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ORIGINAL RESEARCH ARTICLE

Phytochemical Investigation & Antimicrobial Activity of Methanolic Extract of *Sonneratia apetala* Buch-Ham. Areal parts

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ABSTRACT

The present study was attempt to investigate the phytochemical constituents and to explore & antimicrobial activity of methanolic extracts of *Sonneratia apetala* arial parts. all the tests for phytochemical constituents of methanolic extracts of *S. apetal,*were found tobe presence of terpenoid , steroid, alkaloid , flavonoid ,tannins, saponins& polysaccharide Antimicrobial activities of the plant extracts were determined by the well diffusion method. In vitro screening of *S. apetala* plant extracts showed species specific activity ininhibiting the growth of bacteria &fungi.The tested extract showed to varying degrees of antibacterial potential against tested gram-negative as well as gram-positive bacteria.These promising findings suggest antibacterial activity of the plant material. Methanol extracts showed good activity against all the pathogens, The anti-microbial activity of the methanolic extract of *S. apetala at* 10 mg/ml was found to be dose dependant and significant compare to standard anti-microbial agent ciprofloxacin & clotrimazol at 10µ g/disc.

Key words: Antimicrobia**l** Activity,S*onneratia apetala* ,Dsmo, Mic, Ciprofloxacin.,Cotrimazol, Arial part Extract.

INTRODUCTION

Sonneratia apetala Buch-Ham. popularly known Mangrove apple plant belonging to the Sonneratiaceae family and it grows as tree or shrub also is a creeping, glabrous, succulent herb, distributed thought out India in saline area. The mangrove plant have the anti-HIV, Anti bacterial . antiproliferative and anti estrogenic activities and for the treatment of insanity, epilepsy and asthma[1]. The literature also reports that the leaf part of the of the plant is widely used for dysentery, sprain & bruises, in treatment ofeye troubles (such as cataract) and open sores in children ears andalso in heart troubles,[2] The aerial parts of the part contain a large no. of terpenoid , steroid, alkaloid & polysaccharide[3]. The antimicrobial activity of the mangrove plant extract of *S. apetala* on the various test microorganisms, including clinical multiple antibiotic resistant bacteria and phytopathogens were i n vestigated[4,5]. Antimicrobial activities of the plant extracts were determined by the well diffusion method. In vitro screening of *S. apetala* mangrove plant extracts of showed species specific activity inhibiting the growth of bacteria and fungi,. Hexane, chloroform and methanol extracts showed good activity against all the pathogens, where as methanolic extracts were active against most of the pathogen

MATERIALS AND METHODS

Drugs and Chemicals

Ciprofloxacin & clotrimazol were (Micro Lab. Ltd., Goa), methanol (Merck Pvt. Ltd. Mumbai) and other chemicals were procured from suppliers.

Collection of Plant Material

The plant material were collected from the costal belts of astarang area near the Puri of odisha.The plant was identified, confirmed and authenticated by the taxonomist Dr.N.K.Dhal,Institute of Minerals and Materials Technology Bhubaneswar, Orissa ,India ,Vide Voucher specimen no. (V.N. no -11,600). After authentification plant material were collected in bulk and washed under running tap water to readucing dirt. Then plant materials were shade dried. The dried materials were made

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into coarse powder by grinding in mechanical grinder.

PREPARATION OF EXTRACTS

The coarse powder was taken in Soxhlet apparatus and extracted successively with methanol and water. The extraction was done for 72 hours. The marcof each extract was dried and used for extraction with successive solvent. The liquid extracts were concentrated and separated under vacuum reduced pressure to obtain a sticky mass. The yield of the methanol extract was found to be 11.6% w/w with respect to dried plant material. The extracts were stored in desiccator for further use [6].

Phytochemical investigation

Chemical tests were carried out on all the extracts for the qualitative determination of phytochemical constituents [7].

EXPERIMENTAL

Preparation of extract

The powered plant material was extracted with 95% methanol using Soxhlet apparatus. The solvent was removed under reduced pressure, which gave a greenish-black colored sticky residue .The dried extract was then mixed with dimethyl sulfoxide (DMSO) for antimicrobial study[9,10].

Standard Drugs used

Ciprofloxacin & clotrimazol were used as reference standards or the antimicrobial studies respectively.

Microorganisms used

For the present study the microorganisms used include *Sathylococcus aureus, Bacillus subtilis, Bacillus polymexia, Streptococcus faecalis, Pseudomonas aerguenosa, salmonella typhi, Vibrio cholera, Shigella dysenteriae, Escherichia coli, Asperillus niger and Candida albicans* respectively suitable strains of these microorganisms were produced form microbiology laboratory of. University Department of Pharmaceutical Sciences, Utkal University, Vani vihar, Bbsr-751004, Odisha, India

Antimicrobial Activity

Determination of Minimum inhibitory concentration (MIC)

Minimum inhibitory concentration (MIC) of the extract was performed by both dilution methods at concentration of the extract ranging from 25mg/ml to 500mg/ml in DMSO against all the test microorganisms [11].

Determination of zone of inhibition

The zone of inhabitation of the extract was performed by agar disc diffusion method at concentrations of 2,5 and 10mg/ml of the extract in DMSO. Ciprofloxacin (5mg/ml) and Clotrimzole (2.5mg/ml) were used as reference controls only DMSO) was also maintained though out the experiment.

Method

Different methods are there for antimicrobial activity study. For zone of inhabitation and Minimum inhibitory concentration (MIC) different methods are followed.

I. Agar diffusion method

II. Disc Diffusion method

For the MIC study the methods are

a. Tube dilution method

b. Agar dilution method

For zone of inhibition study the agar disc diffusion method and for MIC study the Tube dilution method was applied.

Preparation of Nutrient Medium for Sub-culture of Bacteria

Requirements for Liquid Culture Media:

Peptone 1%

Sodium Chloride -0.5%

Beef extract-1%

Distilled water q.s. up to 100ml.

PH-up to 7.4-7.6

Procedure

All the Glassware and other equipment were sterilized .With the above working formula the nutrient broth was prepared for the study of the collected microorganisms from our laboratory. The Procedure was maintained properly. The flask was then plugged and wrapped with foil. Then it was autoclaved for 25 minutes at 15 psi at 121ºc the required glassware also sterilized. Then the flask was allowed to cool in front of Laminar Air Flow. The media was poured in small quantities into pre-sterilized conical flask. The wire loop was sterilized by flaming to red-hot condition in Bunsen flame .Then it was pulled out, cooled and the top of the container containing the culture was flamed and different microorganisms(bacteria) were inoculated in different small conical flasks under laminar Air Flow cabinet under aseptic conditions. The flasks were then incubated at 37ºc for 24 hours[12].

Procedure for zone of Inhibition Study:

Requirement for solid culture media:

Peptone 1%

Sodium Chloride -0.5%

Beef extract-1%

Agar-1%

Distilled water q.s.to 100ml
PH up to 7.4-7.6

Procedure

With the above working formula the media was prepared in the flasks. The flasks were well plagued and wrapped with foil and autoclaved for at least 2.5 minute at 15 psi. at 121ºc

Then the medium was poured in different Petri dishes that were pre-sterilized. Then it was allowed to cool. Then the sub-culture of different bacteria was poured and distributed evenlyover the petri dishes. With the help of a filter paper dishes (6mm)soaked in different concentrations of extract (2,5 and 10mg/ml) were placed on each Petri dish over the solidified medium . Under each disc of very petri dishes marked as 1, 2, 3 and 4 and a sticker was attached to each Petri dishes with the particular name of the bacteria poured in it. Then those were divided into different groups. Ciprofloxacin dissolved in DMSO. After that all the petri dishes were covered and incubated for at least 24 hours at 37ºc the very next day the diameters of zone of Inhibition were measured with the help of transparent scale. The results are depicted in (**Table 2**) [12].

Procedure for Minimum Inhibitory Concentration Study:

Requirements:
Peptone: 1%
Beef extract-1%
Sodium Chloride-0.5%
Distilled water q.s.to 100ml
PH up to 7.4-7.6

Procedure

The nutrient media was prepared with the above working formula. It was well plagued and autoclaved for 25 minutes at 15psi at 121ºC, then it was cooled to RT in front of Laminar Air flow. All the glassware were sterilized in hot air oven. The test tubes were then marked and distributed in stand. Then 8ml of nutrient media is poured and cotton plagued all the test tube. Those are again autoclaved in same conditions. Then it was cooled to RT in front of the laminar airflow. Then1ml of the extract of different concentration was added to different marked test tubes form lower to higher concentration. Then 1ml different concentration was added to different marked test tubes from lower to higher concentration. Then1 ml. of different microorganism was added to the particular markedtest tubes. Then the test tubes were well – plagued and wrapped withpaper. Then those were incubated at 37ºC for at least 24hours. The

turbidity of the test tubes samples were incubated at least 24 hours.The turbidity of the test tubes samples were compared with controls (only nutrient broth). The result are depicted in (**Table 2**)[13] .

Table 1:Phytochemical screening of metanolic extract of Sonneratia apetala

Chemical Constituent	Tests	Methanolic Extract
Alkaloid	Mayers test	+
	Dragendroff's test	+
	Wagners test	+
Carbohydrate	Molisch's test	+
	Benedicts test	+
	Fehling's test	+
Glycosides	Modified Borntragers	--
	Legal test	--
Saponins	Foam test	+
	Froth test	+
Phytosterols	Salkowski test	+
	Libermann Burchard	+
	Fats and Oil Stain test	--
	Resins Acetone water test	+
	Phenols Ferric chloride test	+
	Tannins Alkaline reagent	+
Flavanoids	Gelatin test	+
	Lead acetate test	+
Proteins	Xanthoproteic test	–
	Ninhydrin test	--
Diterpenes	Copper acetate test	+

+ (present)*, -- (abscent)

Table 2: MIC (µ g/ml.) and zone of inhibition (mm) of methanolic extract of *S. apetala* aerial parts.

Micro-organism	MIC(µgm\ml.)	Zone of Inhibition (mm)			
		Extract(mg/ml.)			Standard
		2	5	10	
Gram Positive bacteria					
Staphylococcus aureus	50	8.7	17.3	18.7	27.3
Bacillus polymexia	50	9.0	17.7	20.3	25.0
Gram Negative Bacteria					
Psudomonas aerugenosa	400	7.0	10.0	12.7	24.3
Salmonella typhi	75	9.0	16.3	18.3	23.3
Vibro cholera	150	8.7	11.3	15.3	22.3

Escherichia coli	250	8.0	11.7	15.7	21.0
Fungi					
Phenicillum notatum	300	8.0	11.0	14.3	20.0
Aspergillus niger	225	7.3	12.7	16.3	23.7
Candida albicans	150	8.7	16.3	20.0	28.3

*values are mean of three readings , standards : Anti-bacterial studies – Ciprofloxacin 10µ g/ml.& Anti Fungal studies clotrimazole- 10µ g/disc.

RESULTS AND DISCUSION

The phytochemical screening of methanolic extracts of s.apetal shows presence of terpenoid , steroid, alkaloid , flavonoid ,tannins, saponins& polysaccharide.and Table no. 2 depicts the antimicrobial activity of the methanolic extract of *S. apetala* areal parts . The result of MIC study revealed the anti-microbial activity of the extract against the tested strains of microorganisms between concentration ranges of 50 and 400 mg/ml. The results of zone of inhibition study revealed that the extract possess antimicrobial activity in a concentration dependent manner against the test organisms and was comparable with the slandered drug. The gram.- positive bacteria were observed to be more susceptible than gram negative bacteria. These observations are more likely to be the fact that an outer membrane in gram negative bacteria, which acts as a barrier to many environmental substances including antibiotic. These observations are more likely to be the fact that an outer membrane in gram negative bacteria, which acts as a barrier to many environmental substances including antibiotics. Among the tested strains of bacteria the extract was most effective against *B. polymexia* and least against *P. Aerugenosa*, which is naturally resistant to antibacterial agents. Our results from the present study indicate the potential usefulness of *S. apetala* in the treatment of various pathogenic diseases as mentioned in the Ayurvedic literature.

CONCLUSION

The phytochemical studies indicated the presence of several types of chemical Constituents present in areal parts of *S. apetala*. It could be concluded that all the methanolic extract of *S. apetala* exhibited comparable antimicrobial activity with standard drug. Among all the tested strains of bacteria the extract was most effective against *B. polymexia* and least against *P. Aerugenosa*, which is naturally resistant to antibacterial agents

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