

Bioactivity of mangrove plant *Bruguiera cylindrica* against selected phytopathogens

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ABSTRACT

In this present study antimicrobial activity of *Bruguiera cylindrica* (Rhizophoraceae), the plant parts of were collected from coringa forest near Kakinada, Godavari-krishna delta area were dried and extracted successively with hexane, chloroform and methanol using the soxhlet extraction apparatus. The antimicrobial activities of the organic solvent extracts on the various test microorganisms, including bacteria and fungi investigated using agar well diffusion technique. Methanol extracts exhibited promising antimicrobial activity than chloroform and hexane extracts. Among all tested microorganisms *Pseudomonas syringae* (26 mm) and *Xanthomonas campestris* (22 mm) showed highest growth inhibition where as no activity were found with *Pseudomonas marginales*, *Cladosporium herbarum* and *Macrophomina phaseolina* with concentration (100 mg/ml) . This study, has to some extent, validated the medicinal potential of the mangrove plants.

Key words: *Bruguiera cylindrica*, Soxhlet extraction, antimicrobial activities and mangrove plants.

INTRODUCTION

Medical plants have been used for years in daily life to treat disease all over the world. Natural products can be selected for biological screening based on ethno medical use of plants, because many infectious diseases are known to have been treated with herbal remedies throughout the history of mankind. Numerous studies have been carried out on various natural products screening their antimicrobial activity ^{1, 2, 3, 4}. The potential of antimicrobial properties of plants are related to their ability to synthesize compounds by the secondary metabolism. Secondary metabolites proved to be the most important group of compounds that showed wide range of antibacterial and antifungal activity. Several chemical compounds of relatively complex structure with antimicrobial activity have

been studied. Antimicrobial properties of medicinal plants are being increasingly reported from different parts of the world.

Mangroves are widespread in tropical and sub tropical regions, growing in the saline intertidal zones of sheltered coast lines. Mangroves are biochemically unique, producing a wide array of novel natural products. Mangroves are rich source of steroids, triterpenes, saponins, flavonoids, alkaloids, polyphenols and tannins ^{5, 6, 7}. Mangrove and mangrove associates contain biologically active antiviral, antibacterial and antifungal compounds ⁸. 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.

Bruguiera cylindrica belongs to family Rhizophoraceae, roots are knee pneumatophores with buttress, leaves: are thin, light green, and pointed where as bark is smooth and grey which grows up to 20 m tall. Used as firewood and timber. Young radicles may be eaten as a vegetable or preserve after boiling. Bark produces a peculiar odor which frightens away fish. The scrapped skin of the fruit is used to stop bleeding. The leaves are used to control blood pressure (India).

The present study was to screen the antimicrobial activities of *B. cylindrica* and search for new compounds from mangrove plants.

MATERIAL AND METHODS

Plant and extraction

The material was taxonomically identified and the Voucher specimen is stored. The plant parts were collected from coringa forest near Kakinada, Godavari-krishna delta area, Andhra Pradesh, India. 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

Alternaria alternata (MTCC 2724), *Acremonium strictum* (MTCC 2599), *Aspergillus flavus* (MTCC 463), *Aspergillus niger* (MTCC 272), *Bipolaris bicolor* (MTCC 2105), *Cladosporium herbarum* (MTCC 2143), *Curvularia lunata* (MTCC 2030), *Erwinia carotovora* (MTCC 3609), *Fusarium oxysporum* (MTCC 1755), *Macrophomina phaseolina* (MTCC 2165), *Penicillium expansum* (MTCC 2006), *Pseudomonas syringae* (MTCC 1604), *Pseudomonas marginales* (MTCC 2758), *Rhizoctonia solani* (MTCC 4633), *Tiarosporella phaseolina* (MTCC 2165), *Ustilago maydis* (MTCC 1474), *Xanthomonas campestris* (MTCC 2286) including fungi and bacteria were obtained from Microbial Type Culture Collection (MTCC), IMTECH, 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°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 5×10^5 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 ⁹ modified by ¹⁰.

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 antimicrobial activity were later tested to determine the Minimal Inhibitory Concentration (MIC) for each bacterial and fungal sample.

RESULTS

Table 1 summarizes the antimicrobial activities of zone of inhibition of methanol (9 to 26 mm) with 100 mg/ml. The MIC values varying (15 to 150 mg/ml). 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.

Methanolic MIC (15 mg/ml) shows lowest activity against *P. syringae* where as highest MIC (150 mg/ml) against *A. niger*.

Table 1: Antimicrobial activity of methanol extracts of *B. cylindrica*

Microorganisms	Methanol 100 (Zone of inhibition in mm)	Methanol 300 (Zone of inhibition in mm)	Methanol 500 (Zone of inhibition in mm)	MIC (Extract concentration mg/ml)
<i>Alternaria alternata</i>	19	21	26	60
<i>Acremonium strictum</i>	16	20	22	65
<i>Aspergillus flavus</i>	12	15	16	100
<i>Aspergillus niger</i>	9	11	14	150
<i>Bipolaris bicolor</i>	14	16	20	75
<i>Cladosporium herbarum</i>	-	-	-	-
<i>Curvularia lunata</i>	20	22	25	60
<i>Erwinia carotovora</i>	19	21	24	90
<i>Fusarium oxysporum</i>	20	22	26	50
<i>Macrophomina phaseolina</i>	-	-	-	-
<i>Penicillium expansum</i>	15	16	18	100
<i>Pseudomonas syringae</i>	26	28	31	15
<i>Pseudomonas marginales</i>	-	-	-	-
<i>Rhizoctonia solani</i>	18	19	22	25
<i>Tiarosporella phaseolina</i>	13	15	17	100
<i>Ustilago maydis</i>	17	19	24	75
<i>Xanthomonas campestris</i>	22	23	27	25

Volume per well: 50µl; Borer size used: 6mm; Extract concentrations in 100-500 mg/ml

DISCUSSION

Methanolic extracts showed most active and significant (Zone of inhibition: 26 mm) against *P. syringae* and *X. campestris* (22 mm) followed by *C. lunata*, *F. oxysporum* and *E. carotovora* while weakest activity against *A. niger* (9 mm) on the other hand no activities were found against *C. herbarum*, *M. phaseolina* and *P. marginales* with methanolic extracts. The hexane and chloroform extracts appears to have less antibacterial and antifungal activity than the methanolic extracts hence we are not presenting the results. 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.

CONCLUSION

Overall, the present study provides enough data to show the potential of mangrove *B. cylindrica*. The above results it can be concluded that plant extracts of *B. cylindrica* 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. Such screening of various natural organic compounds and identifying active agents is the need of the hour, because successful prediction of important lead molecule and drug like properties at the onset of drug discovery will pay off later in drug development.

Further studies are needed to identify the pure component and establish the exact mechanism of action for antibacterial and antifungal action of the plant extract.

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