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Susceptibility Of Sesbania grandiflora Root Extract Against Problematic Groups Of Drug Resistant Microbes

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Abstract : Medicinal plants have been considered as important therapeutic aid for alleviating ailments of man kind. In spite of tremendous development in the field of allopathy. Medicinal plants and their derivatives still remain one of the major roles in medicinal therapy. The world's health organization also has recognized the important of traditional medicine and has been active in creating strategies guidelines and standard for botanical medicines. Sesbania *grandiflora Linn* belongs to the family Leguminosae and has been widely used in Ayurveda. Agati is a widely available, fast growing plant, generally popular for animal fodder. The plants parts used are Root, Bark, Leaf, Flower and Fruit. Sesbania *grandiflora* known as agati, syn. Aeschynomene *grandiflora* or hummingbird tree/scarlet wisteria is a small tree in the genus Sesbania, commonly it is known as caturay, katurai, Chamorro, corkwood tree, scarlet wisteria, sesban, vegetable hummingbird in English, agathi, in Tamil, hadga in hindi. In Ayurveda the plant has been used for the treatment of head ache, for fever[1,2] as tonic, in cataract and as astringent. The leaves of the plant have been reported to have anxiolytic[3-9] and anticonvulsant effect while the flowers have been reported to have antimicrobial activity[10], hypolipidemic, antiulcer[11] and anti-inflammatory effects. In view of the above considerations the present study was designed to investigate the antibacterial activity of ethanolic root extract of Sesbania *grandiflora*.
Keywords: Sesbania *grandiflora* Ethanolic Root Extract (SGERE), S.*aureus*, S.*epidermis*, E.*Coli*, B.*subtilis*, Disc diffusion method, MIC and Antimicrobial activity.

Introduction

Host and pathogen co-evolve through continuous contest for survival. In this battle, the pathogen tries to ensure its persistence in the host and the host tries to protect itself from the pathogens harmful and often detrimental influences. The war is not without weapons. The host and pathogen use their strategies to prevail over each other.

Infectious diseases, a global concern, continue to be one of the leading causes of morbidity and mortality. Globally, 90% of the total deaths from infectious diseases are caused by infections of the respiratory tract, HIV/AIDS, diarrhoeal diseases, tuberculosis, malaria and measles. Infectious diseases continue to be a public health problem particularly in developing countries where vaccines are unavailable or unaffordable.

Sesbania *grandiflora Linn* belongs to the family Leguminosae and has been widely used in Ayurveda. Sesbania *grandiflora* known as agate or hummingbird tree/scarlet wisteria is a small tree in the genus Sesbania, commonly it is known as caturay, katurai, Chamorro, corkwood tree, scarlet wisteria, sesban, vegetable hummingbird in English, agathi, in Tamil, hadga in hindi.

Antibacterial studies have not been reported for the root of this plant. The present investigation is therefore undertaken, to study the antibacterial activity of ethanolic root extract of Sesbania *grandiflora.*

Scope Of Study

In our present investigation the use of Sesbania *grandiflora* for the medicinal purpose used locally in the treatment of various diseases and we examined the ethanolic root extract for their antimicrobial activity.

The results of our studies conducted and herewith we report that sesbania *grandifora* root extract is useful in controlling, strengthening the antimicrobial potential. Therefore, the present investigation is also a part of continuing programme related to the phytochemical analysis.

Materials And Methods

Chemicals

All the fine chemicals were purchased from Sigma Chemical Co., USA. All other chemicals used were of good quality and analytical grade.

Sesbania *grandiflora* roots were purchased from the local market from the Ayurvedic shop, Vellore. All the plants were identified taxonomically by Dr.Prof.N.P.M.Mohammed Tariq (Botanist) Department of Biotechnology, Islamiah College (Autonomous) Vaniyambadi.

Phytochemical Analysis

Test for carbohydrates

A small quantity of extract was dissolved separately in 5 ml of distilled water and filtered. The filtrate was tested to detect the presence of carbohydrates.

Molisch's test:

To 2ml of extract, 2 ml of Molisch's reagent was added. Then, 2 ml of concentrated sulphuric acid was added along the sides of the test tubes. Disappearance in color on the addition of excess solution indicated the presence of carbohydrates.

Benedict's test:

To 0.5 ml of extract, 5 ml of Benedict's reagent was added. The mixture is then boiled for 5 minutes. Presence of a bluish green precipitate indicated the presence of carbohydrates.

Test for Glycosides:

To 2ml of extract 1ml of aqueous NaOH solution was added. The appearance of a yellow color indicated the presence of glycosides.

Test for Proteins and Amino acids

Ninhydrin test:

A small quantity extract solution was boiled with 0.2% solution of Ninhydrin. Purple color indicated the presence of free amino acids

Test for Phytosterols and Triterpenoids

Salkowski test:

To 2 ml of the extract, 1 ml of concentrated Sulphuric acid added. Chloroform was added along the sides of the test tube. A red color produced in the chloroform layer indicated the presence of Phytosterols or if it is yellow in color at the lower layer indicated the presence of triterpenoids.

Zinc hydrochloride reduction test:

The extract was treated with mixture of zinc dust and concentrated hydrochloric acid. Red color indicated the presence of flavanoids.

Test for Alkaloids

A small portion of the solvent free extract was stirred separately with a few drops of dilute hydrochloric acid and filtered. The filtrate was tested with Mayer's reagent (Potassium mercuric iodide solution). The cream precipitate indicates the presence of alkaloids.

Dried powder of herb treated with 5% Ammonical Ethanol and the test carried out after 48 hours, the fraction was treated with Mayer's, Wagner's and Dragndroff's reagent.

Test for Tannins

Gelatin test:

To 5ml of extract, few drops of 1 % lead acetate were added. Absence of a yellow or red precipitate indicated the absence of tannins.

5gm of extract in 50ml water and boiled for 45 min. in waterbath and 2% gelatin solution is added dropwise.

Test for Saponins:

To 5 ml of the extract, a drop of sodium bicarbonate was added. It is shaken vigorously and kept undisturbed for 3 minutes. Appearance of a honey comb like froth indicated the presence of saponins.

Flavonoids:

1ml of extract add 10ml of 95% ethanol and kept in boiling waterbath for 15 minutes and after filtration mg ribbon were added along with 2-3 drops of HCl.

Steroids:

1ml of extract was extracted with methanol for 15minutes and then Libermann Burchard reagent was added drop wise.

Preparation Of Ethanolic Root Extract

The Ethanolic root extract[12] of Sesbania *grandiflora* was prepared by soxhletion. The powdered (root) plant material (250g) was repeatedly extracted in a 1000ml round bottomed flask with 500ml ethanol (95%). The reflux time for each solvent was 30-40 cycles for complete extraction. The extracts were cooled at room temperature, filtered and evaporated to dryness under reduced pressure in a rotary evaporator and kept under refrigeration at –4°C till future use. The percentage yield was found to be 5.85% with respect to the initial dried root plant material. The ethanolic root extract of Sesbania *grandiflora* was abbreviated as ERESG.

Antimicrobial Activity

The microorganisms were collected from the Bethesdha Hospital, Ambur, Vellore district, Tamilnadu. The antimicrobial activity was evaluated using disc diffusion method[13-15].

Test Microbes Used

i) Staphylococcus *aureus*
ii) Staphylococcus *epidermis*
iii) Escherichia *coli*
iv) Bacillus *subtilis*

Determination Of Microbial Growth Inhibitory Property

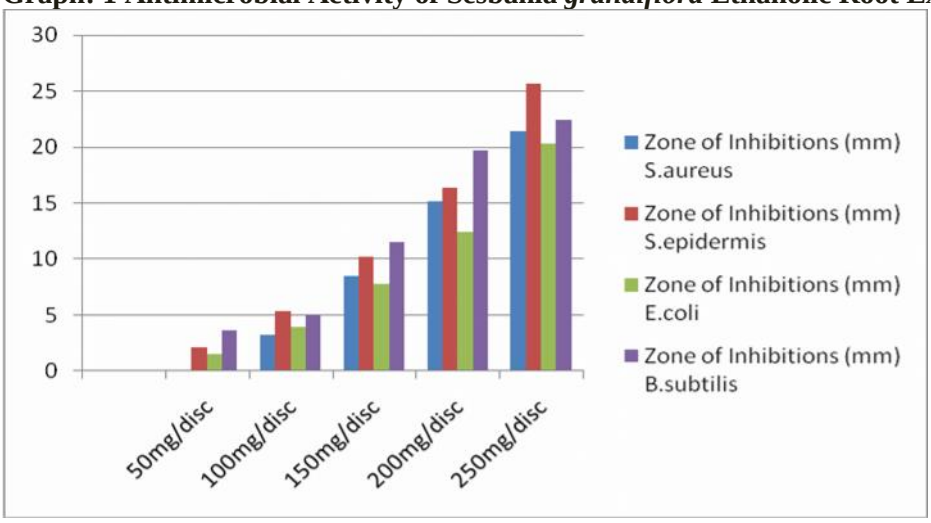
The test compounds were initially dissolved in ethanol as per the solubility of the sample and tested at the concentration of 1000µg/ml against all the micro-organisms. Sterile Nutrient Agar (NA) plates were prepared and 0.1ml of inoculums from the standardized culture of test organisms was spread uniformly. Wells were prepared by using a sterile borer of diameter and 10mm and 100µl of the test sample, standard antibiotic and the solvent control (ethanol) were added in each well separately. A standard antibiotic Ciprofloxacin 100µg/ml was used. The plates were placed at the 40°C for 1hr, in order to diffusion takes place of test solution in to the medium and plates were incubated for 24hrs at 37°C. A period of time sufficient for the growth of at least 15 to 20 generations. The zone of inhibition of microbial growth around the wells was measured in mm[16].

The results are the mean values of triplicate tests repeated two to three times after every 72hrs of inhibition at 37°C, the data statistically significant at P<0.05, Minimum Inhibitory Concentrations.

Table: 1 Antimicrobial Activity & Effect of Disc Diffusion and MIC

Zone of Inhibitions (mm) ERESG				
Microbes/Dose	S.*aureus*	S.*epidermis*	E.*coli*	B.*subtilis*
50mg/disc	0.0	2.1	1.5	3.6
100mg/disc	3.2	5.4	3.9	5.1
150mg/disc	8.5	10.2	7.8	11.5
200mg/disc	15.2	16.4	12.4	19.7
250mg/disc	21.4	25.7	20.3	22.4
MIC (mg/ml)	**50**	**25**	**25**	**12.5**
Ciprofloxacin Standard Antibiotic Disc				
5µg/disc	20.3	14.3	18.5	10.3
25µg/disc	27.4	23.7	20.6	18.5
50µg/disc	36.8	33.5	31.9	25.7
100µg/disc	42.1	41.5	36.4	30.2
200µg/disc	45.7	48.4	41.7	38.5
MIC (µg/ml)	**0.31**	**0.62**	**0.31**	**1.25**

Graph: 1 Antimicrobial Activity of Sesbania *grandiflora* Ethanolic Root Extract



Results And Discussions

The Minimum Inhibitory Concentration (MIC) of Sesbania *grandiflora* ethanolic root extract on gram positive and gram negative bacteria at different concentrations, by disc diffusion method, was determined to access their antimicrobial activity. The highest zone of growth inhibition were exhibited and produces a mean zone diameter of 25.7mm at a dose of 250mg/disc on S.*epidermis* and lowest zone of growth inhibition was observed on E.coli which gave a zone of inhibition measuring 1.5mm and no zone of growth inhibition was seen in S.*aureus* at a dose of 50mg/disc (Table.1).The antimicrobial properties suggested that the phytoconstituents, various bioactive compounds, present in roots are more potent and can be used as traditional medicine in near future.

The zone of inhibition and minimum inhibitory concentrations of extract on the microbes at the different concentrations, by disc diffusion method, was determined to assess their antimicrobial activity of ethanolic root extract of sesbania *grandiflora.* The antimicrobial activity property suggest that the phytoconstituents, the bioactive compounds, present in the root extract are more potent and also corroborate the use of whole plant in the traditional medicine in near future.

Conclusion

There should be a wide range of search for the alternative remedies to prevent and cure the infections and to the antimicrobial resistant microbes. The allopathic forms of antimicrobials give adverse effects to the human system and to the metabolism. So, therefore we need an antimicrobial which has lower side effects and high effectiveness. The antimicrobial drug preparations with the herbal extracts will be the new alternative medicines for synthetic drugs. It is suggested that using the extracts are effective and economic, herbals may be prepared for pathogenic infections. The preliminary phytochemical screening also supported antimicrobial activity. The broad spectrum of antimicrobial activity of Sesbania *grandiflora* root extract is highly promising for further bioactive compounds can be made evaluated and being continued for the further studies.

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