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Fatty Acid Profile, Volatiles and Antibacterial Screening of Lipids of The Sponge *Siphonodictyon coralliphagum* Collected From The Bay of Bengal (Orissa Coast)

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The fatty acid composition as well as volatiles of total lipids in the marine sponge *Siphonodictyon coralliphagum* was analyzed. Most abundant are saturated fatty acid among which the % of docosanoic acid is found to be 15.49. Branched saturated fatty acids are also present in a good amount (23.45 %) and octadecanoic acid, 17 methyl-constitutes 11.62 % of it's total content. Monounsaturated fatty acids constitute only 5.53 % of the total fatty acid. Among poly unsaturated fatty acids, 5,9-hexacosa-dienoic acid is dominant (8.27 %). The lipid extract showed activity against *Escherichia coli* (A fish pathogen).

Key Words: Sponges, *Siphonodictyon coralliphagum*, Fatty acid, Volatiles, Antibacterial.

INTRODUCTION

Siphonodictidine is the major secondary metabolites from *S. coralliphagum* are responsible for maintaining zones of dead corals polyps around the oscular chimneys of these sponges[1]. Compound *bis*(sulfto)-cyclosiphonodictyol A, which inhibits the binding of [3H]-LTB4 to intact human neutrophils with an IC_{50} value of 44 μM was isolated from this sponge[2].

The antimicrobial activity of the sponge *S. coralliphagum* was already reported[3]. Siphonodictyal-A and siphonodictyal-B, isolated from *S. coralliphagum* exhibited antimicrobial activity.

Some unusual fatty acids of sponges exhibit biological activity[4,5]. The biologically active substances are probably involved into protective mechanisms of these animals and defend sponges from predators[6]. It is believed that short fatty acids of symbiotic microorganism can participate in the biosynthesis of some long-chain fatty acids in sponge cells. Complete and correct information on the lipid and fatty acid composition of sponges is necessary for the elucidation of biosynthetic pathways and functional role of fatty acids in these archaic organisms. So, it is interesting to find out the fatty acid composition of the total lipid of the bioactive sponge *S. coralliphagum*.

EXPERIMENTAL

Sponge specimen *Siphonodictyon coralliphagum* (Class Demospongiae Sollas, order Haplosclerida Topsent, family Adociidae de Laubenfels) collected during February-March 2006 from 13 m depth of the Bay of Bengal of Orissa coast, were stored in alcohol and transported to the laboratory. The sample was identified upto genus level by Dr. P.A. Thomas, Ex-Emeritus Scientist (ICAR), Trivandrum, Kerala.

Extraction: The sponge sample was thoroughly washed and air-dried. The sponge sample (10 g) was homogenized and successively extracted three times with chloroform-methanol (2:1, v/v) to isolate lipids[7]. Crude lipid extracts were purified by folch wash[8] to remove non-lipid contaminants. The chloroform phase was separated from combined extract, dried over anhydrous sodium sulphate and concentrated under nitrogen atmosphere.

Preparation of fatty acid methyl esters: The fatty acids so obtained were converted to corresponding methyl esters. Fatty acid (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 ºC 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 ºC in a water bath. These fatty acid methyl esters (FAME) were then analyzed by GCMS for identification.

Fatty acid methyl esters (FAME) analysis: Fatty acid methyl esters analyses were performed on a Shimadzu QP-5000 GC-MS equipped with FID and a 25 m × 0.25 mm, 0.25 µm film thickness, WCOT column coated with 5 % diphenyl siloxane, supplied by J & 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. The column temperature was programmed for fatty acid methyl esters (FAMEs) from 120-300 ºC at 2 ºC/min, 300 ºC for 10 min, with total run time of 100 min 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.

Isolation and analysis of the volatile compounds: Part of the lipophilic extract (100 mg) was subjected to a 4 h distillation-extraction in a Lickens-Nickerson apparatus[9]. Volatiles were extracted from the distillate with diethyl ether (yield: 3 mg) and investigated by Shimadzu QP-5000 GC-MS with a 25 m × 0.25 mm, 0.25 µm film thickness, WCOT column coated with 5 % diphenyl siloxane, supplied by J & 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. The column temperature was programmed from 40 to 280 ºC at a rate of 4 ºC/min using 70 eV ionization voltage (EI).

Antibiotic activity testing of lipid extract of *Siphonodictyon coralliphagum*: The antibacterial assay of lipid extract of *S. coralliphagum* collected from 13 m depth (200 µg /6 mm disc) was carried out against 5 fish pathogens (*Edwardsiella tarda, Staphylococcus aureus, Micrococcus* sp., *Pseudomonas aeruginosa* and *E. coli*), 2 human pathogens (*S. aureus* and *S. typhii*) including 3 MDR (Multi drug resistant) strains (*S. pyogenes, Acinetobacter* sp. and *S. typhii*) by disc-assay method[10].

The test bacterial fish pathogen cultures were obtained from the stock cultures maintained in the Pathology Laboratory of Central Institute of Fresh Water Aquaculture, ICAR, Bhubaneswar.

RESULTS AND DISCUSSION

The total fatty acid methyl esters obtained by saponification followed by methylation of the lipids were analyzed by GC/MS. Present GCMS analysis showed the presence of 33 components in the fatty acid mixture of total lipid (Table-1). The content of linear saturated fatty acid is more than 50 % of the total fatty acid content. All series of saturated fatty acids of linear structure from C14 upto C32 are present in the lipid composition of sponges. Widespread acids are, C14:0, C15:0, C16:0, C17:0, C18:0, C19:0, C20:0 among which C16:0 and C18:0 dominated in most of the sponges. All the fatty acids from C11:0 to C26:0 were found in *S. coralliphagum* except C13:0. Generally the % of C16:0 and C18:0 in sponges are found to be 1.5-33.0, whereas the sp. under investigation contains 5.07 % of C16:0 and 4.82 % of C18:0. At the same time in *S. coralliphagum*, the main saturated fatty was C22:0 (15.49 %) which is a notable observation. Whereas there is a sp. *C. aprica* in which the main saturated acid was C24:0 (16.7%)[11]. The content of C24:0 and C25:0 were found to be 7.55 and 6.09 %, respectively.

In sponges branched saturated iso- and anteiso-acids with a total number of carbon atoms from C14 to C29 were found, among which C15-C20 acids were the most widespread. The ratio of those acids can reach 40-50 % of the total content[12]. The *S. coralliphagum* under investigation contains 16.94 % of those acids of the total fatty acid content. i-C13:0, i-C15:0, ai-C16:0, i-C17:0, i-C18:0 were found among which i-C18:0 dominated (11.62 %).

It is considered that saturated iso and anteiso C15-C20 acids have a bacterial origin[13,14]. The ratio of iso and anteiso FA was much more in some sponges, a significant part of whose biomass was compounded by bacterial symbionts. Thus in sponge *S. coralliphagum* the content of iso and anteiso may be due to symbiotic bacteria.

Lipids of some sponges included significant number of saturated fatty acids with midbranching of their carbon chain[15]. 9-Me-C14:0 and 14-Me-C17:0 were found in *S. coralliphagum* whose % of composition were found to be 2.70 and 0.50, respectively.

Polymethyl branched saturated fatty acids of sponges are presented by usual isoprenoid fatty acids, 4,8,12-trimethyltridenane, phytanic acid and pristanic acids whose content varies from 0.5 to 20 % of the fatty acid total. The *S. coralliphagum* contains only one isoprenoid fatty acid *i.e.*, phytanic acid (0.55%). These isoprenoid fatty acids are the structural components of plasma membranes of sponge cells.

The % of composition of monoenes vary from 2 upto 50 of the fatty acid total content in various sponge species. The content of monoenes in *S. coralliphagum* was found to be 5.53 %. The relative content of C16:1Δ9 (palmitooleic acid) was

TABLE-1
GCMS ANALYSIS OF FAME OF TOTAL LIPID OF *Siphonodictyon coralliphagum*

Retention time	Compound	Total (%)
2.70	Undecanoic acid (C11:0)	0.39
3.90	Dodecanoic acid (C12:0)	0.17
6.13	Tetradecanoic acid (C14:0)	1.02
6.66	Methyl 9-methyl tetradecanoate	2.70
6.68	Tridecanoic acid, 12-methyl	1.19
7.34	Pentadecanoic acid (C15:0)	0.93
7.94	Pentadecanoic acid, 14-methyl	0.37
9.10	Hexadecanoic acid (C16:0)	5.07
9.50	9-Hexadecenoic acid C19:1)	1.18
9.73	Hexadecanoic acid, 14-methyl	4.25
10.39	Heptadecanoic acid (C17:0)	2.07
11.20	Hexadecanoic acid, 3,7,11,15-tetramethyl	0.55
11.30	Cyclopropane octanoic acid, 2-hexyl	0.87
11.51	Heptadecanoic acid, 14-methyl	0.50
11.94	Heptadecanoic acid, 16-methyl	0.61
12.84	Octadecanoic acid	4.82
13.14	7-Octadecenoic acid	1.44
13.29	10-Octadecenoic acid	2.13
13.45	Octadecanoic acid, 17-methyl	11.62
13.85	Nonadecanoic acid	1.60
15.14	Cyclopropane octanoic acid, 2-octyl	0.79
16.53	Eicosanoic acid	2.13
17.07	Heneicosanoic acid	2.86
20.19	Docosanoic acid	15.49
20.64	Tricosanoic acid	3.94
24.47	Tetracosanoic acid	7.55
24.71	15-Tetracosenoic acid	0.78
25.25	Pentacosanoic acid	6.09
28.32	5,9-Pentacosadienoic acid	2.6
31.04	Hexacosanoic acid	1.47
32.49	5,9-Hexacosadienoic acid	8.27
34.91	5,9-Heptacosadienoic acid	3.80

found to be 3-5 % on the average, while in *S. coralliphagum* it's conent was 1.18 %. Generally C18:1Δ9 (oleic acid) and *cis*-vaccenic acid (C18:1Δ11) are the principle isomers of C18 monoene in sponges. But the *S. coralliphagum* contains C18:1Δ7 and C18:1Δ10 whose % of composition were 1.44 and 2.13, respectively. Sponges are specified by a great diversity of monoenic acids with number of carbon atoms more than 22. The presence of C24:1Δ15 was idenitified in this sp. (0.78 %). Polyenoic fatty acids of *S. coralliphagum* are mainly represented by demospongic acids whose major part has a characteristic 5,9-dienoic structural fragment of carbon chain[16]. The total content of linear dienoic fatty acids was 14.67 % of the total fatty acids in *S. coralliphagum* and the C25:2(5,9), C26:2(5,9), C27:2(5,9) non-methylene interrupted fatty acids typical of sponges account for the main of this content among which the % of C26:2 (5,9) was found to be 8.27.

Two cyclopropane acids i.e cyclopropane octanoic acid, 2-hexyl and cyclopropane octanoic acid, 2-octyl were identified from this sponge. The content of cyclopropane acids were found to be 1.66 %, the content of which were usually found to be less than 15 % of the total fatty acid[17,18]. The results of isolated volatiles are presented in Table-2. Volatile compounds often possess valuable biological activities. They serve as allelochemicals defending the organisms from bacteria fungi and viruses.

Analogous to other investigated sponges[19,20], the volatiles in *S. coralliphagum*, appeared to be relatively simple. 1,2-Benzene dicarboxylic acid, *bis*(2-ethylhexyl) was predominated (42.00 % of the total volatile compounds). The % of dihydroxy eicosane was 4.01. The other volatiles were 1-chloro octadecane (5.41 %), 1-dodecene (1.91 %) and tridecene-1-al (6.33 %). Three free fatty acids *viz.*, dodecanoic acid, 2-penten-1-yl ester, hexadecanoic acid and octadecanoic acid were also present (Table-2). The % of composition of dodecanoic acid, 2-penten-1-yl ester was found to be less in amount (0.57). The other two free fatty acids *viz.*, hexadecanoic acid and octadecanoic acid were present in good amount (15.74 and 13.74 %, respectively). Such compounds are often accepted as resulting from hydrolysis during the isolation procedure. These compounds possess some biological activities (antibacterial and insecticidal) that could improve the resistance of the sponge towards pathogens and predators.

TABLE-2
COMPOSITION OF THE VOLATILE COMPOUNDS IN *Siphonodictyon coralliphagum*

Volatile compounds	Composition (%)
1-Dodecene	1.91
Dodecanoic acid, 2-penten-1-yl ester	0.57
Tridecene-1-al	6.33
Cyclopentane undecanoic acid	9.90
Dihydroxy eicosane	4.01
Hexadecanoic acid	15.74
Octadecanoic acid	13.74
1,2-Benzene dicarboxylic acid, *bis*(2-ethylhexyl)	42.00
1-Chloro octadecane	5.41

The antimicrobial activity of the sponge *S. coralliphagum* was already studied by Sullivan *et al.*[3]. The lipid extract of *S. coralliphagum* is taken for antibacterial screening against human pathogens and fish pathogens and it has shown strong activity against *Escherichia coli* (A fish pathogen) with (at 200 µg/6 mm disc) 10 mm zone of inhibition. Antimicrobial activity is exhibited by many lipids of sponges including fatty acids. Hence, the inhibitory activity of the sponge might be partly due to the presence of important fatty acids.

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REFERENCES

1. B. Sullivan, D.J. Faulkner and L. Webb, *Science*, **221**, 1175 (1983).
2. K.B. Killday, A.E. Wright, R.H. Jackson and M.A. Sills, *J. Nat. Prod.*, **58**, 958 (1995).
3. B. Sullivan, P. Djura, D.E. Mcintyre and D.J. Faulkner, *Tetrahedron*, **37**, 979 (1981).
4. N.M. Carballeira, J.E. Betancourt, E.A. Orellano and F.A. Gonzalez, *J. Nat. Prod.*, **65**, 1715 (2002).
5. P. Ciminiello, E. Fattorusso, S. Magno, A. Mangoni, A. Ialenti and M. Dirosa, *Experientia*, **47**, 739 (1991).
6. P. Proksch, *Toxicon*, **32**, 639 (1994).
7. W.W. Christie, In: Lipid Analysis, Pergamon Press, Oxford, p. 22 (1982).
8. J. Folch, M. Lees and G.H.S. Stanely, *J. Biol. Chem.*, **226**, 497 (1957).
9. H. Hendriks, J. Geerts and Th. Malingre, *Pharm. Weekblad, Sci.*, **116**, 1316 (1981).
10. J.F. Acar, in ed.: V. Lorian, The Disc Susceptibility Test: Antibiotic in Laboratory Medicine, Williams and Wilkins, Baltimore, London, p. 24 (1980).
11. N.M. Carballeria, M.E. Maldonado, E. Rivera and B. Porras, *Biochem. Syst. Ecol.*, **17**, 311 (1989).
12. N.M. Carballeira and J. Rodriguez, *Lipids*, **26**, 324 (1991).
13. J. Dalsgaard, M. John, G. Kattner, D. Müller-Navarra and W. Hagen, *Adv. Mar. Biol.*, **46**, 225 (2003).
14. T. Kaneda, *Microb. Rev.*, **55**, 288 (1991).
15. G. Barnathan, E. Genin, N.E. Velosaotsy, J.M. Kornprobst, S. Al-Lihaibi, A. Al-Sofyani and R. Nongonierma, *Comp. Biochem. Physiol. Ser. B*, **135**, 297 (2003).
16. Y. Ando, Y. Kawabata, K. Narukawa and T. Ota, *Japan Fish. Sci.*, **64**, 136 (1998).
17. W.W. Christie, E.Y. Brechany, I.N. Marekov, K.L. Stefanov and S.N. Andreev, *Comp. Boichem. Physiol. Ser. B*, **109**, 245 (1994).
18. F.T. Gillan, I.L. Stoilov, J.E. Thompson, R.W. Hogg, C.R. Wilkinson and C. Djerassi, *Lipids*, **23**, 1139 (1988).
19. S. De Rosa, C. Iodice, J. Nechev, K. Stefanov and S. Popov, *J. Serb. Chem. Soc.*, **68**, 249 (2003).
20. S. De Rosa, S. De Caro, G. Tommonaro, K. Slantchev, K. Stefanov and S. Popov, *Marine Biol.*, **140**, 465 (2002).

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