



Asian Journal of Plant Sciences

ISSN 1682-3974

science
alert

ANSI*net*
an open access publisher
<http://ansinet.com>

Antibacterial Activity and GCMS Analysis of the Extract of Leaves of *Finlaysonia obovata* (A Mangrove Plant)

Pravat Manjari Mishra and A. Sree

Department of Natural Product, Regional Research Laboratory, Bhubaneswar-751013, India

Abstract: The antibacterial screening of hexane, chloroform and alcohol extracts of leaves of *Finlaysonia obovata* was carried out against fresh water fish pathogenic bacteria viz., *Aeromonas hydrophila*, *Pseudomonas aeruginosa*, *Vibrio alginolyticus*, *Staphylococcus aureus*, *Escherichia Coli*, *Edwardsiella tarda* and *Micrococcus* Sp. (multidrug resistant strain) by disc-assay method. The hexane and chloroform extracts were found 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 acids composition of the leaf is also studied by FAME 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.

Key words: Antibacterial, *finlaysonia obovata*, FAME, fish pathogen, hydrocarbons, mangrove, sesquiterpene, triterpene

INTRODUCTION

Latex bearing plants are found in some 20 botanical families. The most prominent families include Sapotaceae, Moraceae, Compositae, Apocynaceae, Asclepiadaceae 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 fish pathogens.

Latex bearing plants were found to show Anthelmintic 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 (Kalita *et al.*, 2004).

Finlaysonia obovata is a lofty climber with stout branches and obovate or oblong leaves with a common name Lata Rai and belongs to the family Periplocaceae. It is a mangrove plant exuding white latex and found in the tidal flats in India, Burma and Malay, the leaves of which are reported to be eaten as salad in Moluccas.

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 some fish pathogenic bacteria. This paper presents the antimicrobial activity of *F. obovata* against fish pathogens.

MATERIALS AND METHODS

Plant material: *F. obovata* is collected from Bhitarkanika area of Orissa (during late winter season) and was identified by Mr. K.S. Murthy, I/C SMP unit, Central Research Unit (AY), Bhubaneswar.

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 seven fresh water fish pathogenic bacteria viz., *Micrococcus* sp. (multidrug resistant strain), *Aeromonas hydrophila*, *Pseudomonas aeruginosa*, *Vibrio alginolyticus*, *Staphylococcus aureus*, *Escherichia coli*, *Edwardsiella tarda* by disc-assay method (Acar, 1980). The test bacterial pathogen cultures were obtained from the stock cultures maintained in the Pathology Laboratory of Central Institute of Fresh Water Aquaculture, ICAR, Bhubaneswar (Vimala *et al.*, 2000).

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 (10^6 cfu mL⁻¹) in Brain Heart Infusion (BHI) broth. The plates were incubated for 48 h 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* and *E. tarda*. So, this extract was further taken up for fractionation, 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 monitored 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(2:1,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 (10 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 FAME were analyzed on a Shimadzu GC-17A gas chromatographed equipped with FID and a 25 m×0.25 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⁻¹ 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 fish pathogens (Table 1). 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⁻¹ 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 fish 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 (Ebrahimzadeh *et al.*, 2006) etc.

The GCMS analysis of the chloroform extract, hexane extract and FAME of Folch extract are given in the Table 3-5, respectively. Hexane extract is found to contain

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

Extracts	<i>Micrococcus</i>	<i>A. hydrophila</i>	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>V. alginolyticus</i>	<i>S. aureus</i>	<i>E. tarda</i>
Ethyl 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 *F. obovata* leaves. (Zone of inhibition in mm including 6 mm disc)

Sample	<i>Micrococcus</i> sp.	<i>A. hydrophila</i>	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>V. alginolyticus</i>	<i>S. aureus</i>	<i>E. tarda</i>
FR 1(EtA:H-5:9.5)	+ve	-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(EtOAc:H-1:9)	-ve	-ve	-ve	-ve	-ve	-ve	-ve
FR 3(EtA:H-1.5:8.5)	-ve	-ve	-ve	-ve	-ve	-ve	-ve
FR 4(EtA:H-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

GCMS RT	Compound	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-Octanol	2.81
63.825	Isooctyl vinyl ester	1.43
65.683	2-ethyl-1-decanol	8.64
66.692	1,1-Heptanediol, diacetate	0.26
67.500	Pentacosane	1.44
69.858	Hexadecane	3.17
75.958	4,4,6a,6b,8a,11,11,14b-Octamethyl-1,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,14,14a,14b-octadecahydro-2H-picen-3-one	4.47
78.142	Urs-12-ene	27.44
78.358	Perhydrocyclopropa[e]azulene-4,5,6-triol,1,1,4,6-tetramethyl	15.79
78.550	Sesquilandulyl acetate	4.45

RT- Retention time

saturated hydrocarbons, unsaturated hydrocarbons (3.33%), sesquiterpens (3.76%), triterpenes (10%). Chloroform extract is found to contain mostly saturated hydrocarbons, unsaturated hydrocarbons, alcohols (10%), sesquiterpens (4.45%), triterpenes (30%). Longiborneol acetate, sesquilandulyl 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 triterpenes, 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 *F. obovata* were found to contain triterpene hydrocarbon, urs-12 ene

Table 4: GCMS analysis of hexane extract of leaf

GCMS RT	Compound	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-[1,1-Dimethyl]-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	Lauric acid	0.27
45.833	Stearic acid	0.18
55.692	1,2-Benzenedicarboxylic acid, dioctyl ester	10.22
61.542	Sesquilandulyl acetate	1.25
61.717	2,6,10-Dodecatrien-1,al,12-(acetoxy)-2,6,10-trimethyl,(E,E,E,)	1.65
62.758	Longiborneol acetate	2.51
63.450	13-Docosenoic acid, methyl ester	1.40
65.308	n-pentadecane	2.62
65.500	2,7-Dimethyl-1-octanol	5.38
65.383	pentacosane	0.68
66.092	1-Hexene,3,5,5-trimethyl	0.94
67.142	4-methyl-1-undecene	0.69
69.375	2-methyl-octadecane	3.03
73.158	Hexadecatetraen-1-ol, 3,7,11,15-tetramethyl-, Acetate,(E,E,E)	0.24
75.417	4,4,6a,6b,8a,11,11,14b-Octamethyl-1,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,14,14a,14b-Octadecahydro-2H-picen-3-one	2.45
77.381	Eudesmol acetate	3.87
78.063	Urs-12-ene	9.15

RT- Retention time

(>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-12-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

Table 5: GCMS analysis of FAME of Folch extract

GCMS RT	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-Octadecadienoic acid/ linoleic acid	1.45
13.57	8-Octadecenoic acid	3.19
13.96	Heptadecanoic acid, 16-methyl	6.83
14.87	9,11-Octadecadienoic 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	Triacosanoic acid/ Melissic acid	2.94

RT- Retention time

contains palmitic acid as major constituent (54.65%). Two important poly unsaturated fatty acids i.e., Linoleic acid (ω -6, 1.54%) and 9,11-Octadecadienoic acid (3.25%) are also present along with arachidic acid (2.56%).

The non polar extracts of mangroves have shown antibacterial activity against fish pathogens earlier (Choudhury *et al.*, 2005). The present study provides enough data to show the potential of mangrove extracts for the development of anti-pathogenic agents against aquaculture pathogens.

ACKNOWLEDGMENTS

The authors thank Director, RRL, Bhubaneswar for facilities, Mr. K.S. Murthy, I/C SMP Unit, Central Research Institute (AY), Bhubaneswar for identification of the plant. One of us (PMM) thanks DOD for research fellowship. We express our thanks to Dr. Subhashree Choudhury for helping in antibacterial screening.

REFERENCES

- Acar, J.F., 1980. The Disc Susceptibility Test: Antibiotic in Laboratory Medicine. Lorian, V. (Ed.), Williams and Wilkins: Baltimore, London, 24-54.
- Amorin, Alziro de, R.B. Helcio, P.P. Jorge, Carauta, Daise Lopes, Maria A.C. Kaplan, 1999. Anthelmintic activity of the latex of *Fiscus* species. J. Ethnopharmacol., 64: 255-258.
- Asekun, O.T. and A.B. Adeniyi, 2003. The leaf essential oil of *Costus afer* Ker-Gawl from Nigeria. Flavour and Fragrance J., 18: 309-311.
- Bandaranayake, W.M., 2002. Bioactivities, bioactive compounds and chemical constituents of mangrove plants. Wetland Ecol. Manage., 10: 421-452.
- Bhatt, D.C., K.D. Mitaliya, U.S. Baxi and M.M. Prajapati, 2002. Medicinal aspects of some latex-bearing plants from Saurashtra. Adv. Plant Sci., 15: 39-41.
- Bryon, F. Brehm-Stecher and A.J. Eric, 2003. Sensitization of *Staphylococcus aureus* and *Escherichia coli* to antibiotics by the sesquiterpenoids Nerolidol, Farnesol, Bisabolol and Apritone. Antimicrob Agents Chemother., 47: 3357-3360.
- Choudhury, S., A. Sree, S.C. Mukherjee, P. Pattnaik and M. Bapuji, 2005. Antibacterial activity of organic extracts of selected marine algae and mangroves against fish pathogens. Asian Fisheries Sci., 18: 287-296.
- Christie, W.W., 1982. In: Lipid analysis. Pergamon Press: Oxford, pp: 22.
- Ebrahimzadeh, M.A., M. Mahmoudi and E. Salimi, 2006. Antiinflammatory activity of *Sambucus ebulus* hexane extracts. Fitoterapia, 77: 146-148.
- Elzaawely, A.A., T.D. Xuan and S. Tawata, 2005. Antioxidant and antibacterial activities of *Rumex japonicus* HOUTT. Aerial parts. Biol. Pharm. Bull., 28: 2225-2230.
- Folch, J., M. Lees and G.H.S. Stanely, 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem., 226: 497-509.
- Fourie, T.G., E. Matthee and F.O. Snyckers, 1989. Pentacyclic triterpene acid, with anti-ulcer properties, from *Cussonia natalensis*. Phytochemistry, 28: 2851-2852.
- Kalita, D. and C.N. Saikia, 2004. Chemical constituents and energy content of some latex bearing plants. Bioresour. Technol., 92: 19-22.
- Katerer, D.R. and J.N. Eloff, 2005. Antibacterial and antioxidant activity of *Sutherlandia frutescens* (Fabaceae), a reputed ant-HIV/AIDS phytochemistry. Phytother. Res., 19: 779-81.
- Kokpal, V., D.H. Miles, A.M. Payne and V. Chittawong, 1990. Chemical constituents and bioactive compounds from mangrove plants. Studies in Natural Products Chem., 7: 175-199.
- Kumar, V.L. and N. Basu, 1994. Anti-inflammatory activity of the latex of *Calotropis procera*. J. Ethnopharmacol., 44: 123-125.
- Kunle, O., J. Okogun, E. Egamana, E. Emojevwe and M. Shok, 2003. Antimicrobial activity of various extracts and carvacrol from *Lippia multiflora* leaf extract. Phytomedicine, 10: 59-61.

- Mahato, S.B. and S. Sen, 1997. Advances in triterpenoid research, 1990-1994. *Phytochemistry*, 44: 1185-1236.
- Vimala, A., S. Mishra, A. Sree, M. Bapuji, P. Pattnaik, S.C. Mukherjee and C.C. Rath, 2000. A note on *in vitro* antibacterial activity of bacterial associates of marine sponges against common fish pathogens. *J. Aquacul.*, 8: 61-65.
- Yamagishi, T., D.C. Zhang, J.J. Chang, D.R. Mcphail, A.T. Mcphail and K.H. Lee, 1988. The Cytotoxic principles of *Hyptis capitata* and the structures of the new triterpenes hyptatic acid-A and-B. *Phytochemistry*, 27: 3213-3216.