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Research Article

ANTIMICROBIAL ACTIVITY OF *SESBANIA GRANDIFLORA (L.) PERS* .

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ABSTRACT
Sesbania grandiflora (L.) Pers is a plant that is used in traditional medicine for the treatment of cold,fever, stomach disorder, diarrhea and jaundice and as skin cleanser. To evaluate the scientific basis for the use of the plant, the antimicrobial activities of extracts of the stem bark and leaves were evaluated against some common gram negative and gram positive bacteria and fungi. The study also investigated the chemical constituents of the plant and the effect of temperature and pH on its antimicrobial activity. Sesbania grandiflora (L.) Pers has broad spectrum antibacterial activity and a potential source ofnew classes of antibiotics that could be useful for infectious disease chemotherapy and control.The phytochemical constituents of the dried powdered plant parts were extracted using aqueous and organic solvents (acetone and ethanol). The antimicrobial activity of the concentrated extracts was evaluated by determination of the diameter of zone of inhibition against both gram negative and gram positive bacteria and fungi using the disc diffusion method.
KEYWORDS: *Sesbania grandiflora* (L.) Per, Antimicrobial activity, minimum inhibitory concentration

INTRODUCTION

Sesbania grandiflora (L.) Pers also called *agati*, an open braching tree up to 15 m tall and 30 cm in diameter[1], belongs to the family *Fabaceae* under the subfamily Faboideae. *Sesbania grandiflora (L.) Pers* native range through Tropical Asia including, india, Indonesia, Malaysia, Myanmar and Philippines with possibly Indonesia as the center of the diversit and Southeast Asia is non-contiguous[2]. The chemical constituents found are galactommannans, linoleic acid, beta-Sitosterol and Carbohydrates[3]. Traditionally the bark is used as astringent and utilized for the treatment of smallpox, ulcers in the mouth and alimentary cannal, inbitter, injava ,infantile disorders of stomach, scabies, the juice of the leaves are utilized for the treatment of epileptic fits and clinical research supports the anticonvulsive activity of Agatileaves, astringent, bitter, termogenic, styptic, alexeteric, anthelmintic, vulnery, demulcent, constipating, expectorants thermogenic, styptic, alexeteric, anthelmintic, vulnerary, demulcent, constipating, expectorant and antipyretic, vulnerary, demulcent, constipating, expectorants and antipyretic, vulnerary, demulcent, bronchitis, cough, vomiting, wounds, ulcers, diarrhoea, dysentery, internal and external haemorrhages, dental caries, oral ulcers, proctoptosis, stomatitis and intermittent fevers[4].The literature survey also revealed that there are no reports on correlation between chemical constituents and their pharmacological properties. Antibacterial studies also have not been reported for the bark of this plant. The present study is therefore undertaken, to study the antibacterial activity of the stem bark of *Sesbania grandiflora (L.) Pers.*

MATERIALS AND METHODS

Materials

Dried steam bark of ***Sesbania grandiflora (L.) Pers*** was collected and authenticated from Sheikh international Mfg & Exp of medicinal herbs 65B/1, Periyar Nagar, Dindigul-624001,Tamil Nadu, India vide letter no TCN/41/2009 dated 09/09/2009.

Antibacterial activities of different extracts and isolated compounds were studied by the disc-diffusion method[1,2,3].

Preparation of Extracts

50g of each of the plant parts were coarsely powdered using a mortar and pestle and were further reduced to powder using an electric blender. The powder was transferred into closed containers. Each of the powdered air-dried plant material was extracted with water, acetone and ethanol. 25g of each powdered sample was mixed in a conical flask with 100ml of de ionized distilled water or organic solvent, plugged, then shaken at 120 rpm for 30 minutes and kept for24 h. After 24 h, each of the extracts was filtered rapidly through four layers of gauge

and then by a more delicate filtration through Whatman no1filter paper. The resulting filtrates were then concentrated in a rotary evaporator and subsequently lyophilized to dryness. The yield of powder was Solvents used with increasing polarity: Petroleum ether, Benzene, Chloroform, Methanol and finally with water**.**

Test organism

All the microbial cultures, used for antimicrobial screening were procured from National Centre for Industrial Microorganisms (NCIM), Pune, India.

The test organisms used are:-*Eschericia coli, Bacillus subtilis, Pseudomonas aeruginosa, Staphylococcus aureus.*

Determination of microbial growth inhibitory properties

The test compounds were initially dissolved in chloroform and benzene as per the solubility of the sample and tested at concentrations of 1000 µg/ml against all the microorganisms.Sterile NA plates were prepared and 0.1ml of the inoculum from the standardized culture of test organism was spread uniformly. Wells were prepared by using a sterile borer of diameter 10 mm and 100 µl of the test substance, standard antibiotic and the solvent control were added in each well separately. A standard antibiotic, ciprofloxacin100 µg/ml was used. The plates were placed at 40C for 1hr to allow the diffusion of test solution in to the medium and plates were incubated for 24hrs at 370C, a period of time sufficient for the growth of at least 10 to 15 generations. The zone of inhibition of microbial growth around the well was measured in mm[5].

Determination of Minimum inhibitory concentration of different extracts of Sesbania grandiflora (L.) Pers.

I. Preparation of inoculum by subculturing

Stock cultures were maintained at 4°C on slant of nutrient agar and in nutrient broth. Active cultures for experiments were prepared by transferring a loopful of cells from the stock cultures to test tubes of Nutrient broth for bacteria and that were incubated for 24 hrs at 37°C.

II. Preparation of Media

Nutrient agar medium (NA) was used for preliminary antibacterial study. The medium was prepared by dissolving the different ingredients in distilled water and autoclaving at 1210C for 15 minutes[6]**.**

III. Determination of Minimum inhibitory concentration

The disc diffusion method was used for the determination of minimum inhibitory concentration. In vitro antimicrobial activity was screened by using Nutrient agar (NA). The NA plates were prepared by pouring 15 ml of molten media into sterile petriplates. The plates were allowed to solidify and 0.1 % inoculum suspension was swabbed uniformly and the inoculum was allowed to dry for 5 minutes. The different extracts of different concentrations were loaded on 3 mm sterile disc till saturation. The loaded disc was placed on the surface of medium and the compound was allowed to diffuse for 5 minutes and the plates were kept for incubation at 370C for 24 hrs. At the end of incubation, inhibition zones formed around the disc were measured with transparent ruler in millimeter and the lowest concentration of each extract which is showing inhibition of growth of bacteria was determined. These studies were performed by using standard drugs (10 µg/disc Penicillin and 80 µg/disc gentamicin)[7]

The results are the mean values of triplicate tests repeated three times after every 72 hours of inhibition at 37°C; data statistically significant at $p < 0.05$; MIC minimum inhibitory concentration.Figures showing zone of inhibition of different extracts of *Sesbania grandiflora* (L.) Pers (Antibacterial activity of different extracts) Refer table no 1.

RESULTS AND DISCUSSION

MIC and ZOI of two different extracts on gram positive and negative bacteria at different concentrations, by disc diffusion method, was determined to access their antimicrobial effect. The leaves and barks extracts were active (weak to moderate) against all the microbes tested. The highest zones of growth inhibition were exhibited by bark extract against all the microorganisms compared to leaves extracts. It produced a mean zone diameter of 13.2 mm at a dose of 250 mg/ml on E. coli and lowest zone of growth inhibition was observed on P. aeruginosa, which gave a zone of inhibition measuring 27.1 mm. The lowest minimum inhibitory concentration (MIC) was calculated for E. coli at 75.0 mg/ml while the highest MIC calculated was for P. aeruginosa (50 mg/ml). The antibacterial properties suggest that the phytoconstituents present in bark extracts are more potent than leaves extracts, and also corroborate the use of plant in traditional medicine for itch and, common skin problems.

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Table1. Effect of disc diffusion and MIC antimicrobial bioassay

Zone of inhibition (mm)				
Microorganisms **Dose**	***E. coli***	***B. subtilis***	***S. aureus***	***P. aeruginosa***
Leaves extract				
50 mg/disc	0.0	2.1	1.5	3.6
100 mg/disc	3.2	5.4	3.9	5.1
150 mg/disc	8.5	10.2	7.8	11.5
200 mg/disc	15.2	16.4	12.4	19.7
250 mg/disc	21.4	25.7	20.3	22.4
MIC (mg/ml)	**50**	**25**	**25**	**12.5**
Bark extract				
50 mg/disc	0.0	3.5	0.8	4.3
100 mg/disc	0.0	7.2	4.5	6.2
150 mg/disc	3.9	9.5	9.2	10.5
200 mg/disc	8.4	17.6	15.7	20.3
250 mg/disc	13.2	23.3	24.5	27.1
MIC (mg/ml)	**75**	**12.5**	**25**	**6.25**
Ciprofloxacin				
5 µg/disc	20.3	14.3	18.5	10.3
25 µg/disc	27.4	23.7	20.6	18.5
50 µg/disc	36.8	33.5	31.9	25.7
100 µg/disc	42.1	41.5	36.4	30.2
200 µg/disc	45.7	48.4	41.7	38.5
MIC (µg/ ml)	**0.31**	**0.62**	**0.31**	**1.25**

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