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Bioactivity of Marine Mangrove Plant *Avicennia alba* on Selected Plant and Oral Pathogens

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ABSTRACT: In this present study *Avicennia alba* was screened for antimicrobial activity against some clinical and Phytopathogens. The plant parts of *A. alba* were collected from coringa forest near Kakinada area, dried and extracted successively with hexane, chloroform and methanol using the soxhlet extraction apparatus. The antimicrobial activities of the plant extracts on the various test microorganisms, including multiple antibiotic resistant bacteria, were investigated. Antimicrobial activities of the extracts were determined by the well diffusion method. The experimental results concluded that plant extracts of *A. alba* have greater potential as antimicrobial compounds against microorganisms and that they can be used in the treatment of infectious diseases caused by resistant pathogenic microorganisms.

Keywords: Antibiotic resistant bacteria, antimicrobial activity, folkloric medicine, *Avicennia alba.*

INTRODUCTION

Historically, plants have provided a source of inspiration for novel drug compounds, as plant derived medicines have made large contributions to human health and well-being. The use of plant extracts, as well as other alternative forms of medical treatments, is enjoying great popularity in the late 1990s. Their role is twofold in the development of new drugs: first they may become the base for the development of a medicine, a natural blueprint for the development of new drugs, or second: a phytomedicine to be used for the treatment of diseases. It is estimated that today, plant materials are present in, or have provided the models for 50% Western drugs[1]. The primary benefits of using plant derived medicines are that they are relatively safer than synthetic alternatives, offering profound therapeutic benefits and more affordable treatment. The beneficial medicinal effects of plant materials typically result from the combinations of secondary products such as alkaloids, steroids, tannins, and phenol compounds, which are synthesized and deposited in specific parts or in all parts of the plant. A large number of plants in different location around the world have been extracted and semi-purified to investigate individually their antimicrobial activity [2].

Mangroves are widespread in tropical and sub tropical regions, growing in the saline intertidal zones of sheltered coast lines and contain biologically active antiviral, antibacterial and antifungal compounds [3]. [4] Have reported the presence of compounds like tannins, alkaloids, and polyphenols in mangroves which play an important role in the suppression of deleterious microorganisms [5, 6, 7].

Mangrove plant extracts have been used for centuries as popular method for treating several health disorders plant-derived substances have recently become of great interest owing to their versatile applications. Numerous studies have been carried out on various natural products screening their antimicrobial activity [8, 9, 10, 11]. The present study was to screen the antimicrobial activities of *A. alba* and search for new compounds from mangrove plants.

MATERIALS AND METHODS

Plant and extraction:

Mangrove plant *Avicennia alba* classified in the plant family Avicenniaceae. The bark and seeds are used

as a fish poison and resin used in birth control. The plant parts were collected from Coringa Mangrove Wetland, Andhra Pradesh, India. The material was taxonomically identified and the Voucher specimen is stored. The plant material were dried under shade with occasional shifting and then powdered with a mechanical grinder and stored in an airtight container. The powder obtained was subjected to successive soxhlet extraction with the organic solvents with increasing order of polarity.

Test microorganisms:

The microorganisms that were studied including *Acremonium strictum* (MTCC 2599), *Aeromonas hydrophila* (MTCC 646), *Aspergillus flavus* (MTCC 4633), *Bipolaris bicolor* (MTCC 2105), *Erwinia caratovara* (MTCC 3609), *Fusarium oxysporum* (MTCC 1755), *Lactobacillus acidophilus* (MTCC 447), *Pseudomonas marginales* (MTCC 2758), *Rhizoctonia solani* (MTCC 4633), *Streptococcus mitis* (MTCC 2696) and *Streptococcus mutans* (MTCC 890) including fungi and bacteria were obtained from Microbial Type Culture Collection (MTCC), Chandigarh were used as test organisms. The strains are maintained and tested on Nutrient Agar (NA) for bacteria and Potato Dextrose Agar (PDA) for fungi. Active cultures were generated by inoculating a loop full of culture in separate 100mL nutrient broths and incubating on a shaker at 37 ^{o}C overnight. The cells were harvested by centrifuging at 4000 rpm for 5 min, washed with normal saline, spun at 4000 rpm for 5 min again and diluted in normal saline to obtain 5 x 10^{5} cfu/mL.

Determination of antibacterial activity:

The crude extracts of the different plant parts of different species were subjected to antimicrobial assay using the agar well diffusion method of (Murray *et al*., 1995) [12] modified by (Olurinola, 1996) [13].

20 ml of nutrient agar was dispensed into sterile universal bottles these were then inoculated with 0.2 ml of cultures mixed gently and poured into sterile petri dishes. After setting a number 3-cup borer (6mm) diameter was properly sterilized by flaming and used to make three to five uniform cups/wells in each petri dish. A drop of molten nutrient agar was used to seal the base of each cup. The cups/wells were filled with 50µℓ of the extract concentration of 100mg/ml and allow diffusing for 45 minutes. The solvents used for reconstituting the extracts were similarly analyzed. The plates were incubated at 37°c for 24 hours for bacteria. The above procedure is allowed for fungal assays but except the media potato dextrose agar instead of nutrient agar and incubates at 25°c for 48 hours. The zones of inhibition were measured with antibiotic zone scale in mm and the experiment was carried out in duplicates. The extracts and the phytochemicals that showed prominent antimicrobial activity were later tested to determine the Minimal Inhibitory Concentration (MIC) for each bacterial and fungal sample.

RESULTS

(Table 1 and fig 1) summarizes the antimicrobial activities zone of inhibition of chloroform varies from (7 to 19 mm) where as with methanol (11 to 27 mm) at 100 mg/ml concentration. The variation of antimicrobial activity of our extracts might be due to distribution of antimicrobial substances, which varied from fraction to fraction of the crude extract.

(Table 1 and fig 2) Both methanolic MIC (5 mg/ml) and chloroform MIC (15 mg/ml) extract shows lowest activity against *S. mutans* whereas highest methanolic MIC (90 mg/ml) and chloroform MIC (100 mg/ml) shown against *P. marginales*. Chloroform followed by MIC (90 mg/ml) *R. solani* and MIC (65 mg/ml) against *L. acidophilus.*

DISCUSSION

Methanolic extracts showed most active and significant (Zone of inhibition 27 mm) against Gram-positive *S. mutans* and it was found to be resistant to many of the antibacterial agents viz., Penicillin, amoxicillin, cefuroxin, tetracycline and erythromycin [3] and causes dental caries in humans [14] while weakest activity against *P. marginales*. On the other hand no activity was found against *S. mitis* and *F. oxysporum* with both chloroform and methanolic extracts. The hexane extract appears to have less antibacterial and antifungal activity than the chloroform and methanolic extracts. The above results it can be concluded that plant extracts have greater potential as antimicrobial compounds against microorganisms and that they can be used in the treatment of infectious diseases caused by resistant pathogenic microorganisms.

Overall, the present study provides enough data to show the potential of mangrove *A. alba.* The above results it can be concluded that plant extracts of *A. alba* have greater potential as antimicrobial compounds against microorganisms and that they can be used in the treatment of infectious diseases caused by resistant pathogenic microorganisms. The present study was conducted to develop newer lead for better and safer chemotherapeutic agents from mangroves. Further studies are needed to identify the pure component and establish the exact mechanism of action for antibacterial action of the plant extract.

Table 1: Antimicrobial activity of chloroform and methanol extracts *A. alba.*

Microorganisms	Chloroform 100 mg/ml	MIC (Extract concentration mg/ml)	Methanol 100 mg/ml	MIC (Extract concentration mg/ml)
Acremonium strictum	11	75	14	50
Aeromonas hydrophila	10	90	12	65
Aspergillus flavus	-	-	13	60
Bipolaris bicolor	-	-	11	75
Erwinia carotovora	7	100	13	65
Fusarium oxysporum	-	-	-	-
Lactobacillus acidophilus	12	65	14	55
Pseudomaonas marginales	8	100	10	90
Rhizoctonia solani	9	90	12	65
Streptococcus mitis	-	-	-	-
Streptococcus mutans	19	15	27	5

Volume per well: 50µl, Borer size used: 6mm.

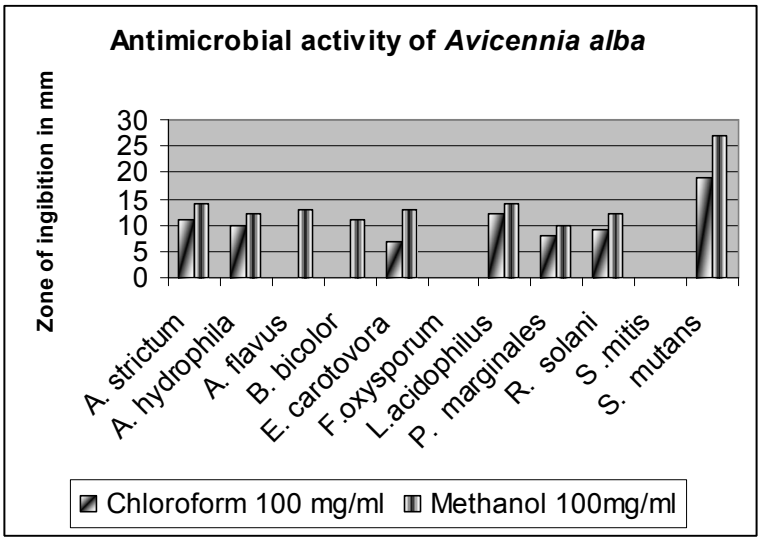

Fig 1

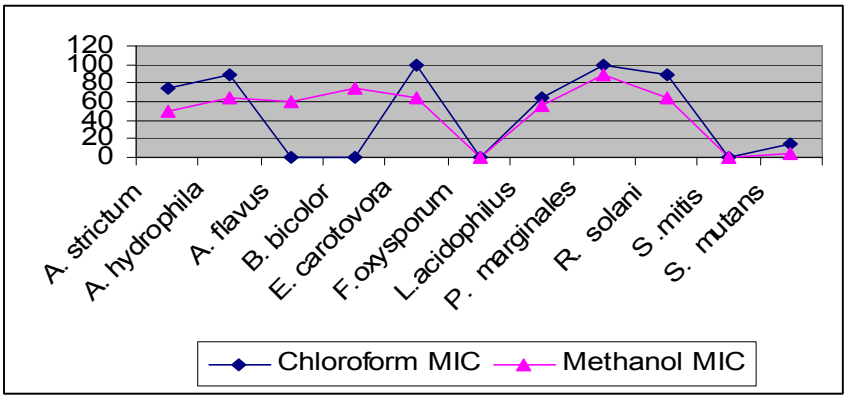

Fig 2

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