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Research Article

PHYTOCHEMICAL SCREENING AND ANTIMICROBIAL ACTIVITY OF *SESBANIA SESBAN* (L.) MERR.

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ABSTRACT

Sesbania sesban (L.) Merr. is an erect, branched small tree up to 6 m tall with soft wood and paripinnate leaves. Flowers are yellow with brown streaks on the corolla. Fruits are sub cylindrical and shortly beaked. Seeds are green or brown and usually mottled. The plant is used as astringent, anti-inflammatory, carminative, demulcent, anthelmintic and antimicrobial. The phytochemical analysis of the methanol and ethanol extracts of both stem and root of *Sesbania sesban* revealed the presence of alkaloids, carbohydrates, proteins, phytosterol, phenol, flavonoids, fixed oil and gum. The leaf extract showed the presence of alkaloids, carbohydrates, protein, phytosterol, flavonoids and fixed oil. In vitro biological screening effects of the methanol stem extract was tested against ten bacterial species and five fungal species. Highly significant activity was observed against the bacteria *Erwinia amylovora* followed by *Escherichia coli*. In the case of fungi *Curvularia lunata* and *Fusarium oxysporum* were inhibited completely.

Keywords: *Sesbania sesban*, alkaloids, phytosterol, phenol, flavonoids, methanol stem extract.

INTRODUCTION

Medicinal plants are nature's gift to human beings for disease free healthy life. In India, different parts of several medicinal plants or their extracts are used for the treatment of various diseases. More than a hundred species of therapeutically important higher plants are listed and described in ancient Indian treatise possessing antimicrobial activity[1].

According to World Health Organization (WHO), traditional medicine is estimated to be used by 80% of the population of most developing countries. These plant-based medicines are used for primary health care needs[2]. Although plants are unique in their activities, it has also been found that a particular plant may be used by different tribes or countries for different ailments. This shows that plants possess a wide range of healing powers which are attributed to their chemical composition. Despite the wealth of human experience and folklore concerning the medicinal uses of plants, proper scientific investigation has only been applied to a small fraction of the world's plants[3]. Tamil Nadu is under strategic geographical location and possesses an invaluable treasure of herbal medicinal plants holding a major share in cultivation and export of more than fifty medicinal plants species. Medicinal plants are cultivated in Tamil Nadu in isolated patches each being grown in favourable soil and agro climatic region[4].

The medicinal value of plants lies in some chemical substances or group of compounds that produce a definite physiological action in the human body. These chemical substances are called secondary metabolites. The most important of these bioactive groups of plants are alkaloids, terpenoids, steroids, flavonoids, tannins and phenolic compounds[5].

Sesbania sesban Linn. is a soft, slightly woody, 1-6 m tall perennial nitrogen fixing small tree. The leaves are compound 12-18 cm long made up of 6-27 pairs of leaflets. The raceme has 2-20 flowers which are yellow with purple or brown streaks on the corolla. Pods are subcylindrical, straight or slightly curved up to 30 cm long and 5 mm wide containing 10- 50 seeds. The plant is used as carminative, anthelmintic, astringent, anti-inflammatory, antimicrobial, antifertility, demulcent and purgative. It is also given as a medicine against fever, ulcers etc.,[6]

MATERIALS AND METHODS

Plant materials

Different plant parts (leaf, stem and root) of *S.sesban* were collected and the authenticity of the plant was confirmed by the Botanical Survey of India, Coimbatore, India.

Preparation of extracts

The plants were cleaned, washed, shade dried and powdered for the phytochemical study. The parts used were leaf, stem and root. Extraction of the plant parts were done with different solvents based on the polarity of the solvents. The solvents used were hexane, chloroform, methanol, ethanol and water. The extract of the leaf, stem and root were obtained through the cold percolation method. The powdered plant material was weighed and then soaked in hexane for 72 hrs. Then the extract was taken by filtering the content. The same procedure was repeated again and the extract was collected. The extracts were pooled together and concentrated on a water bath by keeping the temperature below the boiling point of the solvent used. The concentrated extract was kept in the desiccator for further evaporation of the solvent. Then the extract was weighed and the yield was recorded. The same procedure was repeated for all the solvents. The extracts taken were used for further phytochemical analyses of the plant. The standard qualitative phytochemical tests given for the respective compounds were performed.

Qualitative phytochemical analysis

The concentrated extracts were subjected to qualitative test for the identification of various phytochemical constituents as per standard procedures[7, 8, 9, 10]. Based on the results obtained in the qualitative phytochemical analysis, the methanol extract of the stem was taken for antimicrobial study.

Antibacterial assay

The antibacterial assay was performed by agar well diffusion method. The nutrient agar was inoculated with 100 µl of the inoculum (10^6 CFU/ml) and poured into the Petri plate. A well was prepared in the plates with the help of a cork-borer (6 mm). About 50 µl of the extract (100, 250 and 500 µg/ml) was dispensed into the well. The flavonoid quercetin 100 µg/ml was used as the standard. The plates were incubated overnight at 37ºC. For each bacterial strain, bacitracin 100 µg/ml as positive control and pure solvent (methanol) as the negative control were maintained. The diameters of the inhibition zones were measured in mm. The bacterial species used were *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922), *Salmonella typhi* (MTCC 733), *Enterococcus faecalis* (ATCC 29212), *Erwinia amylovora* (MTCC 2760), *Proteus vulgaris* (MTCC 1771), *Pseudomonas aeruginosa* (MTCC 424), *Klebsiella pneumoniae* (ATCC 15380), *Shigella dysenteriae* (MTCC 5151) and *Bacillus subtilis* (ATCC 441).

sAntifungal activity

The antifungal activity was performed by Poison plate method. Different concentrations (100, 250, 500 and 1000 µg/ml) of the methanol extract was added to the potato dextrose agar and poured into the petriplate. The flavonoid quercetin 100 µg/ml was used as the standard. Carbendazim 100 µg/ml was used as positive control and pure solvent (methanol) as the negative control. A disc (6 mm in diameter) of actively growing mycelium of the test fungi was obtained using a sterile cork borer. These fungal discs were placed on the potato dextrose agar which was mixed with the extract. The plates were maintained at a temperature of 28±2°C. After 48 hrs, the plates were observed and the diameter of the fungal growth was measured. The zone of inhibition was measured for pathogenicity of the extract. Fungal species taken for study were *Aspergillus fumigatus, Colletotrichum gloeosporioides, Curvularia lunata, Fusarium oxysporum* and *Verticillium glaucum.*

RESULTS AND DISCUSSION

The phytochemical test of the crude methanol and ethanol extracts of both stem and root of *Sesbania sesban* revealed the presence of alkaloids, carbohydrates, protein, phytosterol, phenol, flavonoids, fixed oil and gum. Saponin was found only in the aqueous extract of the plant. These metabolites are similar to those found in *F. sycomorus*[11, 12]. The alkaloids, tannins and flavonoids are known to have curative activity against several pathogens and therefore could be used for the treatment of various illnesses[13, 14].

Table 1: Qualitative Phytochemical analysis of *Sesbania sesban*

Tests	Leaf					Stem					Root				
	H	C	M	E	A	H	C	M	E	A	H	C	M	E	A
Alkaloids	+	+	+	++	++	+	++	++	+	+	+	++	++	++	+
Carbohydrates	-	-	+	+	+	-	-	+	+	+	-	-	+	+	-
Protein	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Phytosterol	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Phenol	-	-	+	+	+	-	-	+	+	-	-	-	-	-	-
Flavonoids	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+
Saponin	-	-	-	-	+	-	-	-	-	+	-	-	-	-	+
Fixed Oil	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gum	-	+	-	+	+	+	+	+	+	-	+	+	+	+	+

H- hexane, C- chloroform, M- methanol, E- ethanol, A- aqueous
++ = strong, + = present, - = absent

Table 2: Antibacterial activity of methanol stem extract of *Sesbania sesban* (in mm)

Bacteria	Extract 100 µg/ml	Extract 250 µg/ml	Extract 500 µg/ml	Extract 1000 µg/ml	Quercetin 100 µg/ml	Bacitracin 100 µg/ml
B.subtilis	8.75±0.43	9.25±0.43	15.5±0.50	9.75±1.0	15.0±0.71	8.75±0.43
E.coli	8.5±0.50	16.0±1.0	9.25±0.43	9.0	12.25±1.48	8.75±0.43
E.faecalis	9.25±0.43	12.75±0.43	9.0	-	12.25±0.43	-
E.amylovora	11.25±1.48	17.25±1.30	-	9.75±1.30	15.25±0.83	9.75±0.83
P.vulgaris	-	-	-	-	-	-
P.aeruginosa	8.25±0.43	7.5±1.12	9.5±0.87	7.5±0.50	12.25±0.43	9.0±0.71
K.pneumoniae	8.5±1.30	-	8.0±1.30	-	19.25±0.83	9.0
S.typhi	-	-	-	-	14.25±0.83	-
S.dysenteriae	-	-	10.25±1.30	10.0±2.0	10.0±1.0	10.0±1.0
S.aureus	9.5±0.50	13.0±0.71	8.5±0.50	-	14.25±0.83	9.5±0.87

Values are mean inhibition zone (mm) ± S.D of four replicates

Table 3 : Antifungal activity of methanol stem extract of *Sesbania sesban* (in mm)

Fungi	Extract 100 µg/ml	Extract 250 µg/ml	Extract 500 µg/ml	Extract 1000 µg/ml	Quercetin 100 µg/ml	Carbendazim 100 µg/ml
Aspergillus fumigatus	-	-	10.5±5.76	-	27.75±5.49	27.75±5.49
Colletotrichum gloeosporioides	10.75±6.09	-	11.0±5.09	9.25±6.37	14.25±5.11	19.75±5.58
Curvularia lunata	12.75±3.63	10.5±4.03	18.75±2.94	15.75±2.48	18.75±2.94	18.75±2.94
Fusarium oxysporum	23.25±5.62	13.75±5.53	23.25±5.62	23.25±5.62	23.25±5.62	23.25±5.62
Verticillium glaucum	10.5±2.17	-	14.75±2.16	2.5±0.5	29.75±1.08	31.75±1.63

Values are mean (mm) ± S.D of four replicates

Phytochemical analysis is the characterization of an active principle responsible for some toxic or beneficial effect shown by a crude plant extract. Alkaloids are heterogeneous group compounds which contain one or more nitrogen atom in acyclic system. These are widely used for medicinal purposes and have positive or negative effects to human beings[15]. Alkaloids are reported to have analgesic, anti-inflammatory function and help to alleviate pain, develop resistance against diseases and endurance against stress[16]. A better precipitation of alkaloids was obtained in the methanol and ethanol extracts of both stem and root extracts of *Sesbania sesban*. The result coincides with the view of Jain *et al.,* (2004) who found high degree of alkaloid precipitation in the methanol extract of *Cocculus hirsutus*[17].

Harborne (1973) qualified flavonoids as being probably the most useful class of secondary plant constituents from a systematic point of view[7]. The flavonoids are the compounds structurally derived from the parent substance flavone, and contain conjugated aromatic systems[15]. Flavonoids have been referred to as nature's biological compound because of their inherent ability to modify the reaction taking place in the body due to allergies, virus and carcinogens. They show anti-inflammatory, antimicrobial and anticancer activity[18].

Flavonoids are found in chloroform, methanol and ethanol extracts of leaves, stem and root extracts of *S.sesban* and show different degree of precipitation. Highest degree of colour development was observed in the methanol extracts of leaves followed by the stem of *S.sesban*. This result correlates with the result of Siciliano *et al.* (2004) who detected and quantified eight flavonoids, three C-

glycosyl and five O-glycosyl flavones in roots, leaves, stems and fruits of *Sechium edule*[19].

Phenols are reported as antitumour agents and exhibit antioxidant properties[20]. The methanol and ethanol extracts of stem and leaf of *S.sesban* showed a better precipitation of phenolic content. Similar results were reported for methanol leaf extracts of *oxalis corniculata*[21].

Phytosterols were found to be present in all the five extracts of the plant parts. Sterols and triterpenes are based on the cyclopentane perhydrophenanthrene ring system. In recent years, an increasing number of these compounds have been detected in plant tissues. These phytosterols are probably ubiquitous in occurrence in higher plants and occur as both free and as simple glucosides[15]. Similar observations are made from the plant parts of *Ichnocarpus frutescens*[22]. Today, natural products derived from plants are being tested for the presence of new drugs with new modes of pharmacological action, utilizing the special feature of higher plants to produce a large number of secondary metabolites[23].

Highly significant degree of activity was observed against the test bacteria *Erwinia amylovora* with 17.25 mm in diameter followed by *Escherichia coli* with 16 mm in diameter at 250 µg/ml of the extract. The carbon tetrachloride partitionate of the methanol leaf extract of *S.sesban* showed the strongest inhibitory activity against *E. coli* having the zone size 12 mm[24]. In most of the bacteria examined, a better zone of inhibition was obtained at 250µg/ml and 500 µg/ml of the extract. When compared to the standard flavonoid quercetin, the plant extract showed a substantial amount of inhibition in the case of *Bacillus subtilis* (15.5 mm), *Escherichia coli* (16 mm), *Enterococcus faecalis* (12.75 mm), *Erwinia amylovora* (17.25mm) and *Shigella dysenteriae* (10.25 mm). A fluctuating trend of inhibition zone was found against some pathogens in the analysis. Similar fluctuation trend of inhibition zone was reported by Kunjal Bhatt *et al.,* (2003) and Uma and Sasikumar (2005)[25, 26]. This may be due to the fact that at higher concentrations, the rate of diffusion may perhaps be varied and hence, it might not be available to react with the microorganisms.

Plants are important source of potentially useful structures for the development of new chemotherapeutic agents. The first step towards this goal is the *in vitro* antibacterial activity assay and in the recent years several reports available on the antibacterial activity of plant extracts on human pathogenic bacteria[27]. The beneficial effects of treatment can be achieved with the stem extract of *S.sesban* for various bacterial infectious diseases like pneumonia, diarrohea, urinary tract infection and even some skin disease. The broad antibacterial activities could be as a result of the plant secondary metabolites like alkaloids, flavonoids, tannins, phytosterols etc., present in the extracts. Usman and Osuji (2007) reported that tannins had been widely used topically to sprains, bruises and superficial wounds as such, it could be probable that tannins and other plant phenols from this extract were responsible for these broad activities[14].

In the case of *Aspergillus fumigatus*, *Curvularia lunata*, and *Verticillium glaucum* a higher degree of inhibition was obtained with 500 µg/ml of the methanol stem extract. *Fusarium oxysporum* and *Curvularia lunata* were inhibited completely at 100µg/ml and 500 µg/ml of the extract. Carbon tetrachloride and chloroform soluble fraction of the methanol extract of *Sesbania sesban* leaf strongly inhibited the growth of *A. niger*[24]. The methanol extract of the plants *Grewia arborea, Moringa heterophylla, Strychnos nuxvomica* etc. exhibited varying degrees of inhibition activity against the fungi[28]. Some of these observations have helped in identifying the active principle responsible for such activities and in developing drugs for the therapeutic use in human beings.

CONCLUSION

Phytochemical study showed the presence of phytochemicals such as alkaloids, flavonoids, phenols and phytosterols in *Sesbania sesban* which might be responsible for their therapeutic effects. 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. In conclusion, the stem extract of *Sesbania sesban* possess a broad spectrum of activity against a panel of bacteria and fungi responsible for the most common bacterial and fungal diseases.

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