

## ***In vitro* antimicrobial potentiality of mangrove plant *Myriostachya wigtiana* against selected phytopathogens**

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### **ABSTRACT**

In this present study antimicrobial activity of *Myriostachya wigtiana* (Poaceae), 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 highest activity showed against *B. bicolor*- MTCC 2105 (18 mm) with MIC (75 mg/ml) followed by *C. lunata* (15 mm), *E. carotovora* (15 mm) and *P. marginales* (16 mm), where as lowest activity with *A. strictum* (9 mm) with 100 mg/ml and no activity were found against *C. herbarum* and *M. phaseolina* with all extract concentrations (100, 300 and 500 mg/ml). This study, has to some extent, validated the medicinal potential of the mangrove plants.

**Key words:** *Myriostachya wigtiana*, Soxhlet extraction, antimicrobial activities and mangrove plants.

### **INTRODUCTION**

Various medical plants have been used for years in daily life to treat disease all over the world throughout the history of mankind. Even today, plant materials continue to play a major role as therapeutic remedies in many developing countries (Czgan, 1993; Ody, 1993). As a consequence of an increasing demand for biodiversity in the screening Marine halophytes are the specialized groups of plants adopted for high saline conditions which include mangrove. Finding natural "eco-friendly" plant products that prevent or treat these diseases could be an alternative treatment process. Mangroves are widespread in tropical and sub tropical regions, growing in the saline intertidal zones of sheltered coast lines. Until now, more than 200 bioactive metabolites have been isolated from true mangroves of tropical and subtropical populations (Wu *et al.*, 2008. According to their chemical

structure, most of the isolated compounds belong to steroids, triterpenes, saponins, flavonoids, alkaloids, tannins and phenolics which having a wide range of therapeutic possibilities (Bandaranayake., 1998). Recent research evidenced that Indian mangroves contained antibacterial (Chandrasekaran *et al.*, 2009) and antifungal (Bose, S. and A. Bose, 2008) properties.

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

### **MATERIAL AND METHODS**

#### **Plant and extraction**

*Myriostachya wigtiana* (Poaceae) , 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), *Ustilago maydis* (MTCC 1474), 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  $5 \times 10^5$ 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, 1995)<sup>7</sup> modified by (Olurinola, 1996)<sup>8</sup>.

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.

**Table 1: Antimicrobial activity of methanol extracts of *M.wigtiana***

<i>Microorganisms</i>	Zone of inhibition in ( mm)			
	100 mg/ml	300 mg/ml	500 mg/ml	MIC mg/ml
<i>Alternaria alternata</i>	11	13	14	100
<i>Acremonium strictum</i>	9	14	16	150
<i>Aspergillus flavus</i>	12	14	17	85
<i>Aspergillus niger</i>	14	16	19	100
<i>Bipolaris bicolor</i>	18	20	23	75
<i>Cladosporium herbarum</i>	-	-	-	-
<i>Curvularia lunata</i>	15	21	24	90
<i>Erwinia carotovora</i>	15	17	20	85
<i>Fusarium oxysporum</i>	11	13	15	100
<i>Macrophomina phaseolina</i>	-	-	-	-
<i>Penicillium expansum</i>	9	13	15	150
<i>Pseudomonas syringae</i>	10	11	13	150
<i>Pseudomonas marginales</i>	15	16	22	80
<i>Rhizoctonia solani</i>	11	12	16	85
<i>Ustilago maydis</i>	12	13	15	100

Volume per well: 50µl; Borer size used: 6mm;

Extract concentrations in 100, 300 and 500 mg/ml, No zone (-)

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 18 mm) with 100 mg/ml. The MIC values varying (75 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.

## DISCUSSION

Methanolic extracts showed most active and significant (Zone of inhibition: 18 mm) against *B. bicolor* followed by *C. lunata*, *E. carotovora* and *P. marginales* while weakest activity against *P. expansum* (9 mm) with 100 mg/ml where as on the other hand no activities were found against *C. herbarum* and *M. phaseolina* with all extract concentrations (100, 300 and 500 mg/ml). The hexane and chloroform extracts appears to have less antibacterial and antifungal activity than the methanolic extracts hence we are not presenting the results.

*M. wigtiana* methanolic extract MIC (75 mg/ml) shows lowest activity against *B. bicolor* and where as highest MIC (150 mg/ml) against *P. expansum*, *P. syringae* and *A. strictum*

Molecules derived from natural products have an excellent record of providing novel chemical structure for development as new therapeutic agents. Many of the worlds most valuable and

successful medicines have been derived from nature. Ten of the world's twenty-five top selling pharmaceuticals were derived from natural products and accounted for global sales of almost US\$14billion in 1995. An antimicrobial agent from marine halophytes is immediate need of ethno pharmacological science in developing novel marine pharmaceuticals.

A number of mangroves and mangrove associates proved to have antibacterial, antifungal properties. Highest antibacterial activities are believed to be the presence of high content of phenols which includes tannins (Ravi kumar *et al.*, 1993)<sup>9</sup>, coumarines and their glycosides, anthraquinones and their glycosides, naphthaquinones flavones and related flavonoides, polysaccharides (Trease and Evans, 1997)<sup>10</sup> in mangrove halophytes. Further studies are needed to elucidate the structure and mechanism of action of these marine halophytic 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.

## CONCLUSION

Overall, the present study provides enough data to show the potential of mangrove *M. wigtiana*. The above results it can be concluded that plant extracts of *M. wigtiana* 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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