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BIOACTIVITY AND PHYCOCHEMICAL STUDIES ON *MICROSPORA FLOCCOSA* (CHLOROPHYCOTA) FROM SINDH

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Abstract

Microspora floccosa (Vaucher) Thuret [= *Prolifera floccosa* Vaucher], a filamentous green alga was collected from River Indus at Power House Station, near Hyderabad and extracted with methanol. The extract displayed a strong antimicrobial activity against 14 bacterial and 20 fungal species. Eleven saturated and 11 unsaturated fatty acids were found in the extracts where former were lesser in quantity (47.4 %) than the latter ones (52.6 %). Palmitic acid (C 16:0) was present in the highest amount (12.3 %), while oleic acid (C 18:1) was detected in an appreciable proportion (6.1 %). Two sterols and two terpenes were also obtained from the extract, which were chemically elucidated as cholesterol, 24-isopropyl-5-cholesten-3β-ol, *trans*-phytol and cyclopterospermol.

Introduction

Some interesting results were obtained in the present lab. by investigating the bioactivity and phycochemistry of marine and estuarine algae of Pakistan (Shameel, 1987, 1990, 1993; Aliya & Shameel, 1993, 1998, 2003; Aftab & Shameel, 2006, 2008, 2010). Therefore, a research program was established to compare these results with the freshwater algae of Sindh (Khalid *et al.,* 2010a-d). The present work is a continuation of these studies, where *Microspora floccosa* (Vaucher) Thuret 1850 [= *Prolifera floccosa* Vaucher 1803], an alga of the family Microsporaceae, order Microsporales, class Ulvophyceae, phylum Chlorophycota (*fide* Shameel, 2008) was investigated for its bioactivity and phycochemistry and compared with its brackish and marine water inhabitants.

Materials and Methods

Microspora floccosa commonly occurs in slowly flowing and stagnant waters. It is found particularly in spring but grows throughout the year attached with grasses. Specimens were collected in September 1993 from slow running water of River Indus at Power House Station, Hyderabad attached with the stones and grasses, where it was growing at the margin of water and on water saturated soil along with *Riccia* and mosses. The algal extraction and procedures for different tests of bioactivity have already been described recently in detail (Khalid *et al*., 2010 a). The methods used for the saponification, esterification and identification of fatty acids, as well as extraction, purification an chemical elucidation of other natural products have also been elaborated earlier. The techniques and instumentations such as GLC, GC-MS, ^{1}H- and ^{13}C-NMR spectroscopy may also be found there.

Results

Biological activities: The crude extract showed a strong antibacterial activity against all 14 tested bacterial organisms (Table 1) and exhibited strong antifungal activity against all 20 tested fungal species including 7 human pathogens, 5 plant pathogens and 8 saprophytes (Table 2). Therefore, methanol extract of *Microspora floccosa* revealed very promising results of antimicrobial activities.

Detection of fatty acids: Four fractions obtained from column chromatography were analysed for fatty acids, where fraction A was eluted from column in *n*-hexane (100), fraction B in *n*-hexane:chloroform (95:05), fraction C in *n*-hexane:chloroform (90:10) and fraction D in *n*-hexane:chloroform (85:15). All of them were methylated by diazomethane and analysed initially by GLC and finally by GC-MS. Identification of the individual fatty acids was carried out by matching their mass spectra with the NBS mass spectral library (Helles & Milne, 1978). As a result of that 22 different fatty acids were detected, including 11 saturated and 11 unsaturated acids (Table 3).

Extraction of sterols: Two sterols were identified from the fractions eluted from the silica gel column, where cmpound 1 was eluted in pure form in solvent system of *n*-hexane:chloroform (70:30). Its purity was checked on TLC card in solvent system *n*-hexane:chloroform (60:40) and then by spraying with $Ce(SO_4)_2$. On heating it produced a single dark red spot. After using different spectroscopic methods it was identified as cholesterol (Fig. 1[1]. The compound 2 was eluted in mixture form in *n*-hexane:chloroform (60:40) from column and purified on preparative thick layer silica gel glass plates in solvent system *n*-hexane:chloroform (1:1). Its purity was checked on TLC card (same system as above) and after spraying with $Ce(SO_4)_2$ a reddish purple spot was observed. After using various spectroscopic techniques it was identified as 24-isopropyl-5-cholesten-3β-ol (Fig. 1[2]. Some physical properties of the identified sterols are given in the Table 4.

Isolation of a diterpene: A diterpene was purified and eluted from column in *n*-hexane:chloroform (70:30). It was further purified on preparative silica gel glass plates in solvent system of *n*-hexane: $CHCl_3$ (60:40). Its purity was then checked on TLC card in the solvent system *n*-hexane:chloroform (40:60) and a purplish spot was found after spraying with $Ce(SO_4)_2$. After using various types of spectroscopic techniques it was identified as 3,7,11,15-tetramethyl-hexadec-2-en-1-ol, commonly called *trans*-phytol (Fig. 1 [3]. Some of its physical properties are given in the Table 4.

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Table 1. Antibacterial activity shown by the methanol extract of *Microspora floccosa.*

Bacterial culture	Zone of inhibition (mm)	Reference drugs	Zone of inhibition (mm)
Bacillus cereus	22	Amoxicillin $(H_2O)_3$	19
		Ampicilln $(H_2O)_3$	19
Corynebacterium diphtheriae	18	Amoxicillin $(H_2O)_3$	-
		Ampicillin $(H_2O)_3$	16
Escherichia coli	13	Ainoxicillin $(H_2O)_3$	12
		Ampicillin $(H_2O)_3$	14
Klebsiella pneumoniae	7	Amoxicillin $(H_2O)_3$	-
		Ampicillin $(H_2O)_3$	9
Listeria monocytogenes	9	Amoxicillin $(H_2O)_3$	12
		Ampicillin $(H_2O)_3$	12
Proteus mirabilis	19	Amoxicillin $(H_2O)_3$	20
		Ampicillin $(H_2O)_3$	20
Proteus valgaris	10	Amoxicillin $(H_2O)_3$	10
		Ampicillin $(H_2O)_3$	10
Pseudomonas aeruginosa	14	Amoxicillin $(H_2O)_3$	-
		Ampicillin $(H_2O)_3$	12
Salmonella typhi	23	Amoxicillin $(H_2O)_3$	20
		Ampicillin $(H_2O)_3$	21
Shigella boydii	24	Amoxicillin $(H_2O)_3$	21
		Ampicillin $(H_2O)_3$	21
Staphylococcus aureus	23	Amoxicillin $(H_2O)_3$	22
		Ampicillin $(H_2O)_3$	22
Streptococcus faecalis	19	Amoxicillin $(H_2O)_3$	17
		Ampicillin $(H_2O)_3$	20
Streptococcus pyogenes	9	Amoxicillin $(H_2O)_3$	11
		Ampicillin $(H_2O)_3$	11
Vibrio choleriae	13	Amoxicillin $(H_2O)_3$	11
		Ampicillin $(H_2O)_3$	11

- = Not tested

Table 2. Antifungal activity exhibited by the methanol extract of *Microspora floccosa.*

Fungal culture	Colony Sample	Diam. (mm) control	Inhibition %	MIC µg/mL miconazole	Ketoconazole
Human pathogens:					
Allescheria boydii	13	82	90.24	0.05	0.1-4
Candida albicans	13	95	90.52	0.1-2.0	0.1-8.0
Epidermophyton floccosum	15	108	89.81	0.5-1.0	0.1-8.0
Microsporum canis	12	53	75.47	0.5-10	0.05-12.8
Trichophyton longifusus	12	35	45.71	2.54	5.20
Trichophyton mentagrophytes	14	102	42.15	2.59	5-19
Trichophyton semi	12	95	92.63	2.59	5.19
Plant pathogens:					
Fusarium oxysporm	18	89	88.76	-	-
Macrophomina phaseolina	13	98	85.71	-	-
Pythium aphanidermatum	17	58	72.41	-	-
Pythium oedochilum	12	47	38.29	-	-
Rhizoctonia solani	15	66	72.72	-	-
Saprophytes:					
Aspergillus flevus	18	98	76.53	-	-
Drechslera rostrata	12	60	70.00	0.3	0.3
Gliocladium virens	18	102	88.23	-	-
Nigrospora oryzae	13	65	81.53	0.3	0.3
Paecilomyces lilacinus	17	86	80.23	-	-
Stachybotrys atra	14	84	67.85	0.3	0.3
Trichoderma hamatum	15	60	73.33	-	-
Trichoderma harzianum	10	104	82.69	-	-

MIC = Minimum inhibitory concentration of standard drugs, - = Not tested

Table 3. Fatty acids detected in the methanol extract of *Microspora floccose*.

Systematic name	Common name	Molecular formula	Mol. Wt.	Rel. % age
Saturated acids:				**47.39**
Dimethyl propane-dioic	Dimethyl malonic	$C_4H_7O_4$	118	1.68
n-Heptanoic	Heptylic	$C_7H_{15}O_2$	130	2.72
n-Tetradecanoic	Myristic	$C_{14}H_{28}O_2$	228	0.81
n-Hexadecanoic	Palmitic	$C_{16}H_{32}O_2$	256	12.26
n-Heptadecanoic	Margaric	$C_{17}H_{34}O_2$	270	6.30
n-Octadecanoic	Stearic	$C_{18}H_{36}O_2$	284	2.76
n-Ecosanoic	Arachidic	$C_{20}H_{40}O_2$	312	6.18
n-Heneicosanoic	Heneicosoic	$C_{21}H_{42}O_2$	326	1.56
n-Docosanoic	Behenic	$C_{22}H_{44}O_2$	340	4.78
n-Tricosanoic	Tricosoic	$C_{23}H_{46}O_2$	352	2.72
n-Heptacosanoic	Heptacosoic	$C_{27}H_{54}O_2$	410	5.62
Unsaturated acids:				**52.56**
10-Undecenoic	Undecylenic	$C_{11}H_{20}O_2$	184	6.76
9-Dodecenoic	Lauroleic	$C_{12}H_{22}O_2$	198	6.34
Tridecadienoic	—	$C_{13}H_{22}O_2$	210	0.90
Tridecenoic	Decylacrylic	$C_{13}H_{24}O_2$	212	12.10
9-Tetradecenoic	Myristoleic	$C_{14}H_{26}O_2$	226	2.65
Pentadecenoic	Pentadecylenic	$C_{15}H_{28}O_2$	240	0.95
9-Hexadecenoic	Palmitoleic	$C_{16}H_{30}O_2$	254	2.86
Heptadecenoic	Heptadecylenic	$C_{17}H_{32}O_2$	268	1.27
9-Octadecenoic	Oleic	$C_{18}H_{34}O_2$	282	6.08
Nonadecenoic	Nonadecylenic	$C_{19}H_{36}O_2$	296	7.12
Tricosenoic	—	$C_{23}H_{44}O_2$	352	5.53

Mol. Wt. = Molecular weight, Rel. % age = Relative percentage

Table 4. Natural products obtained from methanol extract of *Microspora floccose*.

Str. No.	Common name	Systematic name	Molecular formula	Molecular weight
Sterols:				
1.	Cholesterol	Cholest-5-en-3β-ol	$C_{27}H_{46}O$	386
2.	24-Isopropyl-5- cholesten-3β-ol	Same	$C_{30}H_{52}O$	428
Terpenes:				
3.	*Trans*-phytol	3, 7, 11, 15- Tetramethyl- hexadec-2-en-l-ol	$C_{20}H_{40}O$	296
4.	Cyclopterospermol	22-Methylene-cyclo-artan-3β-ol	$C_{31}H_{52}O$	440

Str. No. = Structure number in Fig. 1.

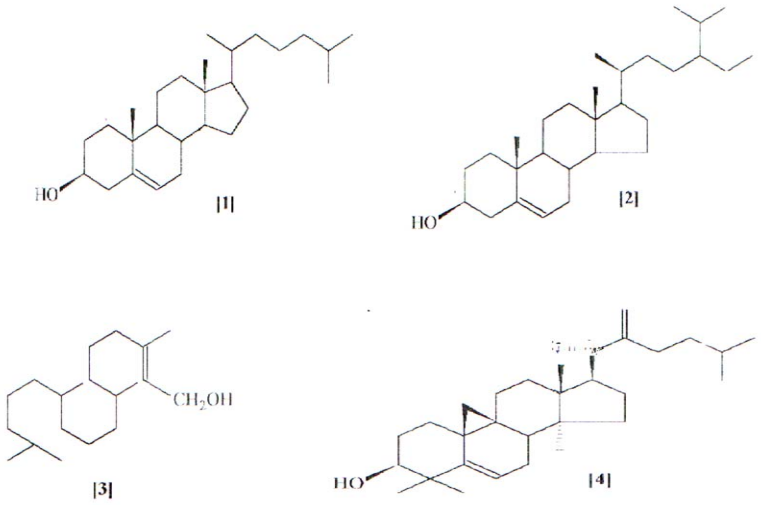

Fig. 1. Natural products extracted from *Microspora floccosa*: **[1]** = Cholesterol, **[2]** = 24-Isopropyl-5-cholesten-3 β-ol, **[3]** = *Trans*-phytol, **[4]** = Cyclopterospermol.

Separation of a triterpene: A triterpene was purified and eluted from column in *n*-hexane:chloroform (20:80). It was further purified on preparative silica gel glass plates in solvent system *n*-hexane: chloroform (10:90). Its purity was then checked on TLC card in the solvent system of pure $CHCl_3$ and a purplish spot was observed after spraying with $Ce(SO_4)_2$. After using various types of spectroscopy it was identified as cyclopterospermol ***i.e.***, 22-methylene cycloartan-3β-ol (Fig. 1 [4]. Some of its physico-chemical properties are shown in the Table 4.

Discussion

Crude extract of the filamentous green alga, *Microspora floccosa* exhibited a strong antimicrobial activity against bacterial and fungal species (Tables 1 & 2). Similar results were shown by the brackish water inhabitants of this species (Aftab & Shameel, 2008). This indicates that the antibiotic properties of this alga are its genetic properties and do not simply depend on its environment. The methanol extract of *Microspora* inhibits the release of histamine from mast cells (Price *et al*., 2002), where it showed anti-inflammatory activity.

The extract of *M. floccosa* contained 22 fatty acids (Table 3), which included 11 unsaturated acids in slightly larger amount (52.56 %) than 11 saturated ones, present in small quantity (47.39 %). This proportion is similar as found in other green algae collected from the freshwater habitats of Sindh (Ghazala *et al.,* 2005) as well as brackish water inhabitant of this species (Aftab & Shameel, 2008). Palmitic (C 16:0) and oleic (C 18:1) acids were present in large and appreciable amounts (12.26 & 6.08 % respectively) in this alga. These two acids were detected in the largest proportion in most of the green seaweeds of Karachi (Qasim, 1986; Shameel, 1990, 1993). Therefore, this alga resembles freshwater green algae in some respects and green seaweeds in the other.

From methanol extract of *M. floccosa* two sterols and two terpenes were extracted, one being cyclic triterpene and the other acyclic diterpene (Table 4). Such compounds may be responsible for its antibiotic activities. Such natural products have been detected in several green seaweeds (Aliya & Shameel, 1993, 1998, 2003) and estuarine algae of Pakistan (Aftab & Shameel, 2006, 2008, 1010). In this way this freshwater alga resembles its marine and estuarine counterparts in the biosynthesis and accumulation of its secondary metabolites.

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