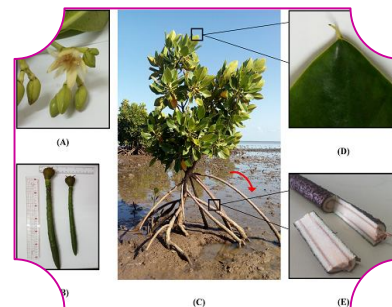




ANTIBACTERIAL ACTIVITY OF MANGROVE PLANT *RHIZOPHORA MUCRONATA* LAMK. FROM THE COAST OF RAIGAD DISTRICT, MAHARASHTRA

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ABSTRACT

The objective of present study is to investigate the antibacterial activities of n-hexane, ethyl acetate and methanol extracts of the leaves of *Rhizophora mucronata* Lamk. against six human pathogenic microbes. The antibacterial activity was evaluated using disc diffusion and microdilution methods. The antibacterial activities of the crude extracts were increased with increasing in the concentration. It is clear that n-hexane extract was the most effective extract. Additionally, gram positive *Bacillus cereus* (*B. cereus*) appear to be the most sensitive strain while *Pseudomonas aeruginosa* (*P. aeruginosa*). The inhibition of bacterial growth at concentration as low as 0.04 mg/mL indicated the potent antibacterial activity of *R. mucronata* extracts. This concludes that the obtained results are considered sufficient for further study to isolate the compounds responsible for the antibacterial activity and suggesting the possibility of finding potent antibacterial agents from *R. mucronata* extracts.

KEYWORDS: Mangroves, *Rhizophora mucronata* Lamk., antibacterial activity, Raigad.

1. INTRODUCTION

Infectious diseases represent a serious public health problem and they remain the leading cause of death throughout the world^[1-3]. Currently, the problems of microbial drug resistance, an increase of opportunistic infections and the toxicity effect of continued use of several antimicrobial drugs^[4] have necessitated a search for new antimicrobial drugs from other sources including natural sources like plants which are the good sources of novel antimicrobial chemotherapeutic agents. Furthermore, plants have been a major source for drug development^[5-7]. Plant extracts and products are used in the treatment of infectious disease^[8-10]. *Rhizophora mucronata* Lamk. (*R. mucronata*) -locally known as *Kandal*- is large mangrove tree (up to 10 – 12 m tall) belongs to the Rhizophoraceae family. It is distributed throughout the Indian coast^[11]. However, *R. mucronata* has been known traditionally as an important remedy for sprue^[11]. Up to date, there are no study has been conducted on the evaluation of the antibacterial activity of this plant. Therefore, this study aims to investigate the antimicrobial activities of *R. mucronata* extracts against four human pathogenic bacteria including two Gram-positive (*Staphylococcus aureus* (*S. aureus*) ATCC25923, *Bacillus cereus* (*B. cereus*) ATCC11778) and two Gram-negative (*Pseudomonas aeruginosa* (*P. aeruginosa*) ATCC27853, *Escherichia coli* (*E. coli*) ATCC35218). The efficacy of n-hexane, ethyl acetate and methanol extracts from the leaves of *R. mucronata* were also investigated and described.

2. MATERIALS AND METHODS

2.1. Plant Collection

R. mucronata was collected from Maharashtra coast at Raigad district in August 2017. The Voucher of the specimen was deposited in the Department of Botany, Doshi Vakil Arts College and G.C.U.B. Science & Commerce College, Goregaon-Raigad. The taxonomic identification of this plant was done by using Flora of Maharashtra.

2.2. Plant preparation and extraction

The fresh plant was washed under running tap water and dried in a warm room for 3 to 5 d. The samples were grinded into fine powder and extracted by Soxhlet with n-hexane, ethyl acetate and methanol successively to get n-hexane, ethyl acetate and methanol extracts. Then, all the crude was kept at -20 °C until further use.

2.3. Samples preparation

A sample of 100 mg from each extract was dissolved in 1 mL DMSO. The extract was then sterilized by filtration through sterile syringe filter (0.2 µm pore). Finally the filtered extract was stored as aliquots until it was used.

2.4. Microbial strains

Four reference strains of human pathogenic bacteria were used in this study including two Gram-positive (*S. aureus* ATCC25923, *B. cereus* ATCC11778) and two Gram-negative (*P. aeruginosa* ATCC27853, *E. coli* ATCC35218).

2.5. Antimicrobial assay

2.5.1. Disc diffusion method

The agar disc diffusion method was employed for the determination of antibacterial activities of the extracts according to Qaralleh *et al.*^[12] with some modification. Briefly, inoculum containing 10⁷ CFU/mL was spread on Mueller-Hinton agar plates for four bacteria. Using sterile forceps, the sterile filter papers (6 mm diameter) containing the crude extracts (1 or 1.5 mg), standard antibiotics (30 µg of chloramphenicol) or negative control (DMSO) were laid down on the surface of inoculated agar plate. The plates were incubated at 37 °C for 24 hours. Each sample was tested in duplicate and the zone of inhibition was measured as millimeter diameter.

2.5.2. Microdilution method

Minimum inhibitory concentration (MIC) was measured by determining the smallest amount of extract or standard antibiotic needed to inhibit the visible growth of a test bacterium. This was done using 96-well plates. The assay plates were filled with Mueller-Hinton broth medium (MHB) containing different concentrations of extracts, tetracycline or solvent control and the test bacterium (10⁷ CFU/mL). Each sample was tested in triplicate and the observation was recorded by naked eyes after 24 h incubation periods at 37 °C. Minimal bactericidal concentration (MBC) was determined by transferring and spreading the treated culture broth of the wells containing the concentrations equal to and higher than the MIC on agar plates. The lowest concentration of the extract or the standard antibiotic required to completely destroy test bacteria after incubation at 37 °C for 24 h was reported as MBC.

3. RESULTS

The antibacterial activity of *R. mucronata* extracts are shown in Table 1. Generally, the results showed that the antibacterial activities of the crude extracts were increased with increasing the concentration. Although the antibacterial activity of the extracts tested is variable, two Gram-positive bacteria (*S. aureus* and *B. cereus*) and only gram negative (*E. coli*) were inhibited by the extracts.

Quantitative analyses on the antibacterial properties were obtained through the determination of bacteriostatic and bactericidal concentrations of *R. mucronata* extracts. Table 2 shows the MIC and MBC of the extracts that produce inhibition zone more than 12 mm. The results of inhibition zone were reflected in lower MIC values. The MIC and MBC values for bacterial strains, which sensitive to the extracts, were in the range of 0.04-1.11 mg/mL and 0.04-10 mg/mL, respectively. Furthermore, in most cases, the MBC values were higher than the MIC values, except for n-hexane extract against *B. cereus* (MIC = MBC). According to the disc diffusion results, MIC and MBC values, it is clear that n-hexane extract was the most effective extract (Table 1 and 2). Additionally, Gram positive *B. cereus* appears to be the most sensitive strain with inhibition zone of 19 mm (1.5 mg/disc) and the MIC value is 0.04 mg/mL. The inhibition of microbial growth at concentration as low as 0.04 mg/mL indicated the potent antibacterial activity of *R. mucronata* extracts.

Table 1: Antimicrobial activity of *R. mucronata* extracts

Microorganisms	Zone of Inhibition		Ethyl acetate		Methanol		Positive control	Negative control
	n-hexane							
	1 mg	1.5 mg	1 mg	1.5 mg	1 mg	1.5 mg		
<i>S. aureus</i>	14.5	14.5	11	16	13	17	21	00
<i>B. cereus</i>	16.00	19	09	09	9	9.5	27	00
<i>E. coli</i>	11.5	13.5	13.5	16.5	9.5	11	30.5	00
<i>P. aeuroginosa</i>	00	00	00	00	00	00	30.5	00

Positive control: tetracycline (100 µg); Negative control: DMSO.

Table 2: MIC and MBC of *R. mucronata* extracts and standard antibiotic

Extracts	<i>S. aureus</i>		<i>B. cereus</i>		<i>E. coli</i>	
	MIC	MBC	MIC	MBC	MIC	MBC
n-hexane	1.11	>10	0.04	0.04	0.04	0.37
Ethyl acetate	0.37	>10	-	-	0.37	>10.00
Methanol	1.11	10	-	-	-	-
Tetracycline	30.0	-	2.00	-	30.0	-

All data were expressed as (mg/mL) except for tetracycline (µg/mL); -: not determined.

4. DISCUSSION

Traditionally, plants were known as the main sources for drugs. Interest in this area continues and many new potent drugs have been isolated. Tropical and sub-tropical areas of the world are rich with many plant species which have effective properties, such as antimicrobial, antiviral and anti-fungal. Many medicine plant extracts have been known to possess antibacterial effects. Mangroves possess novel biologically active compounds. The extracts from different mangrove plants and mangrove associates have been reported to possess inhibition action against human and plant pathogens^[13-22]. In this report, three different polarity extracts have been tested for antimicrobial activity. With respect to the inhibition panel and the MIC and MBC concentrations, n-hexane extract of *R. mucronata* was the most effective extract. The methanol and dichloromethane extracts of *R. mucronata* also demonstrated antibacterial effect, although they were lower than the antibacterial effects of the n-hexane extract. The presence of the activity in n-hexane, dichloro-methane and methanol extracts might be represented by existence of more than one active compound. Chemical analysis of the species belongs to the genus *Rhizophora* have shown the presence of various bioactive ingredients including alkaloids, steroids, triterpenoids and flavonoids^[14,23]. On the other hand, it is interesting to note that the plant extracts showed bacteriostatic and bactericidal actions against *S. aureus*, *B. cereus* and *E. coli*. This suggests that they may possess remarkable therapeutic action in

the treatment of infectious disease caused by these species. The obtained results suggest the possibility of finding potent antibacterial agents from *R. mucronata* extracts and considered sufficient to isolate the compounds responsible for the activity.

Conflict of interest statement: I declare that I have no conflict of interest.

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