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Isolation and characterization of secondary metabolites from the mangrove plant *Rhizophora mucronata*

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ABSTRACT

Objective: To evaluate the antibacterial activity of foliar extracts of *Rhizophora mucronata(R. mucronata)* against pathogens belonging to human origin and to identify the compound hitherto unprecedented in nature by GC-MS analysis. **Methods:** Soxhlet extraction method was used to get the corresponding extracts of ethanol, petroleum ether, acetone, methanol and ethyl acetate. The antimicrobial activities of the organic solvent extracts on the various test organisms using agar well diffusion technique were carried out. Ethyl acetate extract exhibited promising antimicrobial activity and hence minimum inhibitory concentration (MIC) was performed for the same. Column chromatography was done for partial purification of crude extract and fractions were analyzed by GC-MS. **Results:** A column chromatographic fractionation of the extracts and further UV visible and GS-MS analysis suggested the active principle compound were a mixture of squalene (19.19%), n-Hexadecanoic acid (6.59%), phytol (4.74%), 2-cyclohexane-1- one, 4-hydroxy-3,5, (4.20%) and oleic acid (2.88%). **Conclusions:** The results are good enough to serve to transform the practice of research in this sub field across a range of different benefit streams that include drug development. By and large this type of structure analyses are most important as aids to more rational decision taking in safety models versus effectiveness. In general, structural data provide prima facie support for drug hypothesis.

1. Introduction

Mangroves need no introduction in today's world. Apart from the resources those flourishes in the dense tangle of roots, mud and tidal water, mangroves are known for its medicinal wealth. For generations, the mangroves have proven its use worldwide. The various parts of *Rhizophora mucronata (R. mucronata)* find its use to treat human ailments like angina, dysentery, hematuria and many more. Also, according to the available scientific literature, documentation of the trees many uses dates back to the 17th century.

Mangroves, the hot spots of coastal diversity gained prominence after the tsunami of December 2004. Given its unique physical environment mangroves have evolved a suite of adaptations to cope with extreme environmental conditions that include high salinity, strong winds, tidal variations, high temperature and anaerobic tidal swamps. As per the volumes of available data, we need to understand that the mangrove ecosystem encompasses vast number of species that are a rich source of bioactive metabolites and enzymes. Be it the extensive supporting roots of *Rhizophora* or the breathing roots of *Avicennia* or the salt excreting leaves or the viviparous water dispersed seedlings, everything harbors notable potential to produce bioactive metabolites for obvious reasons of survival and propagation, thereby giving them 'chemical signals' to respond, thwart or defend 'environmental clues'.

Approximately 55 species of mangroves from 22 genera are distributed in Indian Ocean region[1]. As much as the habitat of mangrove plants takes different names as mangrove swamps, tidal forests, tidal swamp forests or mangals, so are evidences for its antibacterial[2], antiviral [3], Antifungal[4] and antioxidant activity[5].

In light of this, the present study was initiated to investigate the leaf extracts of *R. mucronata* collected from Parangipettai, Chidambaram District for its biological activity against certain bacteria that play havoc on human health.

2. Materials and methods

Leaves of *R. mucronata* were collected from mangrove

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forest of parangipettai, chidambaram located in Tamilnadu. Prior to the extraction, the leaves of respective species were washed with sterile water to remove any associated debris, shade dried with occasional shifting in order to prevent photolysis and thermal degradation, chopped into small pieces, powdered with a mechanical grinder and stored in an air tight container.

For extraction of crude bioactives, the powder obtained was subjected to successive soxhlet extraction with organic solvents like ethanol, petroleum ether, acetone, methanol and ethyl acetate. The extracts are further concentrated by recovering excess solvents to a thick oily natured crude in a rotary evaporator at reduced pressure. The extract was stored at 4 ℃ in air- tight plastic vials for further studies.

The antibacterial activity of the extracts was assessed against *Eschericia coli(E. coli)*, *Staphylococcus aureus(S. aureus)*, *Klebsiella pneumonia*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Pseudomonas fluorescens*, *Salmonella typhii*, *Bacillus subtilis* by agar well diffusion method. The assay system was prepared with gel punctured Muller Hinton Agar plates. Test culture was swabbed aseptically and inoculated on the surface of the Muller Hinton Agar so as to make a lawn and left to dry for the wells to be perfect. 1g of leaf sample is dissolved in 1 mL of the solvent and 1 mL of filtrate is mixed with 1 mL of the solvent from which various (15 μL, 25 μL, 50 μL/well) concentrations were taken and loaded in the well using micropipette and one well was loaded with the respective solvent as control. Plates were incubated for 16 to 18 hrs at 37 ℃. The percentage of mortality was determined by observing the zones of inhibition. The extracts that showed antimicrobial activity were later tested to determine the minimal inhibitory concentration (MIC) for each test sample.

For column Chromatography the ethyl acetate extract of *R. mucronata* (1 g) was loaded on a silica gel column packed with hexane and eluted with hexane and chloroform (9:1 to 1:9 & 100% chloroform) followed by ethyl acetate and methanol ((9:1 to 1:9 & 100% methanol) to yield 26 fractions. Individual fractions when collected and tested revealed that the 24th fraction that was eluted using ethyl acetate and methanol (8:2) had maximum activity. The absorbance of the fractions eluted from the column chromatography was measured at a resolution from 200-800 nm using UV-Visible spectrophotometer and the readings were recorded. The fractions that were eluted in column chromatography using chloroform and ethyl acetate (2:8) exhibiting activity was subjected to GC-MS equipped with Agilent 5975 inert XL MSD to find out the active principle of the extracts.

3. Results

The plant material was subjected to an extraction process with solvents like ethanol, petroleum ether, acetone, methanol and ethyl acetate. The crude ethyl acetate extracts (50 μL) of *R. mucronata* showed remarkable antibacterial activity with zones of inhibition that is revealed in Table 1. The test organisms were based on the choice of their ubiquitous presence[6,7]. Elution of individual fractions of foliar extracts of *R. mucronata* by column chromatography and their absorbance at 200 to 300 nm in a UV-visible spectrum revealed fraction 24 (ethyl acetate: methanol 8:2) as the most potent one (Table 1). The GC-MS results (Figure.1) of active column fraction (F24) revealed that the active principals were a mixture of squalene (19.19%), n-Hexadecanoic acid (6.59%), phytol (4.74%), 2-cyclohexane-1-one, 4-hydroxy-3,5, (4.20%) and oleic acid (2.88%). Recent research also reports that many fatty acids from mangroves posses antimicrobial property (40-43).

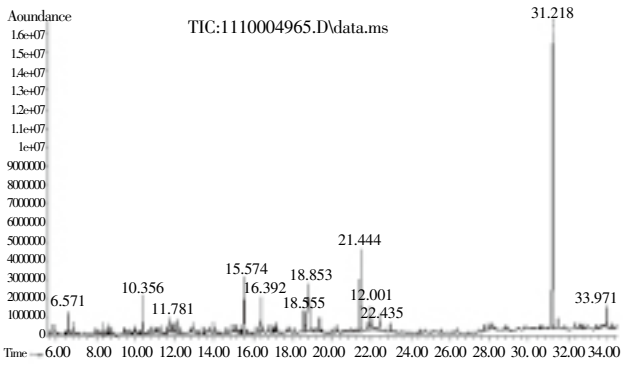

Figure 1. GC-MS analysis of *R. mucronata*.

Table 1
Antibacterial activity of crude ethyl acetate extracts of *R. mucronata*.

Sample	Organism	Zone of inhibition (mm)			MIC
		15 μL	25 μL	50 μL	
Crude extract	*E. coli*	8	11	15	8
	S. aureus	9	12	18	9
	Klebsiella pneumonia	12	13	9	8
	Proteus vulgaris	10	8	11	15
	Pseudomonas aeruginosa	18	10	13	8
	Pseudomonas fluorescens	12	15	9	13
	Salmonella typhii	15	12	13	11
	Bacillus subtilis	12	8	6	13
Fractions eluted from CC	*E. coli*	15	28	35	15
	S. aureus	14	19	28	14
	Klebsiella pneumonia	15	22	21	31
	Proteus vulgaris	12	18	15	26
	Pseudomonas aeruginosa	21	33	12	18
	Pseudomonas fluorescens	27	19	22	18
	Salmonella typhii	18	21	26	15
	Bacillus subtilis	19	15	27	22

4. Discussion

It's no news that the mangrove produce a plethora of bioactive secondary metabolites drawing many a curious researcher to the tidal swamps. The search for bioactive metabolites is a result of curiosity and interest as they prove to be new lead structures as generation next medicines bypassing the age-old antibiotics.

The present study has planned to find out the newer antibacterial compounds from the most unexplored mangrove plant. *R. mucronata* appear to have broad spectrum of antibacterial activity. Of the five solvent tested, ethylacetate was determined to be the best solvent for isolation of bioactive secondary metabolites. The sensitivity of ethyl acetate to all of the mangroves extracts could be attributed due to the presence of common bioactive compounds that had inhibitory effects on the microorganisms. These results indicate that the ex traction had definite effects for the isolation bioactive principles. Bioactivity guided fractionation can be performed using various analytical methods to separate mixture compounds from the targeting extracts[8]. At first the crude extracts of ethyl acetate was confirmed to posses some activities. This is followed by the separation of the compounds using Column chromatography and from the GC-MS result we found that the main phycoconstituent of the active fraction was squalene, n-Hexadecanoic acid and oleic acid.

Conflict of interest statement

We declare that we have no conflict of interest.

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