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Research Article

ANTIMICROBIAL ACTIVITY OF CRUDE METHANOLIC EXTRACTS OF *RHIZOPHORA MUCRONATA*

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ABSTRACT

Mangroves are salt tolerant plants able to grow in extremely saline habitats and tolerate salinity by various eco-physiological mechanisms. This study focus on screening the plant for Antimicrobial activity and FTIR on Methanolic extract of *Rhizophora mucronata.* The fresh leaves of *Rhizophora mucronata* were collected from Krusadai Island, Mandapam, Rameswaram. The Methanol extracts were prepared with the help of Soxhlet apparatus. The Methanolic extract of *R. mucronata* extracts exhibited a maximum growth inhibition against bacterial pathogens *K. oxytoca* (23 mm) and *S. aureus* (21 mm) at the concentration of 75ug/ml. In fungal pathogens *R. mucronata* showed maximum inhibition of 14mm was observed in *Acremonium* sp and *A. niger* at the concentration of 75ug/ml. The FT - IR spectrum confirmed the presence of alcohols, phenols, alkanes, aromatics, carboxylic acids, esters, ethers and alkenes

Keywords: *Rhizophora mucronata,* Methanol, FT – IR, Antimicrobial activity.

INTRODUCTION

Mangrove forests, the world's most productive ecosystems that enrich coastal waters, protect coastlines and enrich coastal waters with a yield of diversified commercial forest products, protect coastlines, and support coastal fisheries. However, the mangroves strives under extreme conditions such as highly fluctuating salinities, extreme tide actions, strong winds, high temperatures, muddy and anaerobic soils. There may be no other group of plants with such highly developed morphological, biological, ecological and physiological adaptations to extreme environmental conditions (Kathiresan and Bingham, 2001). Mangrove environments hold a rich source for discovery of the new microbiota with extensive applications in pharmaceutical science (Gayathri et al., 2010; Boopathy and Kathiresan, 2010; Atri and Sharma, 2012). A number of mangroves and associates contain substances which show biological activities such as antiviral, antibacterial and antifungal properties (Bandaranayake, 1995 & 2002).

Infectious diseases are responsible for 14 million global deaths annually (Walsh, 2003) and amongst them, bacterial infections are a major threat (Westh, 2004). The only solution to this problem is use of antibiotics or chemicals. However, the increasing failure of chemotherapy and antibiotic resistance exhibited by bacterial pathogens has prompted researchers for screening of plants for their antimicrobial activity (Scazzocchio, 2001). Thus, there is an urgent need to discover new antimicrobials for new and re‐emerging bacterial diseases.

The present study attempted to test the antibacterial and antifungal constituents present in Methanolic extract of *R.mucronata* against different pathogens and analyze the functional group by FTIR technique.

MATERIALS AND METHODS

Collection and authentication of *Rhizophora mucronata* Leaves:

The fresh leaves of *Rhizophora mucronata* were collected from Krusadai Island, Mandapam, Rameswaram and authenticated in the herbarium of C.A.S. in Marine Biology, Annamalai University, Parangipettai, India. Fresh, Healthy and Uninfected leaves were washed with running tap water and spread out.

Preparation of plant extract:

The leaves were air dried in the laboratory and made into powder by grinding. The Methanol extracts were prepared with the help of Soxhlet apparatus. After the extracts were obtained it will kept for evaporation to remove the excessive solvents and it was collected and stored for the analysis of further studies.

Anti bacterial activity of the plant extracts:

The different leaf extract of *Rhizophora mucronata* was used throughout the study. The different extracts were tested against different bacterial pathogens such as *V. parahaemolyticus, K. oxytoca, P. aeruginosa, B. subtilis, K. pneumonia* and *S. aureus* for their antimicrobial activity. It was demonstrated by disc diffusion assay.

Antifungal activity of the plant extract:

The both extract were tested against different fungal pathogens such as *A. niger, Acremonium sp., A. flavus, Aspergillus sp., P. digitatum* and *Penicillium sp.* for their antifungal activity. It was demonstrated by disc diffusion assay.

Fourier Transform Infrared Spectroscopy (FTIR) (Griffiths and Haseth, 2007):

The functional groups were characterized by using Fourier transform infrared spectrophotometer (FT-IR; FTIR 8400, Shimadzu, Tokyo, Japan). The methanolic extracts were ground with KBr powder and pressed into pellets for FT-IR spectra measurement in the frequency range of 400 to 4,000 cm^{-1}.

RESULT

Antimicrobial activity of *Rhizophora mucronata:*

In the present investigation, the Methanolic extract of *R. mucronata* extracts exhibited a promising antibacterial activity. In this assay the Methanolic extract of *R. mucronata* were screened for their ability to suppress/inhibit the growth of 6 human bacterial pathogens viz., *V. parahaemolyticus, K. oxytoca, P. aeruginosa, B. subtilis, K. pneumonia* and *S. aureus.* The respective zone of inhibitions was depicted in **Table 1**.

The Methanolic extract of *R. mucronata* extracts exhibited a maximum growth inhibition against *K. oxytoca* (23 mm) and *S. aureus* (21 mm) at the concentration of 75ug/ml. They showed moderate activity against *K. pneumonia* (16mm), *V. parahemolyticus* (14mm) and *P. aeruginosa* (13mm). Minimum zone of inhibition were recorded in *V. parahaemolyticus* (11 mm) at a concentration of 100 µg. The tetracycline exhibited a prominent susceptibility against *S. aureus* (30mm), *K. pneumonia* (29mm), *K. oxytoca* (28mm), *P. aeruginosa* (28mm), *B. subtilis* (27mm) and *V. parahaemolyticus* (26mm).

S.No	Microorganisms (Bacteria)	Methanolic extract of *Rhizhophora mucronata*				Antibiotic Control (Tetracycline)
		25ug/ml.	50ug/ml.	75ug/ml.	100ug/ml	
1	*V. parahaemolyticus*	20mm	16mm	14mm	11mm	26mm
2	*K. oxytoca*	19mm	18mm	23mm	19mm	28mm
3	*P. aeruginosa*	20mm	19mm	13mm	12mm	28mm
4	*B. subtilis*	20mm	15mm	20mm	15mm	27mm
5	*K. pneumonia*	19mm	14mm	16mm	14mm	29mm
6	*S. aureus*	21mm	19mm	21mm	18mm	30mm

Table 1: Antibacterial activity of Methanolic extract of *R. mucronata*

Antifungal activity of *Rhizophora mucronata:*

In antifungal assay nanoparticles were tested against six fungal strains viz., *A. niger, Acremonium sp., A. flavus, Aspergillus sp., P. digitatum* and *Penicillium sp.* The respective zone of inhibitions was depicted in Table 2. Methanolic extract of *R. mucronata* showed maximum inhibition of 14mm was observed in *Acremonium* sp and *Aspergillus niger* at the concentration of 75ug/ml. Moderate level of inhibition was recorded in *Penicillium digitatum* (13mm) followed by *Aspergillus* sp (12mm) at a concentration of 75 µg. Flucnazole displayed the maximum susceptibility of 13mm in *Acremonium sp.* followed by *A. flavus* (11mm).

S.NO	Microorganisms (Fungi)	Methanolic extract of *Rhizhophora mucronata*				Antibiotic control (Flucnazole)
		25ug/ml.	50ug/ml.	75ug/ml.	100ug/ml	
1.	*A. niger,*	9 mm	7 mm	14 mm	11 mm	10 mm
2.	*Acremonium sp.*	10 mm	7 mm	14 mm	12 mm	13 mm
3.	*A. flavus*	_	_	_	_	11 mm
4.	*Aspergillus sp,*	13 mm	12 mm	12 mm	9 mm	10 mm
5.	*P. digitatum,*	11 mm	13 mm	13 mm	8 mm	9 mm
6.	*Penicillium sp.*	_	_	_	_	10 mm

Table 2: Antifungal activity of Methanolic extract of *R. mucronata*

FTIR Analysis:

The FT - IR spectrum was used to identify the functional group of the active components based on the peak value in the region of infrared radiation. The FT - IR spectrum profile was illustrated in the **Figures 1.** The FT - IR spectrum confirmed the presence of alcohols, phenols, alkanes, aromatics, carboxylic acids, esters, ethers and alkenes

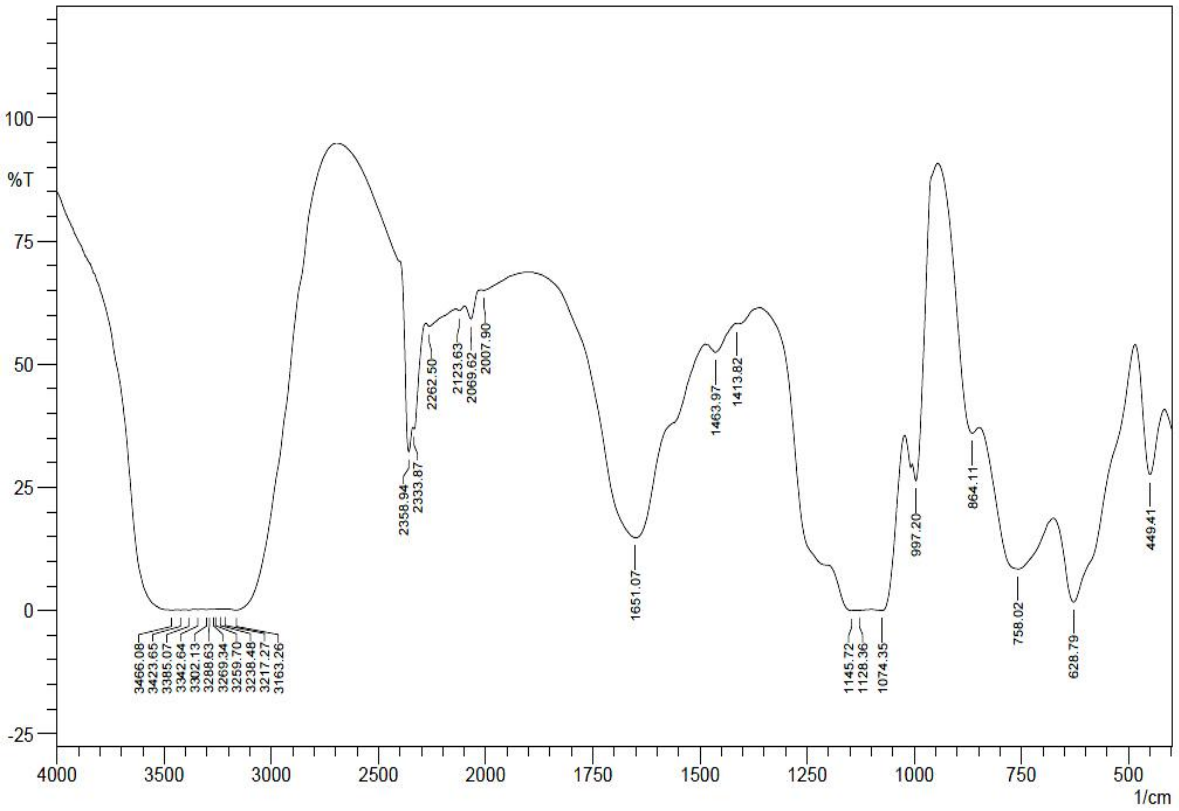

Figure 1: FTIR Spectrum of Methanolic extract of *Rhizophora mucronata*

Wavenumber, cm^{-1}	**Bond**	**Interacting functional groups**
3441.01	O–H stretch, H–bonded	alcohols, phenols
2922.16	C–H stretch	Alkanes
1643.35	–C=C– stretch	Alkenes
1411.89	C–C stretch (in–ring)	Aromatics
1101.35	C–O stretch	alcohols, carboxylic acids, esters, ethers
651.94	=C–H bend	Alkenes

Table 3: Key in FT-IR peaks of Methanolic extract of *Rhizophora mucronata*

DISCUSSION

India has a great diversity of plants used in folk medicine and only few of these have been studied for antimicrobial studies (Premanathan, 1992). Several mangroves and halophytes are extensively used in traditional medicine, only some of them were tested for biological activities (Funnel, 2004). The antimicrobial activity exhibited by the mangrove plant parts could be due to the presence of phytochemicals like alkaloids, tannins, flavonoids and sugars present in the plant extract. The variation of antimicrobial activity of present study might be due to distribution of antimicrobial substances, which varied from fraction to fraction of the crude extract. Further studies are needed to identify the pure compound and establish the exact mechanism of action for antibacterial action of the plant extract.

Similar results are noted in various other mangroves occurring along Mumbai. Both the cold stem extract showed comparable results but there was no significant difference (P<0.5).The highest zone of inhibition (20 mm) exhibited by both the plants against *S.typhi* and *E.coli* while *R.apiculata* also shows the presence of antifungal compound in it as it inhibits the *C.albicance* (18 mm) (Pimpliskar Mukesh, 2012). These authors have recorded negative resuls in *Acanthus ilicifolus* and *Ipomea percarpae*, this indicates that the presence of Mangrove plants are species specific. According to HPTLC and IR analysis of potent fractions showed the presence of bioactive principles like coumarin, anthrones, essential oils and tannins. Further gelatin precipitation test also proves the presence of tannins. Similarly biochemical constituents recorded by Bandaranayake (1998), Pimpliskar (2004) and Cowman (1999) shows the presence of tannins, coumarin,anthrone and essential oils in the mangrove plants.

CONCLUSION

From these results, it is clearly apparent that *R.mucronata* holds potent bioactive compounds that can efficiently repress the growth of human and animal pathogens. Further bioassay monitored purification will open perspectives on the discovery of bioactive that could be utilized in the field of human and veterinary grade bio-therapeutics.

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