the leaves are used for the treatment of epileptic fits and clinical research supports the anticonvulsive activity of Agati leaves. Agati is valued as fodder throughout Indonesia, particularly in dry season for feeding cattles and goats.

In recent years, secondary plant metabolites or phytochemicals previously with unknown pharmacological activities, have been extensively investigated as a source of medicinal agents.[14] Thus it is anticipated that phytochemicals with adequate antibacterial efficiency could be used for the treatment of bacterial infections.[15] The increasing failure of chemotherapeutics and antibiotic resistance exhibited by pathogenic microbial infectious agents has led to the screening of several medicinal plants for their potential antimicrobial activity.[16,17] The present study aims in exploring the phytochemical constituents, antibacterial and antifungal properties of the leaf extracts of *sesbania grandiflora* {L}.

MATERIALS AND METHODS

Collection of plant material

The fully matured fresh leaves of *S. grandiflora* (L) were collected from kudapanakunnu area and were identified in the Post graduate department and research centre of botany, Mahatma Gandhi College, Thiruvananthapuram. The leaves were washed thoroughly, shade dried and finely powered. The dried powdered leaves were extracted with four different solvents such as water, acetone, methanol and ethanol. For aqueous extraction, ten grams of the powdered leaves were mixed with 100ml of distilled water, boiled for about two hours and filtered. Whereas acetone, methanol and ethanol extracts were prepared by mixing ten grams of powdered leaf samples with 100ml of each solvent separately in mechanical shaker for about 48 hours at room temperature. Extracts were filtered, concentrated, dried and were stored in the refrigerator at 4°C for future use.

Phytochemical Analysis

The prepared plant extracts were analyzed for the presence of alkaloids, carbohydrates, glycosides, sapponins, proteins, fixed oils, steroids, terpenoids, tannins, flavonoids, gum and mucilages.[18]

Preparation of plant extract for antimicrobial screening

For antimicrobial screening the concentrated, dried and powdered ethanol leaf extract was dissolved in 10 % dimethyl sulfoxide (DMSO) and were stored at 4^0 C for further use.

Antibacterial activity

Antibacterial activity was carried out against two selected gram negative pathogens (such as *Escherichia coli and Pseudomonas aeroginosa*). The strains used for the present study were obtained from Biogenix Research centre, Valiyavila, Thiruvananthapuram. In order to access the biological significance and ability of the plant part, minimal inhibitory activity was determined by Agar cup method.

Petri plates containing 20ml of Muller Hinton medium were seeded each with 24hr old culture of bacterial strains such as *E.coli and P. aeroginosa*. Wells of approximately 10mm diameter were bored using a well cutter and extracts of 25 µl, 50 µl and 100µl concentrations were added to the wells from a stock concentration of 0.1g/1ml.

The plates were then incubated at 37°C for 24 hours. Antibacterial activity was assayed by measuring the diameter of the inhibition zone in millimeters formed around the wells.[19] Gentamycin (standard antibacterial agent, concentration: 20mg/ml) was used as the positive control.

Antifungal activity

Antifungal activity was also determined by Agar cup method. Potato Dextrose agar plates were prepared and overnight grown isolates of fungi such as *Candida albicans* and *Aspergillus niger* were swabbed.

Wells of approximately 10mm diameter were bored using a well cutter and extracts of 25 µl, 50 µl and 100 µl concentrations were added and the zones of inhibition were measured after overnight incubation which were then compared with that of standard antibiotics. Clotrimazole was used as the positive control.

RESULTS AND DISSCUSION

Phytochemicals have been found to possess a wide range of activities, which may help in protection against chronic diseases.

The powdered leaf extracts of *S. grandiflora* (L) have been screened for phytochemical constituents in four different solvents such as methanol, acetone, ethanol and water. Preliminary phytochemical analysis revealed the presence of total 11 compounds such as carbohydrates, tannins,

streroids, terpenoids, alkaloids, flavanoids, cardiac glycosides, oils, sapponins, coumarins, gum and mucilage (Table 1).

Table 1: Phytochemical analysis of crude leaf extract of *S.grandiflora* (L).

Solvents	M	A	E	W
Test				
Detection of carbohydrates				
Molischs test	-	-	+	+
Fehling's test	-	-	-	+
Test for tannins	-	-	-	+
Test for steroids	+	-	-	+
Test for terpenoids				
Solkowiski test	-	-	-	+
Detection of Alkaloids				
Mayer's Test	+	+	+	+
Detection of Flavanoids	-	-	-	+
Test for protein				
Biuret test	-	-	-	-
Xanthoprotein	-	-	-	-
Test for cardiac glycosides				
Keller killani test	+	-	-	+
Test for fixed oils	-	-	-	+
Test for sapponins				
Foam test	-	+	-	+
Test for phenol compounds				
FeCl3 test	-	-	-	-
Detection of coumarins	+	+	-	-
Test for amino acids				
Ninhydrin test	-	-	-	-
Gum and mucilage	-	-	-	+

*M-Methanol,*A-Acetone,*E-Ethanol,*W-Water,* **'-'** =absent,* **'+'** =present

Phytochemical studies of all the four different extracts conclude that methanol and aqueous extracts of leaf samples had more positive results for glycosides, steroids, cardiac glycosides, coumarins and alkaloids. With acetone and ethanol solvents only alkaloids, sapponins, coumarins, and carbohydrates were detected. Traditionally sapponins have been extensively used as detergents, pesticides as well as mollucicides in addition to their industrial application such as foaming, surface active agents etc and also found to have beneficial health effects.[20] The role of tannins is to protect from predation, pesticides and also in plant growth regulation. Previous studies by various other workers prove that flavanoids provide health benefits through cell signaling pathways and antioxidant effects.

Antibacterial activity

Antibacterial activity of *S. grandiflora* (L) (leaf ethanol extract with DMSO) was assayed *in vitro* by agar cup method against two clinical gram negative isolates viz. *E.coli* and *P.aeroginosa.* Standard antibiotics were tested for their activity and their zones of inhibition were recorded. Table 2 shows the zone of inhibition produced by the extracts on Muller Hinton agar against the respective bacterial pathogens.

Table 2: Zone diameter of inhibition of ethanol leaf extract of *S. grandiflora* (L).

Test organisms	Zone of inhibition in mm			Positive Control
	Concentration of leaf extracts			
	25	50	100	
E.Coli	**Nil**	**Nil**	**Nil**	**28**
P.aeruginosa	**Nil**	**Nil**	**12**	**38**

The plant extract had shown no activity in all the three different concentrations against *E.coli* (Table 2) (Plate 1). Whereas 100µl leaf extract concentration had produced a 12mm inhibition zone *against P.aeroginosa* (Table 2) (Plate 2). The result obtained might be considered sufficient for further studies for isolation and identification of active principle and for the evaluation of possible antimicrobial activity of other extracts from other parts of the plant.

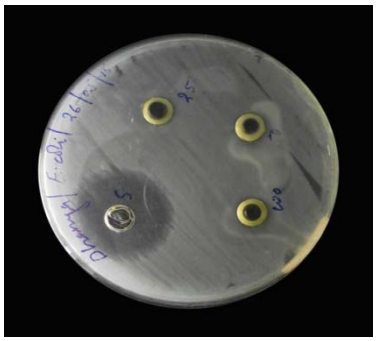

Plate 1: Antibacterial activity of the leaf extracts of *S. grandiflora* (L) against *E.coli.*

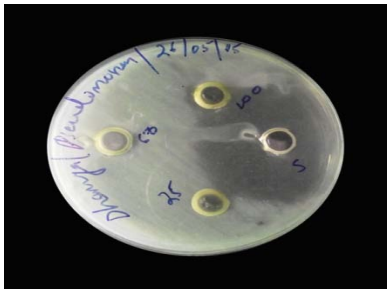

Plate 2: Antibacterial activity of the leaf extracts of *S. grandiflora* (L) against *Pseudomonas aeruginosa.*

Antifungal activity

In order to access the biological significance and ability of the plant extract, antifungal activity of *S. grandiflora* (L) (leaf ethanol extract with DMSO) was assayed *in vitro* by

agar cup method against two clinical fungal isolates viz. *Candida albicans* and *Aspergillus niger.* The given table shows antifungal activity of the plant species.

Table 3: Zone diameter of inhibition of ethanol leaf extract of *S. grandiflora* (L).

Test organisms	Zone of inhibition in mm			Positive Control
	Concentration of leaf extracts			
	25	50	100	
C. albicans	14	16	23	29
A. niger	Negligible	Negligible	Negligible	28

The sequence of antifungal activity against *C. albicans* produced a 14mm, 16mm and 23mm zones of inhibition in 25, 50 and 100µl of leaf extract concentrations respectively (Table 3) (Plate 3). Whereas no inhibitory activity was found against *A.niger* at all the various concentrations (Table 3) (Plate 4).

Plate 3: Antibacterial activity of the leaf extracts of *S. grandiflora* (L) against *Candida albicans*

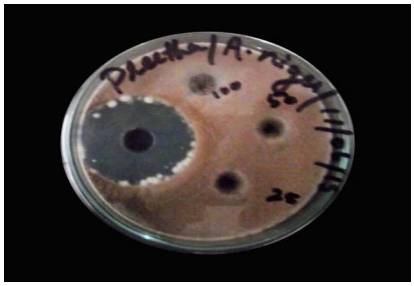

Plate 4: Antibacterial activity of the leaf extracts of *S. grandiflora* (L) against *Aspergillus niger.*

The present study reveals that the ethanol leaf extracts of *S. grandiflora* (L) were more active against the clinical fungal pathogen *Candida albicans*. Anti bacterial activity were found to be very negligible when compared to antifungal activity. In literature it has been reported that the antifungal activity is due to the presence of different chemical agents in the leaf extract including essential oils, flavanoids, terpenoids and other components which are classified as active antimicrobial compounds. The results of the study supports to a certain degree, the use of traditional medicinal plants in human and animal disease therapy and reinforce the concept of ethno botanical approach in screening plants as potential sources of bioactive substances.[21] These findings can form the basis of further studies to isolate active phytochemicals, elucidate them against wider range of bacterial and fungal strains with the goal to find new therapeutic principles.

CONCLUSION

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 conclude that the leaves of *S. grandiflora* (L) 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 and methanol extracts of leaves. The presence of various secondary metabolites such as glycosides, phytosterols, alkaloids, oils, sapponins, phenols and flavanoids were believed to exhibit the antibiotic properties of *S. grandiflora* (L) leaves and confirmed their antimicrobial efficacy against selected pathogens.

The present work highlights the possible use of *S. grandiflora* (L) leaf extracts as a source of antioxidants and as antibacterial agents that can be used to prevent enteric diseases. The study reveals that the results of extraction yield, total phenol and flavonoid compounds and bioactivity tests varied depending upon the type of solvent being used. The leaves of *S. grandiflora* (L) contain a considerable quantity of phenol - flavonoid compounds which were considered to be the major contributor for their antioxidant and antibacterial activities. Hence it can be concluded that the leaves of *S. grandiflora* (L) would direct to the establishment of some compounds that could be used to invent new and more potent anti microbial drugs of natural origin. Therefore future research should be addressed on the application of using *S. grandiflora* (L) leaves as natural remedied and to protect against infectious diseases.

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