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COMPOSITION OF THE LIPOPHILIC EXTRACT FROM THE SPONGE *Axinella carteri* COLLECTED FROM THE BAY OF BENGAL OF THE ORISSA COAST

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The composition of the lipophilic extract from the two specimen of the sponge Axinella carteri *(Dendy) collected from two different depths of the Bay of Bengal of the Orissa coast were investigated. Fatty acids, as well as volatile compounds and sterols, were identified. A high concentration of polyunsaturated fatty acids was identified from depth species compared to the species collected from shallow water. The presence of a high concentration of a polymethyl-branched fatty acid, i.e., phytanic acid, and a demospongic acid (C26:2, Δ 5,9) were identified in both specimen, but the % composition of both acids were more in the species collected from depth than the species collected from shallow water. Important polyunsaturated fatty acids like 5,8,11,14-eicosatetraenoic acid and 5,8,11,14,17-eicosapentaenoic acid (EPA) were also found in depth species which were absent in the species collected from shallow water. Antimicrobial screening of the lipid extracts of* A. carteri *was also studied against different pathogens.*

Key words: sponges, *Axinella carteri* (Dendy), fatty acid, volatiles, sterol, antibacterial.

This work aims at extending knowledge on sponge lipids, especially for the genus *Axinella* (Dendy). Earlier bioactive alkaloids and cyclopeptides have been isolated from the tropical marine sponge *Axinella carteri* (Dendy) [1, 2]. Previously bioactive pyrrole-derived alkaloids and triterpenes have been isolated from other *Axinella* sp. [3, 4]. But much less work has been done in analyzing the fatty acid composition of these bioactive marine sponges. Differences in the lipid composition of the sponge from the two collection sites could have adaptive value. There are no investigations on the fatty acid composition of the *A. carteri* (Dendy) collected from the Bay of Bengal of the Orissa coast. We report here the total fatty acid composition of sponge species *A. carteri* (Dendy) collected from two different depths (13 m depth and 28 m depth) of the Bay of Bengal of the Orissa Coast. Antimicrobial screening of the lipid extracts of *A. carteri* was also studied against different pathogens.

GC-MS analysis of the total lipid of the sponge *A. carteri* (Dendy) collected from two different depths of the Bay of Bengal of Orissa showed marked differences in their fatty acid composition (Table 1). The sponge species collected from 28 m depth contained a greater number of fatty acids when compared to the species collected from 13 m depth.

The sponge species collected from 28 m depth is found to contain 58.94% of unsaturated fatty acids, which includes 52.87% of C26:2(5,9), a polyunsaturated fatty acid (PUFA), while the sponge collected from 13 m depth was found to contain 46.75% of unsaturated fatty acids, which includes 42.75% of C26:2(5,9).

Important PUFAs such as C20:5(5,8,11,14,17) and C20:4(5,8,11,14) were present in the species collected from depth (0.43% and 2%, respectively), while these were not found in the species collected from shallow water. It was revealed that the % composition of saturated straight chain fatty acids was greater in sp. collected from shallow water (30.31%) when compared to the species collected from depth (6.42%), which is an important finding in depth variation. The % of saturated branched chain fatty acids was 29.86% in the sp. species collected from depth, while the shallow water sp. contained 23.83% of saturated branched chain fatty acids.

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TABLE 1. GC-MS Analysis of FAME of Total Lipid of *Axinella carteri* Collected from Two Different Depths (28 m and 13 m)

Acids	RT	Acid content, %	
		28 m depth	13 m depth
14:0	7.75	0.37	2.3
16:1 (7)	10.56	0.75	
16:0	10.90	2.47	11.04
16:0 br	11.17		5.48
16:1 (9)	12.21	0.46	3.42
16:0 (3,7,11,15 tetramethyl)	13.50	24.76	18.35
17:0	12.42		2.21
18:1 (6)	13.65		0.61
18:1 (8)	13.58	0.36	
18:1 (11)	13.65	0.26	
18:0	13.94	0.72	6.70
20:0	17.44		1.15
20:4 (5,8,11,14)	15.88	1.92	
20:5 (5,8,11,14,17)	15.97	0.43	
22:0	16.44	0.37	1.78
22:1 (13)	18.53	0.58	
22:2	19.76	0.99	
24:1 (15)	21.78	0.52	
24:0	21.97	0.69	1.70
24:0 br	22.67	0.99	
25:0	22.70	0.56	
25:0 br	23.31	0.67	
24:0 br	23.69	3.44	
26:2 (5,9)	24.19	52.87	42.72
26:0	24.34	1.24	

RT: retention time; br: branched.

The % of C14:0 and C16:0 (2.3% and 11.04%, respectively) were greater in the sp. collected from shallow water than in the sp. collected from depth (0.37% and 2.3%, respectively).

Among other saturated fatty acids, the sp. from depth contains 0.72% of C18:0, 0.37% of C22:0, 0.69% of C24:0, and 1.24% of C26:0 while the % of these acids were found to be 6.70%, 1.78%, 1.70%, and 3.43%, respectively, in the sp. collected from shallow water.

Most work has been done on analyzing the fatty acid composition of the same species from two different regions [5, 6]. But no work related to the comparative study of fatty acid composition of the same sponge collected from two different depths of the same region could be found in the literature. This is the first analysis of the fatty acid profile of the species *A. carteri* (Dendy) collected from two different depths of the same region of the Bay of Bengal of the Orissa coast.

Significant differences in the structures and relative concentrations suggest that the sponge from the two collection sites could have adaptive values and the symbiotic bacteria in the two species are different [7].

The nature of the lipid profiles of the different marine sponges is reviewed by S. A. Rod′kina [8]. From the literature, we have studied the fatty acid composition of the sponges belonging to the class Demospongiae collected from shallow water and compared their results with our data.

The present investigated sponge from depth contained C24:0 br and C25:0 br. In the literature it is reported that these are the rare acids which were found in Caribbean sponges [9], and it was believed that such acids have bacterial origin [10, 11]. So the presence of symbiotic bacteria cannot be excluded.

TABLE 2. Composition of the Volatile Compounds in *Axinella carteri* (Dendy)

Volatile compounds	% composition
Acids	
2-Nonynoic acid	1.00
Cyclopentane undecanoic acid	3.05
2-Cyclopenten-1-undecanoic acid	2.72
Hydrocarbons	
2,7-Dimethyloctane	3.94
Isooctane	3.25
cis-1-Chlorohept-2-ene	3.36
1,6-Dichlorohexane	9.08
1-Chlorooctadecane	15.09
6-Methyl-octadecane	1.63
2,6,10,14-Tetramethylheptadecane	5.89
Docosane	1.89
Hexacosane	2.18
Octacosane	1.86
Esters	
1,2-Benzene dicarboxylic acid, diethyl ester	13.49
1,2-Benzene dicarboxylic acid, dihexyl ester	3.13
Aldehydes	
n-Caproaldehyde	1.31
n-Valeraldehyde	0.81
2-Nonenal (E)	3.07

Polymethyl-branched saturated FAs of sponges are represented by the usual isoprenoid FAs, 4,8,12-trimethyltridenane, phytanic acid, and pristanic acid. In this investigation we obtained only one isoprenoid FA, i.e., phytanic acid [C16:0(3,7,11,15-tetramethyl)]. It was found that the % composition of the C16:0(3,7,11,15-tetramethyl) varies from species to species of the class Demospongiae. There are species of the Demospongiae class having no C16:0(3,7,11,15-tetramethyl) acid or trace amounts of it [5, 6, 8], while both specimen of *A. carteri* collected from depth and shallow water of our present investigation contained C16:0(3,7,11,15-tetramethyl) 24.76% and 18.35%, respectively, of the total FA mixtures.

Generally six isomers of C16:1 acid, C16:1(9) prevailed in most of the species. The species from depth of our present investigation contained C16:1 (7) (0.75%) and C16:1(9) (0.45%), while the shallow water species contained C16:1(9) (3.42 %).

C18:1(9) and C18:1 (11) are the principal isomers of C18 monoenes in sponges. But the present investigated species from depth contained two C18:1 FAs having Δ8 and Δ11 whose % composition were 0.36 and 0.26, respectively, where the species from shallow water contained only C18:1(6) (0.61%).

Non-methylene interrupted dienoic FAs of various structures were much more characteristic of marine sponges. The C26:2(5, 9) is a major constituent of the FAs of the Demospongiae class. There are species of the Demospongiae class having no or trace amounts of the C26:2(5, 9) [5, 7], but there are the species of the Demospongiae class which contain very high amount of C26:2(5, 9) [6, 11]. Both specimen of *A. carteri* of our investigation contained C26:2(5,9) which constitutes more than 40% of the total FA mixtures.

Volatile Compounds. The volatile fraction, produced by steam distillation, appeared to be of limited interest. Analogous to other investigated sponges [12], there is a limited number of volatile compounds in *A. carteri* (Dendy). It contains mainly saturated hydrocarbons, followed by very few free fatty acids (Table 2). Free fatty acids have different functions in the organisms compared to those included in the lipids. Such compounds are often accepted as resulting from hydrolysis during the isolation procedure. Distillation-extraction is a relatively mild process and degradation of the lipids was not expected, so the identified fatty acids might exist in the free state in the sponges. These compounds possess some biological activities (antibacterial, insecticidal) that could improve the resistance of the sponge towards pathogens and predators. They serve as energy substrates and allelopathic agents [13].

TABLE 3. Composition of Total Sterol Isolated from *Axinella carteri*

Sterol	% composition
3-Chlorocholestanone-6	63.35
Cholest-24-en-16-one	1.29
Cholesta-8,24-dien-3β-ol	1.22
Cholesta-8,24-dien-3β-ol acetate	2.95
B-Homo-A-norcholestanone-6	3.37
Cholestanol	5.05
Ergost-22-en-ol	5.00

Investigation of Sterols. Sterols were analyzed by gas chromatography (qualitative and quantitative analyses) and by GC-MS. The results obtained are summarized in Table 3. It is evident that in contrast to most of the marine sponges investigated till now [14], the sterol composition of this sample is very simple, in accordance with the earlier results of Sica et al. [15]. 3-Chlorocholestanone-6 is the main constituent of the species collected at 28 m accompanied by low concentration sterols typical of marine sponges, while the sample collected at 13 m depth (shallow water) contained two sterols, which are cholestanol and ergost-22-en-3-ol having very small concentrations. There are variations in their composition at different locations, which are sometimes found in the sterol composition of the same species collected from different location sites and periods [12]. Some of the minor sterols are characteristic for phytoplankton [16]. So, participation of some phytoplankton species cannot be excluded.

The lipids of *A. carteri* collected from 28 m depth exhibited trace activity against two fish pathogens (*Escherichia coli* and *Micrococcus sp.*) and one human pathogen (*Staphylococcus aureus*), while the sp. collected from shallow water exhibited trace activity against only one human pathogen (*Staphylococcus aureus*) at 200 μg /50 μL per 6 mm disc.

Antimicrobial activity is exhibited by many lipids [17], including fatty acids [18, 19].

Hence, the inhibitory activity of the fatty acid components of the lipid extract of the sponge might be partly due to the presence of important essential polyunsaturated fatty acids.

EXPERIMENTAL

General Experimental Procedures. 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% diphenylsiloxane, 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°C at 2°C/min, 300°C 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.

Sponge Material. Specimens of *A. carteri* (class Demospongiae, order Halichondrida, family Axinellidae) were collected by SCUBA during February 2006 from 13 m and 28 m depth of the Bay of Bengal of the Orissa coast and kept in alcohol tanks during transport to the laboratory.

Extraction. Ten grams of each sponge species was homogenized, air-dried under shade, and successively extracted three times with chloroform-methanol (2:1, v/v) to isolate lipids. Crude lipid extracts were purified by "Folch wash" [20] to remove nonlipid contaminants. The chloroform phase was separated from the combined extract, dried over anhydrous sodium sulfate, and concentrated under nitrogen atmosphere.

Preparation of Fatty Acid Methyl Esters. Part of 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 and simultaneously neutralized and dried over sodium sulfate – sodium biocarbonate mixture. The solvent was evaporated to dryness at reduced pressure at 40°C in a water bath. These fatty acid methyl esters (FAME) were then analyzed by GC-MS for identification.

Isolation and Analysis of Sterols. Part of the lipophilic extract (100 mg) was chromatographed on a silica gel column with mixtures of hexane and acetone in ascending polarity. The fractions containing sterols according to TLC were combined and purified by preparative TLC with hexane-acetone (9:1) as mobile phase. The sterols obtained (3 mg) were analyzed by

GC-MS. The temperature programme was 80-200°C at 2°C/min, 5-min hold, 200-300°C at 10°C/min and a 20-min hold. The injector temperature was 300°C and the detector temperature was 300°C. The carrier gas was helium and the sterols were identified.

Isolation and Analyses of the Volatile Compounds. Part of the lipophilic extract (100 mg) was subjected to a 4 h distillation-extraction in a Lickens-Nickerson apparatus [21]. Volatiles were extracted from the distillate with diethyl ether (yield: 3 mg) and investigated by a Shimadzu QP-5000 GC-MS with a 25 m×0.25 mm, 0.25 μm film thickness, WCOT column coated with 5% diphenylsiloxane, 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°C to 280°C at a rate of 4°C/min using 70 eV ionization voltage (EI).

Antibiotic Activity Testing of Lipid Extracts of *Axinella carteri*. The antibacterial assay of lipid extracts of *A. carteri* collected from two different depths (200 μg /6 mm disc) was carried out against five fish pathogens (*Edwardsiella tarda*, *Staphylococcus aureus*, *Micrococcus* sp., *Pseudomonas aeruginosa*, *Escherichia coli*), two human pathogens (*Staphylococcus* aureus, *Salmonella typhii*) including three MDR (multidrug resistant) strains (*Staphylococcus pyogenes*, *Acinetobacter sp.*, *Salmonella typhii*) by the disc-assay method [22].

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