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ANTIBACTERIAL ACTIVITY AND FATTY ACID COMPOSITION OF THE EXTRACT FROM *HYPNEA MUSCFORMIS* (GIGARTINALES, RHODOPHYTA)

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ABSTRACT

The red alga *Hypnea musciformis* (Wulf.) Lamour., collected from the coast of Karachi, Pakistan has been studied for its fatly acid composition. Sixteen fatty acids were found in its methanolic extract, which were analysed as methyl esters by GC-MS. Altogether 7 satuated, 3 monoenoic, 2 dienoic and 4 trienoic fatty acids could be detected. Two saturated i.e. nonadecyic and heneicasanoic acids and all the 9 unsaturated acids are being reported for the first time from this seaweed. Pentadnytate was present in largest and nonadccyiate in smallest quantity among saturated, while decatrienoam was detected in largest and myristokate in smallest amount among unsaturated ands. The methanolic extract of the waved exhibited strong antibacterial activity against three gram positive and seven gram negative bacteria, the former appeared to be more sensitive than the latter.

INTRODUCTION

Hypnea muscifonnis (Wulfen) Lamouroux is a purple red, filiform, cartilaginous and much branched alga (Anand, 1943). It grows submerged under water and may be collected as drift material from the sandy beaches of Manora, Sandspit, Buleji and Gadani (Shamed and Tanaka, 1992). The specimens collected in the summer exhibited broader tolerance and generally higher photosynthetic rates than the plants collected in winter (Durako and Dawes, 1980). Chemical composition and energy values of *Hypnea muscifonnis have* been studied by Dhargalkar et *al.* (1980). Ascorbic and dehydroascorbic add contents of this seaweed have been reported by Qasim and Barkati (1985). A preliminary survey of its saturated and unsaturated fatty acids has also been made (Qasim, 1986; Shameel, 1990; Hayee-Memon et *at,* 1992). Some of these fatty acids have shown inhibitory effect on *Mycobacterium tuberculosis* (Marolia *et al,* 1982). In the light of these observations, examination of fatty acid composition of marine algae assumes a greater significance. No detailed investigation has so far been carried out on the fatty acids of this alga, this study presents an analysis of both saturated and unsaturated fatty acids of this seaweed. Its methanolic extract has also been tested for antibacterial activity.

MATERIAL AND METHODS

Fresh thalli of *Hypnea muscifonnis* were collected as drift material from the rocky ledges at French Beach of Buleji, near Karachi during November, 1989. The healthy specimens, free from epiphytes and animal castings were selected, thoroughly washed and dried in the shade. The dried thalli were chopped into small pieces and grinded to fine powder. About 140 kg of this powder was extracted thrice with 90% aqueous methanol at room temperature.

The extract was concentrated at reduced pressure and then fractioned in petroleum ether, chloroform, n-butanol and water soluble fractions (Wahidulla *et al,* 1986). The petroleum ether soluble fraction, after chromatography over silica gel and elution with petroleum ether and acetic acid, successively gave two major components designated as A and B, one from each of the eluents. Similarly the chloroform fraction after chromatography over silica gel and elution with petroleum ether; ethyl acetate mixture (9:1) yielded two compounds designated as D_1 and D_2. All these compounds were purified by repeated chromatography over silica gel with eluents of increasing polarity. The crude mixture was treated with diazomethane for 24 hours in cold condition and the fatty acid methyl esters so obtained were directly injected into GC and GC-MS (column SPB-1 fused with silica, carrier gas He, oven temperature 100-240°C, rate 8°C/minute, injector temperature 250°C, ion source temperature 240°C, flow 50 mL/minute).

The methanolic extract of H. *muscifonnis* was concentrated under reduced pressure below 40°C. The dried material was dissolved in sterile distilled water (5 mg/mL) and used to determine antibacterial activity against ten different gram positive and gram negative bacteria. The bacterial cultures were obtained from Department of Microbiology, University of Karachi, all of them were local isolates maintained on stock culture agar. The petri plates (10x10 cm) were prepared with trypticase soy agar (Bhakuni *et aL,* 1974; Naqvi *et al,* 1985), and 1 mL of 1:100 diluted cultures were poured on each plate, which were incubated for 30 minutes at 30°C. Wells of 6 mm diameter were cut with sterile cork borer in the inoculated agar. The wells were filled with algal extract, and the plates were incubated for 24 hours at 37°C. The tests were run in duplicate. At the end of incubation period the zones of inhibition were measured in mm.

RESULTS AND DISCUSSION

The analysis of the fatty acid methyl esters of *Hypnea muscifonnis* through GC-MS revealed the presence of 16 different fatty acids. The significant ions in their mass spectra are as follows:

Methyl nonadienoate: GC-MS, m/z 168 (M^+, $C_{10}H_{16}O_2$, 3%), 136 (M^+- 32, 7%), 125 (M^+ -43, 4%), 111(12%), 97 (24%), 83 (42%), 69 (100%).

Methyl decatrieuoate: 189 (M+, $C_{11}H_{16}O_2$, 3%), 148 (M^+ -32, 2%), 137 (M^+-43, 8%), 125 (5%), 111 (100%), 97 (5%), 83 (5%), 69 (100%).

Methyl uadecadlenoate: 1% (M^+, $C_{12}H_{20}O_2$, 6%), 164 (M^+-32, 5%), 139 (4%), 125 (10%), 111 (20%), 97 (38%), 83 (64%), 69 (100%).

Methyl tridecatrieaoate-. 222 (M^+, $C_{14}H_{22}O_2$, 8%), 190 (M^+ -32, 8%), 148 (M^+-74, 100%),121 (3%),107 (3%), 93 (3%), 79 (100%).

Methyl myristoleate: 240 (M^+, $C_{15}H_{28}O_2$, 10%), 208 (M^+ -32, 2%), 166 (M^+-74, 2%), 155 (5%), 141(4%),127 (6%), 113 (6%), 99 (8%), 85 (26%), 71(100%).

Methyl myristate: 242 (M^+, $C_{15}H_{30}O_2$, 19%), 211 (M^+ -31, 10%), 199 (M^+ -43, 15%), 185 (3%), 157 (4%), 143 (17%), 129 (5%), 115 (2%), 101 (5%), 87 (58%), 74 (100%).

Methyl pentadecatrieaoate: 250 (M^+, $C_{16}H_{26}O_2$, 7%), 218 (M^+ -32, 5%), 176 (M^+ -74, 5%), 155 (5%), 141 (2%), 127 (2%), 113 (5%), 99 (5%), 85 (15%), 71 (100%).

Methyl pentadecenoate: 254 (M^+, $C_{16}H_{30}O_2$, 5%), 222 (M^+-32, 5%), 180 (M^+ -74, 10%),166 (5%),128 (5%),113 (6%), 99 (10%), 85 (22%), 71(100%).

Methyl peatadecylate: 256 (M^+, $C_{16}H_{32}O_2$, 53%), 225 (M^+ -31, 7%), 213 (M^+ -43, 29%),199 (4%),185 (10%), 171 (11%),157 (11%), 143 (5%), 129 (30%), 115 (11%), 101 (3%), 87 (13%),73 (100%).

Methyl hiragoaate: 264 (M^+, $C_{17}H_{28}O_2$ 25%), 221 (M^+ -43, 6%), 190 (M^+ -74, 2%), 181 (11%), 167 (5%), 153 (4%), 139 (7%), 125 (9%), 111 (15%), 97 (39%), 83 (35%), 74 (100%).

Methyl palmitatc 270 (M^+, $C_{17}H_{34}O_2$, 23%), 239 (M^+ -31, 7%), 227 (M^+ -43, 11%), 213 (2%), 199 (3%), 185 (4%), 171 (3%), 157 (15%), 143 (12%), 129 (7%), 115 (5%), 101 (3%), 87 (54%), 74 (100%).

Methyl heptadecyleoate: 282 (M^+, $C_{18}H_{34}O_2$, 10%), 250 (M^+ -32, 9%), 249 (18%), 235 (3%), 208 (M^+ -74, 18%), 194 (3%), 180 (3%), 166 (5%), 152 (2%), 138 (5%), 124 (3%), 110 (2%), 96 (21%), 81 (15%), 68 (100%).

Methyl margarate: 284 (M^+, $C_{18}H_{36}O_2$, 30%), 253 (M^+ -31, 4%), 242 (M^+ -43, 15%), 227 (3%), 213 (4%), 199 (5%), 85 (4%), 171 (3%), 157 (15%), 143 (5%), 129 (3%), 115 (6%), 101(55%), 88 (100%).

Methyl stearate: 298 (M^+, $C_{19}H_{38}O_2$; 30%), 267 (M^+ -31, 8%), 225 (M^+-43, 18%), 241 (3%), 227 (1%), 213 (3%), 199 (7%), 185 (3%), 171 (1%), 157 (3%), 143 (17%), 129 (6%), 115 (2%), 101 (5%), 87 (45%), 74 (100%).

Methyl nonadecylatc 312 (M^+, $C_{20}H_{40}O_2$, 8%), 281 (M^+ -31, 5%), 269 (M^+ -43, 9%), 225 (8%), 241 (2%), 227 (3%), 213 (2%), 199 (5%), 185 (5%), 171 (4%), 157 (17%), 143 (8%), 129 (9%), 115 (10%), 101 (48%), 99 (3%), 85 (15%), 71 (100%).

Methyl heneicosanoate: 310 (M^+, $C_{22}H_{44}O_2$, 25%), 309 (M^+ -31, 5%), 297 (M^+ -43, 2%), 283 (8%), 269 (6%), 241 (8%), 225 (2%), 185 (10%), 171 (5%), 157 (3%), 143 (5%), 129 (85), 115 (5%), 101(75), 87 (7%), 73 (100%).

Table 1 shows the details of various fatty acids obtained from the different components of the extract of *Hypnea muscifonnis.* Altogether 7 saturated 3 monoenoic, 2 dienoic and 4 trienoic fatty acids could be detected. Among saturated fatty esters, methyl pentadecylate was detected in the highest amount (28.19%) and meth nonadecylate in the lowest quantity (3.26%). In the previous studies, palmitic acid has been reported as the most abundant fatty acid in this seaweed (Qasim, 1986; Shameel, 1990; Hayee-Memon *et al*., 1992). Among unsaturated fatty esters, methyl decatrienoate was found in highest proportion (6.32%) and methyl myristoleate in the lowest (0.42%), while previous studies have reported octadecadienoic acid as the most abundant unsaturated fatty add (Qasim, 1986; Shameel, 1990). A total of *nine* unsaturated fatty esters have been detected in this study, while the previous *investigations* have not reported so many unsaturated acids *(lit. cit.).* In the present study two saturated Le. nonadecylic and heneicosanoic acids and all the 9 unsaturated fatty acids are being reported for the first time from *H. muscifonnis.* It is astonishing that oleic acid, although detected previously (Shameel, 1990; Hayee-Memon et *al.*, 1992), could not be found.

Hayee-Memone *et al.* (1991) have worked on the fatty acids of an allied species, *H. valentiae* of Karachi coast. *Hypnea muscifonnis* remarkably differs from this species in its fatty acid composition. In *H. valentiae* arachidic, behenic and pentacosanoic acids are "present, whereas they are absent in *H. muscifonnis.* Heneicosanoic acid is present in *H. muscifonnis* but it is absent in *H. valentine.* Among unsaturated fatty acids tetradecatrienoic, 9-octadecenoic and 17-hexacosenoic acids are present in *H. valentiae* but absent in *H. muscifonnis.* All the unsaturated fatty acids reported in the present study were not found in *H. valen*ti*ae.*

Table 1

Fatty acid composition of *Hypnea musciformis* analysed as methyl esters

Systematic name	Common name	Molecular formula	Molecular weight	R.R.T.	Relative percentage
Saturated fatty acid methyl esters					
Tetradecanoate	Myristate	$C_{15}H_{30}O_2$	242	0.81	7.11
Pentadecanoate	Pentadecylate	$C_{16}H_{32}O_2$	256	1.04	28.19
Hexadecanoate	Palmitate	$C_{17}H_{34}O_2$	270	1.00	9.23
Heptadecanoate	Margarate	$C_{18}H_{36}O_2$	284	1.06	10.96
Octadecanoate	Stearate	$C_{19}H_{38}O_2$	298	1.16	10.93
Nonadecanoate	Nonadecylate	$C_{20}H_{40}O_2$	312	1.35	3.26
Heneicosanoate	Heneicosanoate	$C_{22}H_{44}O_2$	340	1.37	10.30
Unsaturated fatty acid methyl esters					
Nonadienoate	-	$C_{10}H_{16}O_2$	168	0.57	0.63
Decatrienoate	-	$C_{11}H_{16}O_2$	180	0.59	6.32
Undecadienoate	-	$C_{12}H_{20}O_2$	196	0.77	1.26
Tridecatrienoate	-	$C_{14}H_{22}O_2$	222	1.01	1.69
Tetradecenoate	Myristoleate	$C_{15}H_{28}O_2$	240	0.81	0.42
Pentadecatrienoate	-	$C_{16}H_{26}O_2$	250	0.93	1.58
Pentadecenoate	-	$C_{16}H_{30}O_2$	254	1.03	0.74
Hexadecatrienoate	Hiragonate	$C_{17}H_{28}O_2$	264	1.14	5.26
Heptadecenoate	Heptadecylenate	$C_{18}H_{34}O_2$	282	0.58	2.11

The methanolic extract of H. *muscifonnis* has shown strong antibacterial activity against three gram positive and seven gram negative bacteria (Table 2). On the average, gram positive bacteria exhibited a zone of inhibition of 15.67 mm radius and the gram negative ones of 12.57 mm radius. It appears that the former are more sensitive than the latter against antibacterial activity of this seaweed. *Bacillus subtilis* appeared to be the most sensitive bacterium in this regard. The methanolic extract of *H. muscifonnis* was also found to exhibit strong activity against *Bacillus megaterium* and *Streptococcus viridans*, both being gram positive, while it exhibited no activity against four tested gram negative bacteria (Usmanghani and Shameel, 1986). This also confirms the sensitivity of gram positive bacteria.

Table 2

Antibacterial activity of the methanolic extract of *Hypnea musciformis*

Test organism	Zone of inhibition (radius) in mm	
	Control	Algal extract
Gram positive bacteria		
Bacillus subtilis	0	20
Staphylococcus aureus	0	15
Streptococcus faecalis	0	12
Gram negative bacteria		
Escherichia coli	0	10
Salmonella paratyphi A	0	14
S. paratyphi B	0	12
S. typhi	0	12
Shigella flexneri	0	14
Sh. shigi	0	12
Sh. sonnei	0	14

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