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ANTIBACTERIAL ACTIVITY AND GCMS ANALYSIS OF THE EXTRACT OF LEAVES OF RHIZOPHORA APICULATA (A MANGROVE PLANT)

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ABSTRACT

The antibacterial screening of hexane, chloroform and alcohol extracts of leaves of Rhizophora apiculata was carried out against pathogenic bacteria viz., Proteus mirabilis, Klebsiella pneumoniae, Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia Coli, Enterococcus faecalis and Proteus Sp. by disc-assay method. The hexane and chloroform extracts were found to be active against four and five pathogens, respectively. Chromatographic purification of active extracts improved the activity profiles. The GCMS analysis of extracts analysis revealed the presence of triterpenes and hydrocarbons as major constituents. The fatty acid composition of the leaf is also studied by FAlv1E analysis. It contains palmitic acid as a major constituent (54.65%). Two important poly unsaturated fatty acids i.e., Linoleic acid (1.54%) and 9,11–Octadecadienoic acid (3.25%) are also present.

KEYWORDS: Antibacterial activity, Rhizophora apiculata, FA1v1E, hydrocarbons, mangrove plants, triterpenes.

INTRODUCTION

Latex bearing plants are found in some 20 botanical families. The most prominent families include Sapotaceae, Moraceae, Compositae, Apocynaceae, Asclepiadaceae Rhizophoraceae and Euphorbiaceae. In total there are about 18,000 species of latex-producing plants. These are divided into three categories i.e., rubber latex plant, petro corp plant and latex plant

showing bioactivity. The plant under investigation is in the third category of latex plant showing antimicrobial activity against pathogens.

Latex bearing plants were found to show

Antihelmintic activity (Amorin et al., 1999), anti• inflammatory activity (Kumar and basu, 1994) and other medicinal activities (Bhatt et al., 2002). Latex-bearing plants are the renewable sources of energy and chemicals (Kalila el al., 2004).

Rhizophora apiculata Tall-Stilt Mangrove is a tree 20-30 m tall. Leaves are narrowly-elliptic, almost eye-shaped, 8-15 cm long with a common name bakhaw lalaki and belongs to the family Rhizophoraceae. It is a mangrove plant found in the tidal flats in India, Burma and Malay, Mangroves have been a source of several bioactive compounds. Mangrove plants have been used in folklore medicines and extracts from mangrove species have proven activity against human, animal and plant pathogens. Secondary metabolites like alkaloids, phenolics, steroids, terpenoids have been characterized from mangroves and have toxicological, pharmacological and ecological importance (Bandaranayake, 2002; Kokpal et al., 1990).

Taking into account the ever increasing interest of research in the field of natural products we evaluated the efficacy of mangroves and marine algae (Choudhury et al., 2005) for their antibacterial activity against pathogenic bacteria. This paper presents the antimicrobial activity of Rhizophora apiculata against pathogens.

MATERIALS AND METHODS

Plant material: Rhizophora apiculata is collected from Pichavaram bachwaters Mangrove Forest near Chidambaram.

Extraction of plant material: The leaves of the plant (2 kg) were cut, shade dried, powdered and extraction was carried out with different solvents (1 :2 vol./vol., thrice) in the increasing order of polarity like; hexane, chloroform, ethyl acetate and alcohol sequentially by soaking overnight at ambient temperature. The extracts were freed from solvent under reduced pressure. The residue thus obtained are finally dried under vacuum and used for in vitro screening of antibiotic activity.

Antibiotic activity testing of extracts/fractions /compounds: The antibacterial assay of extracts (500 µg/6 mm disc) were carried out against pathogenic bacteria viz., Proteus mirabilis, Klebsiella pneumoniae, Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia Coli, Enterococcus faecalis and Proteus Sp. by disc-assay method (Acar, 1980).

Briefly, each extract, 500 µg/50 µL of appropriate solvent was applied to sterile filter paper discs (6 mm in diameter). After solvent evaporation the discs were placed on nutrient Agar (Himedia, India) test plates inoculated with the overnight culture of the test pathogen (IO' cfu mL) in Brain Heart Infusion (BHI) broth. The plates were incubated for 48 at 37°C. Discs loaded with respective solvent (50 µL) used for dissolution were taken as control after evaporation of the solvent. The zone of inhibition around the disc (average of three experiments) was measured.

Among all extracts, the chloroform extract was found highly active against all except E. coli. So, this extract was further taken up for fractionation, for further screening and isolations of secondary metabolites.

Fractionation of chloroform extract and isolation of pure compounds: The active crude chloroform extract was chromatographed on a column packed with silica gel (100-200 Mesh) and the fractions were mointored by Thin Layer Chromatography (TLC). The antibacterial screening of column fractions of chloroform extract (200 µg/20 µL/6 mm disc) was carried out by the same method as above.

The active column fractions were again rechromatographed to find out the sub fractions which contain maximum purity with major components. These sub fractions are again taken to study the antibacterial activity at 200 µg mL --1.

GC and GCMS analysis of chloroform, hexane extracts and FAME of lipids: The powdered material of dry leaves (10 g) was homogenized and successively extracted three times with chloroform-methanol(2:l,v/v) to isolate lipids (Christie, 1982). Crude lipid extracts were purified by folch wash to remove non lipid contaminants (Folch et al., 1957). The chloroform phase was separated from the combined extract, dried over anhydrous sodium sulphate and concentrated under nitrogen atmosphere. The total lipid (IO mg) were dissolved in 4 mL of 5% hydrochloric acid in methanol and 0.5 mL benzene and then the mixture was refluxed in a

silicone bath at 80-100°C for 2 h. After cooling, the methyl esters were extracted with petroleum ether, simultaneously neutralized and dried over sodium sulphate-sodium bicarbonate mixture.

The solvent was evaporated to dryness at reduced pressure at 40°C in a water bath. These fatty acid methyl esters were analyzed by GC and GCMS for identification.

The hexane extract, chloroform extract and FAlvIE were analyzed on a Shimadzu GC-1 7 A gas chromatographed equipped with FID and a 25 mx0.25 mm, 0.25 µm film thickness, WOT column coated with 5% diphenyl dimethyl siloxane, supplied by J and W (DB-5). Helium was used as the carrier gas at a flow rate of 1.2 mL min, at a column pressure of 42 Kpa. Component separation was achieved following a linear temperature program (120-300°C at 2°C min_, for 90 min), with a total run time of 120 min. The percentage composition was expressed as mean value of three experiments. The samples were then analyzed on a Shimadzu QP-5000 GCMS fitted with the same column and following the same temperature program as above, using 70 eV ionization voltage (EI). Peak identification was carried out by comparison of the mass spectra with those available in the NIST and WILEY libraries.

RESULTS AND DISCUSSION

Results of the screening showed that the chloroform extract and hexane extract exhibited strong antibacterial activity against the pathogens (Table I). The phytochemical screening of this extract showed positive results towards steroids and terpenoids. So, the antimicrobial activity is due to any of these components. The antibacterial screening of column fractions of chloroform extract (200 µg/20 µL/6 mm disc) was carried out by the same method as above is given in Table 2.

The active column fractions were again rechromatographed to find out the sub fractions which contain maximum purity. These sub fractions are again taken to study the antibacterial activity at 200 µg mL -i and results are also presented in Table 2.

The results of present study showed that the chloroform extract of leaf has shown strong antibacterial activity against pathogens compare to other organic extracts. Literature reveals that the hexane and chloroform extract of the plants are found to show strong antimicrobial

activity (Kunle et al., 2003; Katerer et al., 2005; Elzaawely et al., 2005), anti-inflammatory activity (Ebrahirnzadeh el al., 2006) etc.

Table 1: Antibacterial activity screening of leaf extracts of *Rhizophora apiculata* leaves (Zone of inhibition in mm including 6 mm disc).

Extracts	*P. Mirabilis*	*K.pneumoniae*	*P. Aeruginosa*	*S. Aureus*	*E Coli*	*E. Faecalis*	*Proteus* Sp
Ethy I acetate	-ve	-ve	-ve	-ve	Trace	-ve	-ve
Hexane	Trace	**7**	**7**	Trace	Trace	-ve	-ve
Ethanol	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Chloroform	12	7	-ve	**13**	**13**	**13**	-ve
- =no zone							

Table 2: Antibacterial activity screening results of column fractions/subfractions of chloroform extract of leaves of *Rhizophora apiculata* leaves. (Zone of inhibition in mm includin 6mm disc.

Sam2le	*P.mirabilis,*	*K.pneumoniae,*	*P.aeruginosa,*	*S.aureus,*	*E Coli,*	*E.faecalis*	*Proteus* Sp
FR l(F.tkH-5'9.5)	+v	-ve	-ve	-ve	+ve, 7	+ve, 13	-ve
SFR-1.1	-ve	-ve	+ve, 6.5	-ve	-ve	+ve,7	-ve
SFR-1.2	-ve	-ve	+ve, 7	-ve	-ve	+ve,7	-ve
FR 2(Et0Ac:H-1:9)	-ve	-ve	-ve	-ve	-ve	-ve	-ve
FR 3(F.tkH-1S85)	-ve	-ve	-ve	-ve	-ve	-ve	-ve
FR 4(F.tkH-2,8)	-ve	+ve	-ve	-ve	-ve	+ve	+ve
SFR-4.1	-ve	-ve	-ve	-ve	-ve	+ve	+ve

EtA. = Ethyl acetate; H = Hexane; (-) =No zone, FR= Fraction, SFR = Sub fraction.

Table 3: GCMS analysis of chloroform extract of leaf.

GCMSRT	Com2ound	Total (%)
33.933	Tetracosanoic acid methyl ester	0.80
37.308	1,2-Benzene dicarboxylic acid	0.02
	Dibutyl ester	
39.425	Palmitic acid	1.57
51.142	Hexadecene	0.21
62.03	2-butyl-1-0ctanol	2.81
63.825	Isooctyl vinyl ester	1.43
65.683	2-ethy 1-1-decanol	864
66.692	1, 1-Heptanedi ol,diacetate	0.26
67.500	Pentacosane	1.44
69.858	Hexadecane	3.17
75.958	4,4,6a,6b,8a,l l ,l l ,14b-	
	Octamethyl-1,4,4a,5,6,6a,6b, 7,8, 8a,9,l O,l l,12,12a, 14, 14a,l 4b-octadecahydro-2H-picen-3-one	4.47
78.142	Urs-12-ene	27.44
78.358	Perhy drocycl opropa[e] azulene-4,5, 6-tri ol, 1, 1,4, 6-tetramethy I	15.79
78.550	Sesguilavandulyl acetate	4.45

RT- Retention time.

Table 4: GCMS analysis of hexane extract of leaf.

GCMS RT	Com2ound	Total (%)
28.08	Phytol acetate	0.84
30.868	Myristaldehyde	0.33
31.858	1,2-benzene dicarboxylic acid, bis(2-methyl propyl ester)	1.17
32.808	2-[l,1-Dimethy l]-5-oxohexanol	1.40
33.440	Hexadecanoic acid, methyl ester	0.05
33.442	Pentadecanoic acid,14-methyl-, Methyl ester	0.07
36.192	1-Tetradecene	0.05
38.500	Laurie acid	0.27
45.833	Stearic acid	0.18
55.692	1,2-Benzenedicarboxylic acid, dioctyl ester	10.22
61.542	Sesquilavandulyl acetate	1.25
61.717	2,6,1 O-Dodecatrien- l ,al,12-(acetoxy)- 2, 6, 1 0-trimethy l,(E,E,E)	1.65
62.758	Longibomeol acetate	2.51
63.450	13-Docosenoic acid, methyl ester	1.40
65.308	n-pentadecane	2.62
63.500	2, 7-Dimethyl-1-octanol	5.38
65.383	pentacosane	0.68
66.092	1-Hexene,3, 5,5-trimethy I	0.94
67.142	4-methyl-1-undecene	0.69
73.158	Hexadecatetraen-1-ol, 3, 7,11,15-tetramethyl-, Acetate,(E,E,E)	0.24
75.417	4,4,6a,6b,8a,l l ,l l ,14b• Octamethy 1-1, 4,4a,5, 6, 6a, 6b, 7,8,8a,9,l 0,11, 12, 12a,l 4,14a,14b• Octadecahydro-2H-picen-3-one	2.45
77.381	Eudesmol acetate	3.87
78.063	Urs-12-ene	9.15

RT- Retention time.

Table 5: GCMS analysis of FA11E of Folch extract.

GCMSRT	Compound	Total(%)
4.04	Octanedioic acid, dimethyl ester	0.63
5.25	Nonanedioic acid, dimethyl ester	3.95
7.75	Tetradecanoic acid/ Myristic acid	1.51
10.99	Hexadecanoic acid/ palmitic acid	54.65
12.62	Hexadecanoic acid, 15-methyl	0.56
0.30	Octadecanoic acid/ Stearic acid	0.94
13.48	9,12-0ctadecadienoic acid/ linoleic acid	1.45
13.57	8-0ctadecenoic acid	3.19
13.96	Heptadecanoic acid, 16-methyl	6.83
14.87	9,11-0ctadecadienoic acid	3.25
16.81	Eicosanoic acid/ Arachidic acid	2.56
19.49	Docosanoic acid Behenic acid	4.65
20.73	Tricosanoic acid	1.01
21.98	Tetracosanoic acid/ Lignoceric acid	6.32
24.28	Hexacosanoic acid/ cerotic acid	1.64
28.85	Triacontanoic acid/ Melissic acid	2.94

RT- Retention time.

The GCMS analysis of the chloroform extract, hexane extract and F AlvIE of Folch extract are given in the Table 3-5, respectively. Hexane extract is found to contain Saturated hydrocarbons, unsaturated hydrocarbons (3.33%), sesquiterpenes (3.76%), triterpenes (10%). Chloroform extract is found to contain mostly saturated hydrocarbons, unsaturated hydrocarbons, alcohols (10%), sesquiterpenes (4.45%), triterpenes (30%).

Longibomeol acetate, sesquilavandulyl acetate which are also found in essential oils (Asekun et al., 2003) are present in hexane and chloroform extract. Triterpenes are found in latex and resins of some plants and physiological function of these compounds is generally believed to be a chemical defense against certain pathogens causing human and animal diseases.

The widespread reports in recent years on useful biological activities of tritepenes, indicate their potential. Triterpenes are found to show antitumor, anticancer, antiviral, antimicrobial, anti-inflammatory activity (Mahato et al., 1997). In the present investigation hexane and chloroform extracts of Rhizophora apiculata were found to contain triterpene hydrocarbon, urs-12 ene (>30 and 10%, respectively). Ursolic acid was reported to be cytotoxic against A-549, L-1210 and KB tumour cells (Yamagishi et al., 1988). 23-Hydroxy-3-oxo-urs-l 2-en- 28-oic acid was found to exhibit anti-ulcer properties (Fourie et al., 1989). So, the antimicrobial activity of present study may be due to terpenes and sesquiterpenes (Bryon and Eric, 2003). The fatty acids composition of the leaf is also studied by FAME analysis. It mainly contains palmitic acid as major constituent (54.65%). Two important poly unsaturated fatty acids i.e., Linoleic acid (w-6, 1.54%) and 9, 11-0ctadecadienoic acid (3.25%) are also present along with arachidic acid (2.56%).

The non polar extracts of mangroves have shown antibacterial activity against pathogens earlier (Choudhwy et al., 2005).

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