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PHYCOCHEMICAL STUDIES ON *MICROSPORA FLOCCOSA* (VAUCHER), THURET ALGA COLLECTED FROM THE PONDS OF HYDERABAD, SINDH

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ABSTRACT

Microspora floccosa (vaucher) Thuret is a rich source of fibers, vitamins and proteins. Present study was conducted to analyze the fatty acids and sterol composition of the fresh water green alga *Microspora floccosa* present in semi-permanent ponds near Hyderabad Sindh. The acids were isolated and then converted into methyl esters. Subsequently they were identified by GC-MS, which revealed the presence of methyl undecylate, myristate, tridecylate, pentadecylate, palmitate, margarate stearate and nonadecylate among saturated and methyl pentadecatrienoate, hexadecatrienoate and heptadecylenate among un-saturated fatty acids. Besides above two sterols such as 24-methylcholesterol and cholesterol were also identified through EI-mass spectrometry and ^{1}H-NMR spectroscopy.

Key words: *Microspora floccosa*, Fresh water algae, Chlorophycota

INTRODUCTION

Microspora floccosa (vaucher) Thuret is a fresh-water macroalgae belongs to family Microspor-aceae and division Chlorophyta. Being an edible macroalgae, it is a rich source of fibers, vitamins and proteins. In addition to being used as a food source, *M. floccosa* may have a number of addi-tional health benefits such as promoting appetite, rejuvenating, and remedy of many common ailments (Fahprathanchai *et al.,* 2006). In previous studies, the extracts of *M. floccosa* were used to investigate toxicological effects in rates, no morta-lity or abnormality was observed up to sixty days. A range of parameters including body weight, hematology and blood biochemistry (alanine ami-notransferase, ALT; aspartate aminotransferase, AST; blood urea nitrogen, BUN and creatinine, Cre) were investigated in control and treatment groups, which were recorded same in both groups (Fahprathanchai *et al.,* 2006). Crude methanol extracts of *M. floccosa* have been demonstrated to exhibit a strong antimicrobial activity against a variety of bacterial and fungal species (Ghazala *et al.,* 2010). The antimicrobial activity has been sugg-ested to be due to sterols and triterpene compounds (Khalid *et al.,* 2011). Several studies on taxonomy, Biogas production seasonal variation, nutritional properties, limnological studies and ecological survey of the freshwater green algae have been carried out (Leghari *et al*., 2000; Mehwish & Aliya, 2005; Kubra & Leghari, 2008; Ghazala *et al.,* 2009; Adhoni *et al.,* 2015; Tibbetts *et al.,* 2015; Grass *et al.,* 2016). The phycochemistry of the freshwater green algae is less investigated. In this regard, present study has been designed to unravel the phycochemistry of *Microspora floccosa* indigenous to Hyderabad Pakistan.

MATERIALS AND METHODS

Collection of Alga: The freshwater green alga *Microspora floccosa* (vauch) Thuret was collected from freshwater ponds, opposite to Rajputana Hospital Hyderabad, forming a thick mat floating over the surface of water in November 2011. The pH of the ponds was 7.9. It was brought to the Phycology Laboratory, Institute of Plant Sciences, University of Sindh, Jamshoro and thoroughly washed in tap water to remove the external mate-rial and was kept at room temperature for drying.

Isolation of fatty acids: The dried alga (98.08g) was kept for cold percolation for about 20 days in *n*-hexane: chloroform (1:1, v/v), which on evapo-ration under reduced pressure of rotary evapora-tor, afforded a dark green thick syrupy algal extract (1.02g). Out of this (0.75g) was saponified with 10 % KOH in 50% ethanol (EtOH) and refluxed at 100 ^{0}Ċ for six hours. After cooling, the saponified extract of EtOH was evaporated under vacuum and then clarified by partitioning with equal volumes of water and diethyl ether (Et_2O). The experiment was run thrice time. The water solution was made slightly acidic using 6N HCl (pH5-6), after that it was extracted again using same volume of water and Et2O. The experiment was repeated thrice and completely associated Et2O phases were evaporated under reduced pressure, that has given out (0.35 g) of residual substance. At the same time 0.5mL ethereal diazo-methane was introduced and the reaction mixture was kept at room temperature until unless it becomes soluble. The separated amount of aliq-

uots was then run into a gas-chromatograph mass spectrometer (GC-MS) and fatty acid methyl esters were determined. The GC-MS result of eight saturated and three unsaturated fatty acids methyl esters on the mass spectrometry basis is presented in Table 1.

Isolation of Sterols: The extraction of sterols in the form of dried thalli (225g) was left for separation in ethanol for the duration of 21 to 28 days. The separated amount of residue was further filtration using filter paper and concentrated under vacuum and that appeared as a bright green product. The residue was under gone through the solvent extraction of water and ethyl acetate and process was repeated thrice. The most of the combined ethyl acetate layers on volatile expenditure (1.75g) and a concentrated algal material was obtained which was further deposited onto silica gel column (70-230 mesh size) in the chromatographic technique. The portion of the residue was eluted in various solvents such as *n*-hexane, diethyl ether, chloroform and methanol based on their polarity by 2% for each of the solvents. The amount of residue which was eluted in n-hexane: diethyl ether 85:15 given out product of two sterols. i-e Cholesterol and 24-methyl cholesterol (fig.1). They were scrapped off as sterol bands by thin layer chromatography (TLC) plates developed in *n*-hexane: ether: acetic acid (85:15:0.8 v/v). The sterols were first detected under ultra violet (UV) lamp and then by spraying with $CeSO_4$ (Ceric sulphate).

RESULTS AND DISCUSSION

The microscopic examination of the collected alga indicated to be *Microspora floccosa* belonged to the family Microsporaceae, order Microsporales, Phylum Chlorophycota and the class is Ulvophyceae. The alga was identified after Prescott (1962) and revealed the following characteristics. Walls relatively thin, sections are not usually found in the region of the cell, cells in cylindrical shape or little bit seem swollen from 14-17 µ in diameter size, 22-29 (35) µ long chloroplast usually reticulate.

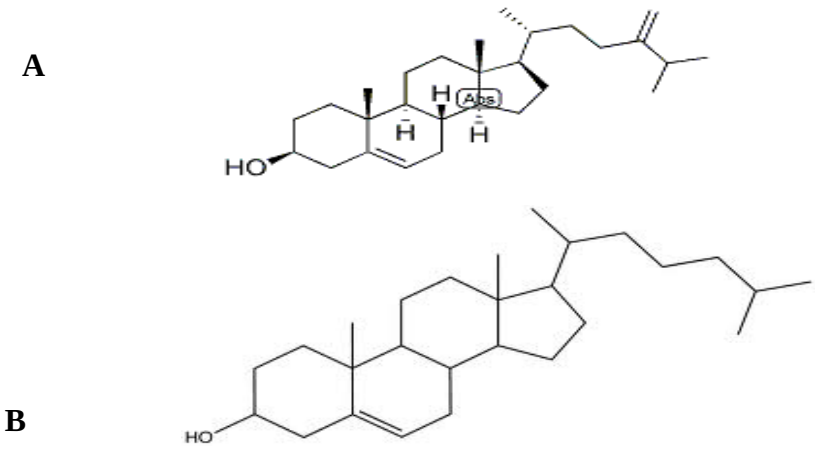

Figure 2: (A) 24-Methyl Cholesterol, (B) Cholesterol

The detected ions in the mass spectra of their methyl esters are shown as under:

Methyl Undecylate GC-MS:m/z 200 (M^+, C_{12} H_{24} O_2, 33%), 157 (M^+-43, 28%), 153(M^+-57, 14%), 143 (17%), 125 (42%), 111 (56%), 97 (49%), 83 (84%), 69 (41%), 55 (100%).

Myristate: m/z 242 (M^+, C_{15} H_{30} O_2, 36%), 199 (M^+-43, 73%), 185 (M^+-57, 30%), 171 (8%), 157 (29%), 129 (53%), 115 (15%), 101 (54%), 83 (58%), 83 (58%), 71 (77%), 57(73%).

Tridecylate: m/z 228 (M^+, C_{14} H_{28} O_2, 8%), 185 (M^+-43, 14%), 171 (4%), 157 (12%), 143 (6%), 129 (7%), 115 (8%), 101 (52%), 87 (100%).

Pentadecylate: m/z 256 (M^+, $C_{16}H_{32}O_2$, 100%), 213 (M^+-43, 42%), 199 (M^+-57, 8%), 185 (16%), 171 (18%), 157 (20%), 143 (8%), 129 (52%), 115(16%), 101(8%), 73 (100%).

Trimethyl-2, 6, 10-dodecatrienoate: m/z 250 (M^+, $C_{16}H_{26}O_2$,25%), 207 (M^+-43,5%), 193 (M^+ 57,1%), 179 (10%), 165 (15%), 151 (5%), 137 (11%), 123 (17%), 109 (39%) 95 (33%), 81 (19%).

Hiragonate: m/z 264 (M^+, $C_{17}H_{28}O_2$, 80%), 221 (M^+-43,10%), 207 (M^+-57,5%), 193 (5%), 193 (5%), 179 (6%), 165 (7%), 151(9%), 137 (20%), 123(35%), 109 (6%), 95 (64%), 81 (78%), 67 (43%).

Palmitate: m/z 270 (M^+, C_{17} H_{34} O_2, 8%), 227 (M^+-43, 47%), 213 (M^+-57, 6%), 199 (12%), 185 (16%), 171 (14%), (157%), 143%), 129 (22%), 115 (8%), 101 (1%), 87 (100 %).

Heptadecenoate: m/z 282 (M^+, $C_{18}H_{34}$ O_2), 207 (M^+-75,7%), 193 (12%), 179 (7%) 165 (9%), 151 (16%), 137 (21%), 123 (2%), 109 (6%), 95 (6%).

Margarate: m/z 284 (M^+, C_{18} H_{36} O_2,99%), 241 (M^+-43,36%), 227 (M^+-57,8%), 213 (4%), 199 (8%), 185 (27%), 171 (10%), 143 (6%), 129 (40%),115 (12%), 115 (12%), 101 (7%), 87 (10%), 78 (36%).

Stereate: m/z 298 (M^+, C_{19} H_{44} O_2, 36%), 225 (14%), 24 (11%), 227 (1%), 213 (8%), 199 (4%), 185 (9%), 171 (5%), 143 (11%), 129 (12%), 115 (10%), 101(6%), 87 (1%), 73 (2%).

Nonadecylate: m/z 312 (M^+, C_{20} H_{40} O_2, 24%) 269 (M^+-43,12%), 255 (M^+-57,4%), 241 (2%), 227, (5%), 213 (9%), 199 (5%), 185 (1%), 171 (1%), 157 (23%), 171(1%), 157 (23%), 143 (9%), 129 (4%), 115 (7%), 101 (64%), 78 (1%).

The identification of the isolated sterols was confirmed by IR, UV, EI-mass (MS) and ^{1}H-NMR spectroscopy as follows:

Cholesterol EIMS:m/z 386 (M^+, C_{27} $H_{46}O$),371 (M^+- CH_3) ,353 (M^+- CH_3- H_2O), 368 (M^+-H_2O),(M^+- H_2O),353 (M^+- H_2 O-CH_3), 325(M^+-H_2O –isopropyl), 311 (M^+- H_2O-CH_3), 301 (M^+-C_6 H_3), 273(M^+-side chain C_8 H_{18}), 255 (M^+-sidechain-H_2 O)231, 213, 199, 178, 161, 149, 119, 107, 95, 81, 69.

^{1}H-NMR (CDl_3 400 MHZ):H-26, 0.86, 3H, d, J=6.6 Hz, H-27,0.91, 3H,d,J=6.4Hz,H-21,1.00, 3H,S,H-19,3.54, 1H.m,H-3, 5.34, 1H, distorted triplet,H-5 ppm.

24-Methylcholesterol EIMS: m/z 400 (M^+, C_{28} H_{48} O), 385 (M^+-CH_3), 382(M^+-H_2 O), 367 (M^+-CH_3-H_2 O), 314, 297, 269, 247, 227, 149, 69.

^{1}H-NMR ($CDCl_3$ 400 MHZ):0.66 (3 H,S,18-Me), 0.89(3H,S,19-Me), 1.24 (1H,D,J-7.0 HZ, 21-Me), 160(6H,m,26,27,H), 3.48(1H,m,3-H), 5.33 (1H,m,6-H) ppm.

In all eleven fatty acids methyl esters were identified from the extract of *Microspora floccosa* (vaucher), Thuret, eight were saturated and three unsaturated (Table 1). The saturated fatty acids were present in greater amount (74.49%) then the unsaturated ones (25.50%). Similar results were also observed on other fresh water algae of Sindh (Valeem and Shameel, 2005; Shahnaz *et al.*, 2006). Among saturated fatty acids hexadecanoate was present in greater proportion (26.75%), while 3, 7, 11-Trimethyle-2, 6-Pentadecatrienoate an unsaturated fatty acid was present in greater amount (12.24%).

Table-1: Fatty acids detected as methyl esters in *n*-hexane: chloroform extract of *Microspora floccose*

Saturated FA methyl esters				
Systematic name	**Common name**	**Mol. Formula**	**Mol. Wt.**	**Rel. %age**
n-Undecanoate	Undecylate	C_{12} H_{24} O_2	200	2.49
n-Tetradecanoate	Myristate	C_{15} H_{30} O_2	242	10.19
n-Tridecanoate	Tridecylate	C_{14} H_{28} O_2	228	11.87
n-Pentadecanoate	Pentadecylate	C_{16} H_{32} O_2	256	12.74
n-Hexadecanoate	Palmitate	C_{17} H_{34} O_2	270	26.75
n-Heptadecanoate	Margarate	C_{18} H_{36} O_2	284	3.84
n-Octadecanoate	Stearate	C_{19} H_{38} O_2	298	4.00
n-Nonadecanoate	Nonadecylate	C_{20} H_{40} O_2	312	2.18
Unsaturated FA esters:				
3,7,11-Trimethyl-2,6 10-dodecatrienoate	Pentadecatrienoate	C_{16} H_{26} O_2	250	12.24
Hexadecatrienoate	Hiragonate	$C_{17}H_{28}$ O_2	264	12.19
Heptadecatrienoate	Heptadecylenate	C_{18} H_{34} O_2	278	1.27
Mol.Wt. = Molecular Weight **Rel. %age** = Relative Percentage.				

Conclusions

The present study reports the phycochemical characterization of *M.floccosa.* The findings revealed that algae were enriched with number of saturated and unsaturated fatty acids Moreover, sterols were also identified from the collected Alga.

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