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Research Article

Evaluation of Antimicrobial principles of *Rhizophora* species along Mumbai Coast

Pimpliskar Mukesh R.[1], R.N.Jadhav[2], B.L.Jadhav[3]

[1]*Dept. of Biotechnology, E.S.A.College of science, Vasai Road 401202 (M.S.)*

[2]*Dept. of Zoology, E.S.A.College of science , Vasai Road 401202 (M.S).*

[3]*Dept of life sciences, Vidyanagari campus, university of Mumbai, Santacruz (E) Mumbai-98 Maharashtra*

**Corresponding author: mukesh227@yahoo.co.in*

ABSTRACT

Mangrove plants are rich source of secondary metabolites like steroids, triterpens, saponins, flavonoids, alkaloids and tannins. Extracts from different mangrove plants are reported to possess diverse medicinal properties. In view of this *Rhizophora apiculata* and *Rhizophora mucronata* were selected to study the antimicrobial and antioxidant potential. For this purpose gram negative, gram positive bacteria and fungi were used to test antibacterial and antifungal activity of these plant extracts. The tested extracts showed to varying degrees of antimicrobial potential against the test microorganisms.These promising finding suggest antibacterial and antifungal activity of plant material indicating presence of bioactive compounds against pathogens.

Keywords: *Rhizophora apiculata, Rhizophora mucronata*, Antibacterial, Antifungal, Bioactive, Agar cup method

1. INTRODUCTION

Tropical and sub-tropical areas of the world are bestowed with abundant flora and herbs which have untapped properties, such as antimicrobial, antiviral and antifungal. According to the World Health Organization, plants are a source of compounds that have the ability to combat disease, antimicrobial, antiviral and antifungal activities [1, 2].

The rise of antibiotic resistant microorganisms is one of the severe problems in health care systems of the world and infectious diseases are the second most serious cause of death worldwide [1, 3]. Mangroves are known for their economic and ecological importance throughout the world. They occupy about one fourth of tropical coastline. Indian mangroves occupy total area of 6,740 km.comprising of 65 species belonging to 39 genera of which 21 species belonging to 20 genera along the Mumbai coastland [4]. The most dominating genera include *Avicinnia, Sonneratia, Rhizophora* and *Acanthus* [5].

Mangroves have been a source of several bioactive compounds. Mangrove plants have been used in folklore medicine and extracts have proven activity against human, animal and plant pathogens.

A number of mangroves and associates contain substances which show biological activities such as antiviral, antibacterial and antifungal properties [6, 7].

Most of the mangroves found along Mumbai coast are used as alternative medicine for the treatment of various diseases. *Rhizophora apiculata* and *R.mucronata* are considered to have astringent, antidiarrhoea, antiemetic and haemostatic properties. [8]. Larvicidal and antiviral properties are also reported of these species [9].

Out of 21 species reported along Mumbai about 10 species have been screened for their antimicrobial properties [10-13]. There are many challenges in drug development with plants which includes the choice of solvent for the extraction and if extraction is to screen plants for antimicrobial components, the effect of the extractant on the separation procedure is not important but it should not inhibit the bioassay procedure. Ethanol as a solvent is widely used by many workers. Its advantages are volatility, miscibility with polar and non-polar solvents and its relatively low toxicity for the test microorganisms.

This study is focuses to test antibacterial and antifungal constituents present in ethanol cold extract of *Rhizophora apiculata* and *R.mucronata* stem against selected human pathogens.

2. MATERIAL AND METHODS

2.1. Plant material

Rhizophora mucronata and Rhizophora apiculata plant was collected from pollution free zone of Godrej Park, Vikroli, Mumbai and Murud coast in the month of March. Stems were separated, cleaned thoroughly and used for the extract

preparation. Fresh plant stem used for the preparation of cold extract.

2.2. Microorganisms

Escherichia coli, Staphylococcus aureus, Salmonella typhi, Steptococcus pyogens, Pseudomonas aeruginosa, Klebsiella pneumoniae, Proteus vulgaris bacteria and fungi *Candida albicans* were used for bioassay. These pathogenic isolates were obtained from Haffkine Institute, Parel, Mumbai. The organisms were maintained on slant of Nutrient media and sub-cultured every month and stored at 4 °C.

2.3. Preparation of extract

Cold ethanol extracts of *Rhizophora apiculata* and *Rhizophora mucronata* were prepared in ethanol solvent as follows: 10 gm of fresh stem were homogenized in mortar and pestle using 10-12 ml of solvent and kept aside for 45 min. for the extraction. It was then filtered through Whattman filter paper no.1, and to the residue again 6-8 ml was added and similarly filtered after 15 min. The final volume of the extract was made to 20 ml by adding required amount of solvent and stored in an airtight borosil glass bottle. All the extracts were preserved in refrigerator at 4 °C.

2.4. Bioassay

All the extracts were first brought to room temperature and then used for the bioassay test against the pathogenic microorganisms by Agar cup method [14]. All the bioassays were carried out in triplicate .The incubation temperature for bacteria and a fungi *C.albicans* was maintained at 37 °C. Results were recorded after 24 and 48 hrs as zones of inhibition were measured in millimeter (mm).Final results were expressed as the arithmetic average of triplicate experiments.

2.5. Minimum inhibitory concentration (MIC) test

The extracts, which showed inhibition zone size above 15 mm, were selected for the MIC test. The extracts were evaporated to dry residue and different concentration was tested against microorganisms by Agar cup method [14].

2.6. Bioassay guided fractionation

Highly potent extracts of plant part exhibiting MIC upto 1mg/ml were selected for bioassay guided fractionation study. Each potent extract was evaporated to dryness and petroleum ether was added to dissolve the residue, it was then centrifuged at 3000 rpm for 20 min. Thereafter residue and filtrate were separated. The filtrate was collected as petroleum ether extract (Fraction 'a'). Residue was dissolved in equal quantity of water and centrifuged at 3000 rpm for 20 min and filtrate was collected as water fraction (fraction 'b'). Finally remaining residue was dissolved in ethanol and centrifuged at 3000 rpm for 20 min and the filtrate was collected and termed as final ethanol extract (fraction 'c'). These three fractions were then used for the bioassay by Agar cup method [14].

2.7. HPTLC (High performance thin layer chromatography) analysis

Highly potent extracts of both the plants under study were analyzed for their fingerprinting by HPTLC method .The analysis was carried out on LINOMAT-IV supplied by CAMAG.The scanning and detection were performed at Anchrome India ltd.,Mulund,Mumbai.

2.8. Gelatin Precipitation Test

To confirm the presence of tannins in the extract of both the plants under study this test was carried. For this 10 ml extract was taken in the test tube, equal volume of 5% gelatin solution was added along with pinch of sodium chloride. White precipitation at the bottom of the test tube indicated the positive results.

2.9. Infra-Red Spectroscopy

The extracts were evaporated and oily semisolid powder residue was used to study IR to detect the functional group. The analysis was carried out at the Department of chemistry, University of Mumbai, using Fourier Transform Infra-red spectroscopy.

3. RESULTS

The antibacterial and antifungal activity of cold ethanol stem extract of *R.apiculata* and *R.mucronata* against human pathogens is represented in Table 1.

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). The result revealed that among the test pathogen *Kl.pneumoniae* and *P.vulgaris* were found to resistant to stem extracts of *R.mucronata* and *R. apicula*ta (Table 1).

Minimum inhibitory concentration (MIC) study shows the inhibition at 0.5 mg/ml in stem extract of *R.mucronata* while 1mg/ml in extracts of *R. apiculata* against the pathogen under study (Table 2). Activity guided fractionation study of both the plants confirms that highly potent extract when subjected to fractionation it exhibits that the bioactive compounds were present in aqueous (Fraction 'b') and ethanol (fraction 'c') while Pet-ether (Fraction 'a') was not found to contain any bioactive principles as it exhibited no lethal activity against the test Pathogen (Table 3)

Infra-Red spectroscopy studies on fraction 'b' and 'c' of stem shows the presence of phenolic, polyphenolic, keto and

carbonyl functional groups [15]. Fraction 'c' of *R.mucronata* along with fraction b and c of *R.apiculata* also shows the presence of amino (-NH) functional groups. Gelatin precipitation test of all the potent ethanol extract indicates the presence of tannins in both the plants under study.

Table 1: Bioassay of cold ethanol extracts on test micro organisms (inhibition zones in mm)

R.mucronata	*E.coli*	*S.aureus*	*S.typhi*	*Str.pyogen*	*Ps.aerug*	*Kl.pneum*	*P.vulg*	*C.alba*
Stem	16	15	20	12	15	-	-	-
R.apiculata								
Stem	20	17	19	18	-	-	-	18

All the experiments were carried out in triplicate by agar cup method.

Table2: MIC (Minimum inhibitory concentrations) results of ethanol cold stem extracts

	R.mucronata stem Conc. in mg/ml				*R.apiculata* stem Conc. in mg/ml			
Microbes	10	5	1	0.5	10	5	1	0.5
E.coli	17	15	13	12	17	15	-	-
S.aureus	16	14	13	10	16	14	13	-
S.typhi	19	17	15	12	16	12	-	-
Sr.pyogen	-	-	-	-	-	-	-	-
P.aerug.	13D	11D	9D	-	-	-	-	-
C.albicans	15	12	-	-	17	14	10	-

All the experiment was carried out in triplicate by agar cup method.

Table 3: Fractionation results of cold ethanol stem extract of R.apiculata

Test culture	Fraction a	Fraction b	Fraction c	Parts
S.aureus	-	13	16	Stem
C.albicans	-	13	12	
Fractionation results of cold ethanol stem extract of *R.mucronata*				
Test culture	Fraction a	Fraction b	Fraction c	Parts
S.aureus	-	12	14	Stem
S.typhi	-	13	-	
Str.pyogens	-	13	15	
C.albicans	-	-	-	

All the experiment were carried out in triplicate by agar cup method

Potent fractions of both the plants were subjected to HPTLC analysis produced several bands with different R_f values in each track. After chromatogram was sprayed with 10%KOH under fluorescent spectra (366 nm) prominant blue and yellow bands were seen which indicates the presence of coumarin and anthrone respectively [16].The fraction 'c' of extract when derivatised with Vanillin sulphuric acid shows the presence of essential oils.

4. DISCUSSION

As antibiotics are increasingly used and misused, the bacterial strains become resistant to antibiotic rapidly. Therefore, screening of antimicrobial activities of medicinal plants has been used for centuries as remedies for human diseases.

On the basis of result obtained in the present investigation, we conclude that the ethanol extract of *Rhizophora apiculata* and *Rhizophora mucronata* had significant *in vitro* antimicrobial activity. The use of phytochemical with known bioactive properties can be of great significance for therapeutic purpose. Both the plant species showed wide range of antibacterial constituents in ethanol stem extract against human pathogen under study. Comparatively, *R.apiculata* exhibited antibacterial as well as antifungal principles while *R.mucronata* could exhibit only antibacterial properties. Similarly a result also reveals that *Kl. pneumoniae* and *P.vulgaris* were found to be resistant to both the plant extracts. Minimum inhibitory concentration (MIC) study and activity guided fractionation study displayed the activity of different potential against *E.coli, S. aureus, S.typhi* by *R.mucronata* by inhibiting at lowest concentration of 0.5 mg/ml while that of *R.apiculata* was upto 1mg/ml against *S.aureus* and *C.albicans* .

The order of potency observed as fraction b> fraction c> fraction a.

Similar results are noted in various other mangroves occurring along Mumbai [11-14, 17]. 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.

Therefore it is suggested that major bioactive constituents in *R.mucronata* and *R.apiculata* stem extract may be tannins, coumalin, anthrone and essential oils.

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