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Fatty acid profile, volatiles and antibacterial screening of lipids of the sponge *Fasciospongia cavernosa* (Schmidt) collected from the Bay of Bengal (Orissa Coast)

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Abstract: The fatty acid composition as well as the volatiles of a lipophilic extract from the marine sponge *Fasciospongia cavernosa* (Schmidt) was analysed. The fatty acids (FA) were characterized by linear saturated fatty acids (33.05 %), branched saturated fatty acids (9.30 %) and mono-unsaturated fatty acids (18.07 %). A significant amount of polyunsaturated fatty acids (PUFA) (30.79 %) was found in the total lipid, which included linoleic acid (18:2 n–6, 11.14 %), 9,12,15-octadecatrienoic acid/α-linolenic acid (18:3 n–3, 1.99 %), di-homo-γ-linolenic acid (20:3 n–6, 2.03 %) and arachidonic acid (20:4 n–3, 0.51 %). An antibacterial assay of the lipid extract of *F. cavernosa* showed broad-spectrum activity against different human and fish pathogens.

Keywords: sponge; *Fasciospongia cavernosa*; fatty acid; volatiles; antibacterial.

INTRODUCTION

Marine sponges are the most primitive multicellular sedentary animals that produce bioactive metabolites. Among the aquatic animals, sponges are specified by the greatest diversity of fatty acids (FA), which have unusual and sometimes unique structures. This generated the idea that there was something unusual in the structure and/or in the way of functioning of sponge membranes.[1,2] Some lipids of sponges are characterized as biologically active.[3–5]

The sterol composition of the sponge *Fasciospongia cavernosa* was previously studied.[6] Literature evidenced that *F. cavernosa* is a rich source of a new class of sesterterpenoids, named cacospongionolides. Cacospongionolide was the first sesterterpene isolated from the Adriatic Sea sponge *F. cavernosa*, which possesses antimicrobial and antitumor activities.[7] This class of marine metabolites are the inhibitors of phospholipase A2 with a potent topical anti-inflamma-

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tory profile and they show high antimicrobial activity against the Gram-positive bacteria *Bacillus subtilis* and *Micrococcus luteus*.[1,8] Cacospongionolide B isolated from this sponge collected in the Northern Adriatic was reported to have anti-inflammatory activity.[9,10] 25-Deoxycacospongionolide B, a bioactive sesterterpene, was also identified as a minor component from this species.[11] Three new derivatives of cacospongionolide which exhibited biological activity were also isolated from this sponge.[12] Two new luffarin derivatives which showed strong anti-inflammatory activity were isolated from the Adriatic Sea sponge *F. cavernosa*.[13] Cavernolide, a novel C_{21} terpene lactone isolated from the sponge *F. cavernosa*, inhibited human synovial $sPLA_2$ in a concentration-dependent manner with an IC_{50} value of 8.8 μM.[14] However, there has been no report on a study of the FA profile and volatiles of the lipid composition of *F. cavernosa*. This is the first report on the analysis of the antibacterial activity, FA and volatile composition of the sponge *F. cavernosa* collected from the Bay of Bengal region of the Orissa Coast.

In this investigation, antibacterial screening of the lipid extract of the sponge *F. cavernosa* was performed against five fish pathogens and two human pathogens, including three MDR (multidrug resistant) strains. The investigation of the FA and volatiles of the lipophilic extract of *F. cavernosa* could give valuable information about its chemotaxonomy. Thus, these minor components appear as biomarkers for such organisms.

EXPERIMENTAL

Sponge material

The sponge *F. cavernosa* (class Demospongiae Sollas, order Dictyoceratida, family Thorectidae Berquist) collected during February–March 2006 from the Bay of Bengal region of the Orissa coast at a depth of 13 m were stored in ethanol and transported to the laboratory. The sample was identified up to genus level by Dr. P. A. Thomas, Ex-Emeritus Scientist (ICAR), Trivandrum, Kerala.

Extraction

The sponge sample was thoroughly washed with distilled water and air-dried in the shade. Ten grams of the sponge sample were homogenised and successively extracted three times with chloroform–methanol (2:1, v/v) to isolate the lipids.[15] The crude lipid extracts were purified by "Folch wash"[16] to remove non-lipid contaminants. The chloroform phase was separated from the combined extract, dried over anhydrous sodium sulphate and concentrated under a nitrogen atmosphere.

Preparation of fatty acid methyl esters

The lipophilic extract (100 mg) was 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 neutralised and dried over a sodium sulphate–sodium bicarbonate mixture. The solvent was evaporated to dryness under reduced pressure at 40 °C on a rotary evaporator (Heidolph, Laborota 4000). These fatty acid methyl esters (FAME) were then analysed by GC–MS.

FAME analysis

The FAME 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 (FAME) from 120–300 °C at a rate of 2 °C/min and held at 300 °C for 10 min, with a total run time of 100 min. The EI ionization voltage was 70 eV. Peak identification was performed by comparison of the obtained mass spectra with those available in the Wiley and NIST libraries (Shimadzu–Wiley Registry™, 8th Edition Mass Spectral Library, Shimadzu and the NIST 08 Mass Spectral Library (NIST/EPA/NIH) – new 2008 version).

Isolation and analysis of the volatile compounds

The lipophilic extract (100 mg) was subjected to a 4 h distillation–extraction in a Lickens–Nickerson apparatus.[17] The volatiles were extracted from the distillate with diethyl ether (yield: 3 mg) and investigated using a Shimadzu QP-5000 GC–MS operating under the same conditions as above except the column temperature was programmed from 40 to 280 °C at a rate of 4 °C/min.

Antibacterial assay

The antibacterial assay of the lipid extract of *F. cavernosa* (200, 100, 50 and 25 μg, all *per* 6 mm disc) was performed against five fish pathogens (*Edwardsiella tarda*, *Staphylococcus aureus*, *Micrococcus* sp., *Pseudomonas aeruginosa* and *Escherichia coli*) and two human pathogens (*Staphylococcus aureus* and *Salmonella typhi*) including three MDR (multidrug resistant) strains (*Staphylococcus pyogenes*, *Acinetobacter* sp. and *Salmonella typhi*) by the disc-assay method.[18]

The test bacterial fish pathogen cultures were obtained from the stock cultures maintained in the Pathology Laboratory of the Central Institute of Fresh Water Aquaculture, ICAR, Bhubaneswar.[19]

The human pathogens (MDR) were obtained from the National Institute of Oceanography, Goa.

Briefly, the lipid extract (200 μg/50μL) in an appropriate solvent was applied to sterile paper discs (6 mm in diameter, Whatman No. 1). After solvent evaporation the discs were placed on nutrient agar (Himedia, India) test plates inoculated with an overnight culture of the test pathogen (10^6 CFU/mL) in Brain Heart Infusion (BHI) broth. The plates were incubated for 48 h at 37 °C. Discs loaded with the respective solvent (50 μL) used for dissolution were taken as controls after evaporation of the solvent. The zone of inhibition around the disc (average of three experiments) was measured. The determination of the minimum inhibitory concentration (*MIC*) of the lipid extract was performed by the same method as above.

RESULTS AND DISCUSSION

Total lipid extract

GC–MS analysis (Table I) showed the presence of 29 components in the mixture of total lipids of *F. cavernosa* with C_{10} to C_{27} FA. The saturated linear FA corresponded to more than 30 % of the total FA content. All of the acids from 10:0 to 24:0 were found, except 21:0. The acids 12:0, 14:0, 18:0 were dominant.

The total content of saturated branched FA was 9.30 %, br-16:0 and br-14:0 being the major among them. The other branched FAs were 3,7,11,15-tetramethylhexadecanoic acid (16:0 br) and br-tetracosanoic acid.

TABLE I. GC–MS analysis of FAME of total lipid of *Fasciospongia cavernosa* (13 m depth)

Retention time, min	Compound	Content, %
2.699	Decanoic acid (10:0)	0.71
3.723	Dodecanoic acid (12:0)	5.77
5.377	Tetradecanoic acid (14:0)	4.40
5.965	Tetradecenoic acid (14:1)	4.86
6.146	12-Methyltetradecanoic acid (14:0 br)	2.04
6.503	Pentadecanoic acid (15:0)	1.52
7.271	Pentadecenoic acid (15:1)	1.22
7.999	Hexadecanoic acid (16:0)	7.86
8.401	*cis*-9-Hexadecenoic acid (16:1)	2.15
8.544	14-Methylhexadecanoic acid (16:0 br)	4.93
8.860	3,7,11,15-Tetramethylhexadecanoic acid (16:0 br)	1.18
9.246	Octadecanoic acid (18:0)	3.46
11.361	8-Octadecenoic acid (18:1)	3.66
11.701	9-Octadecenoic acid (18:1)	1.24
11.933	9,12-Octadecadienoic acid (18:2, n–6)	11.14
13.589	Nonadecanoic acid (19:0)	1.57
14.838	Eicosanoic acid (20:0)	0.43
15.345	11-Eicosenoic acid (20:1)	2.27
15.635	Eicosadienoic acid (20:2)	1.35
16.257	Eicosatrienoic acid (20:3, n–6)	2.03
17.445	5,8,11,14-Eicosatetraenoic acid (20:4, n–3)	0.51
18.221	Docosanoic acid (22:0)	0.51
18.780	Tricosanoic acid (23:0)	5.80
20.389	9,12,15-Octadecatrienoic acid (18:3, n–3)	1.99
22.151	Tetracosanoic acid (24:0)	1.02
22.598	15-Tetracosenoic acid (24:1)	2.67
22.857	br-Tetracosanoic acid (24:0 br)	1.15
23.520	5,9 Hexacosadienoic acid (26:2)	10.69
29.713	5,9-Heptacosadienoic acid (27:2)	3.08

The mass spectrum of the methyl esters of all the saturated FA exhibited the presence of the corresponding $[M]^+$, $[M–31]^+$ and $[M–43]^+$, as well as intensive peaks at *m*/*z* 74, 87 and 143, characteristics of saturated FA methyl esters.[20]

The total content of mono-enoic FA of linear structure was 18.07 %, including the basic 14:1, 16:1(Δ9), 18:1(Δ9) and 20:1(Δ11). It contains a branched mono-enoic FA, *i.e.*, 15-tetracosenoic acid (24:1 br). The mass spectra of the methyl esters of the mono-enoic FA exhibited the corresponding $[M]^+$, $[M–32]^+$ and $[M–72]^+$.

A significant amount (30.79 %) of polyunsaturated fatty acids (PUFA) was found in the total lipid extract of *F. cavernosa*, whereby linoleic acid (18:2 n–6,

11.14 %) was the major component. The other important PUFA present in *F. cavernosa* were 9,12,15-octadecatrienoic acid/α-linolenic acid (18:3 n–3, 1.99 %), dihomo-γ-linolenic acid (20:3 n–6, 2.03 %) and arachidonic acid (20:4, n–3, 0.51 %). A di-enoic acid 20:2 (Δ5,11) was also present in a reasonable amount (1.35 %). The mass spectra of the di-enoic and tri-enoic FA methyl esters exhibited the corresponding $[M]^+$ and $[M–31]^+$, as well as ions with *m*/*z* values of 141 and 150. The residues of specific super long-chain fatty acids (demospongic FA) with 24–30 carbon atoms occur in the lipids of sponge cell membranes.[21] Polyenoic FA of *F. cavernosa* were mainly represented by demospongic acids, the major part of which has a characteristic 5,9-di-enoic structural fragment of the carbon chain.[22] The obtained results showed that 26:2 (5,9) 10.69 % and 27:2 (5,9) 3.08 % acids were dominant.

The FA composition of *F. cavernosa*, including the polyenoic acids, is characteristic of marine sponges.[23]

Volatile compounds

The volatile components of the sponge were isolated by distillation–extraction and investigated by GC–MS. The obtained results are presented in Table II.

TABLE II. Composition of the volatile compounds in *Fasciospongia cavernosa*

Volatile compounds	Content, %
3,5-Dimethyloctane	2.23
Isooctyl vinyl ether	5.68
Decane	8.36
2,7-Dimethyl-1-octanol	2.73
2-Nonenal	13.50
3-*n*-Hexyl-delta-9-tetrahydrocannabinol	2.90
2-Decene-1-ol	2.41
1-Chlorooctane	56.04
1,1-Heptanediol diacetate	3.24
Heptanal	2.29

Volatile compounds often possess valuable biological activities. They serve as allelochemicals defending the organism from bacteria, fungi and viruses. Analogous to other investigated sponges,[24,25] the volatiles in *F. cavernosa* appeared to be relatively simple. Hydrocarbons, alcohols, aldehydes, ketones, esters, ethers, carboxylic acids *etc*. are generally identified in the volatile profile of sponges.[26,27] In the present investigation, the volatile profile of *F. cavernosa* is also characterized by hydrocarbons, ester, aldehydes, alcohols and ether. The content of hydrocarbons, including the halogenated ones, was the highest in the volatiles of *F. cavernosa* (66.63 %). 1-Chlorooctane predominated (56.04 % from the total volatile compounds). 3,5-Dimethyloctane (2.23 %) was also found in the volatiles of *F. cavernosa*. Only one *n*-saturated hydrocarbon, decane, was identified

that was also present in a reasonable amount (8.36 %). 2,7-Dimethyl-1-octanol, 2-decene-1-ol and 3-*n*-hexyl-delta-9-tetrahydrocannabinol were identified in the volatiles (2.73, 2.41 and 2.90 %, respectively). Two aldehydes, 2-nonenal and heptanal, were also found in the volatiles of *F. cavernosa* (13.50 and 2.29 %, respectively). Isooctyl vinyl ether and 1,1-heptanediol diacetate were identified in the volatiles of *F. cavernosa* (5.68 and 3.24 %, respectively). Contrary to most other marine organisms, the investigated sponge contained no esters of fatty acids.

Antimicrobial screening

The results of the antimicrobial screening of *F. cavernosa* are presented in Table III. The lipids exhibited broad-spectrum activity against three fish pathogens (*Edwardsiella tarda*, *Micrococcus* sp. and *Pseudomonas aeruginosa*), three MDR (multi drug resistant) strains (*Staphylococcus pyrogenes*, *Acinetobacter* sp. and *Salmonella typhi*) and one human pathogen (*Salmonella typhi*). The *MIC* values of 50/25 μg showed the significant activity of these lipids. The response of the pathogens to standard antibiotics is provided in Table IV.

TABLE III. Antibiotic activity testing of *F. cavernosa*; the antibacterial assay of the lipid extract (μg/ 6 mm disc) was performed against different fish and human pathogens by the disc-assay method (zone of inhibition in mm, including the 6 mm disc)

Pathogens	Lipid extract, μg			
	200	100	50	25
Staphylococcus aureus[a]	10	Trace	Trace	–[b]
Edwardsiella tarda[c]	10	8.5	–	–
Staphylococcus aureus[c]	8	–	–	–
Salmonella typhi[a]	11	10	9	Trace
Staphylococcus pyogenes (MDR)	10.5	10	7.5	Trace
Acinetobacter sp. (MDR)	11	10	–	–
Salmonella typhi (MDR)	10	9.5	7	–
Micrococcus sp.[c]	11.5	Trace	–	–
Pseudomonas aeruginosa[c]	11	10	Trace	–
Escherichia coli[c]	7.5	Trace	–	–

[a]Human pathogen; [b]no zone; [c]Fish pathogen

Antimicrobial activity is exhibited by many lipids of sponges,[3–5,28] including fatty acids.[29,30] *cis*-9-Octadecenoic and *cis*-9,12-octadecadienoic acids,[28,31] have the maximum antimicrobial activity. The lipid extract of the sponge *F. cavernosa* showed strong activity against all pathogens. This lipid extract contains important polyunsaturated fatty acids, such as linoleic acid (18:2, 11.4 %), α-linolenic acid (18:3, 1.99 %), dihomo-γ-linolenic acid (20:3, 2.03 %) and arachidonic acid (20:4, 0.51 %). It was reported that the bactericidal activity of long chain fatty acids against *Staphylococcus aureus* increases with the degree of unsaturation.[32–34] The antimicrobial activity of arachidonic acid compared to linoleic (18:2) and linolenic (18:3) was found to be higher. The inhibitory activity

was in the following order: arachidonic acid (20:4) > linolenic acid (18:3) > linoleic acid (18:2). Thus, the presence of a good number of PUFA in the lipid content of *F. cavernosa* may be the reason for its strong bioactivity.

TABLE IV. Activity of standard antibiotics against pathogens

Pathogens	Standard antibiotics		
	Gentamycin (10 µg)	Streptomycin (10 µg)	Polymyxin-B (300 U)
Edwardsiella tarda	S[a]	I[b]	R[c]
Pseudomonas aeruginosa	S	I	R
Escherichia coli	S	S	S
Staphylococcus aureus	S	R	R
Micrococcus sp.	S	S	Not done
Salmonella typhi	S	S	Not done

[a]Sensitive (≥ 12 mm); [b]Intermediate (9 mm to 11 mm); [c]Resistant (no zone)

CONCLUSIONS

This study was mainly focused on the lipids of the sponge in the search for new FA structures, evaluation of new sources of major PUFA of biological interest and development of trophic and/or chemotaxonomic biomarkers in the ecosystem. Important essential polyunsaturated fatty acids, such as linoleic acid, α-linolenic acid, dihomo-γ-linolenic acid and arachidonic acid were identified in the lipid composition of *F. cavernosa*. The antibacterial screening of the lipid showed a broad-spectrum activity against different human and fish pathogens. Thus, the study of the lipid composition of *F. cavernosa* is worthwhile.

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И З В О Д

МАСНЕ КИСЕЛИНЕ, ИСПАРЉИВЕ СУПСТАНЦЕ И АНТИБАКТЕРИЈСКА АКТИВНОСТ ЛИПИДА СУНЂЕРА *Fasciospongia cavernosa* (SCHMIDT) ИЗ БЕНГАЛСКОГ ЗАЛИВА (ОБАЛА ОРИСЕ)

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Анализиран је садржај масних киселина и испарљивих супстанци из липофилног екстракта морског сунђера *Fasciospongia cavernosa* (Schmidt). Нађене су линеарне засићене масне киселине (33,05 %), разгранате засићене масне киселине (9,30 %) и мононезасићене масне киселине (18,07 %). Такође је нађена значајна количина полинезасићених масних киселина (PUFA, 30,79 %), укључујући линолну киселину (18:2, n–6, 11,14 %), 9,12,15-октадекатриенску киселину/α-линоленску (18:3, n–3, 1,99 %), дихомо-γ-линоленску киселину (20:3, n–6, 2,03 %) и арахидонску киселину (20:4, n–3, 0,51 %). Липидни екстракт *F. cavernosa* је показао антибактеријску активност спрам широког спектра хуманих и рибљих патогена.

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