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BIOACTIVE COMPOUNDS IN *CHARA CORALLINA* VAR. *WALLICHII* (A. BR.) R. D. WOOD (CHAROPHYTA)

S. M. KHALIQ-UZ-ZAMAN, SIMIN SHAMEEL, MUSTAFA SHAMEEL*, SULTAN MAHMOOD LEGHARI** AND VIQAR UDDIN AHMAD

HEJ Research Institute of Chemistry,
University of Karachi, Karachi-75270, Pakistan.

Abstract

Methanolic (MeOH) extract of *Chara corallina* var. *wallichii* (A. Br.) R. D. Wood has been analysed for its fatty acid and sterol compositions. Using GC-MS technique 23 fatty acids *viz.*, 7 saturated, 6 monoenoic, 5 dienoic and 5 trienoic acids were identified as methyl esters, where 63.5% were unsaturated and 36.5% as saturated acids. With the exception of heptanoic and pentacosenoic, the acids ranged from C10 to C18; longer-chain and polyunsaturated acids beyond trienoic ones were not detected. Hexadecadienoic acid occurred in greatest amount (23.7%), and pentadecylic and palmitic acids appeared to be the major saturated acids. Besides, 4 sterols have been identified by spectroscopic techniques, where ß-sitosterol was 76.8% while cholesterol, clerosterol and stigmasterol were 6.4-8.7%. The alga resembled in certain characters of fatty acid and sterol compositions with chlorophytes and in others with higher plants. The MeOH extract, its EtOAc-soluble part and four isolated sterols showed bioactivity against 10 species of bacteria and 10 species of fungi and phytotoxicity against *Lemna minor*. ß-Sitosterol was least active in its bioactivity as well as phytotoxicity.

Introduction

The algal phylum Charophyta has similarities with several orders of Chlorophyta as well as with the bryophytes and higher plants. Besides several structural and developmental characteristics, the charophytes; their also have numerous biochemical developmental characteristics, the charophytes have also numerous biochemical characters in common with chlorophytes; their fattty acid and sterol compositions have also little information on the natural products of charophytes (Patterson, 1972; Alary-Bernard *et al.*, 1980; Sakano *et al.*, 1983; Sato & Furuya, 1985; Zhang *et al.*, 1989; Patterson *et al.*, 1991). The present study describes the fatty acids and sterols of a charophyte, *Chara corallina* var. *wallichii* (A.Br.) R.D. Wood of River Indus, which might be helpful in relating the phycochemistry and taxonomy of green algae and land plants. Bioactivity of the extracts and of the isolated sterols was also examined for a better insight of aquatic ecosystem of the River Indus.

*Department of Botany/Institute of Marine Sciences, University of Karachi, Karachi-75270, Pakistan
**Department of Botany, University of Sindh, Jamshoro-76080, Pakistan.

Materials and Methods

Fresh specimens of *Chara corallina* var. *wallichii* (A. Br.) R.D. Wood were collected on the 15th, 16th and 20th October 1994 from flood water of River Indus near railway station of Petaro, Sindh, Pakistan. The dioecious algae with red coloured gametes were found growing along with grasses, *Chara zeylanica* and *Nitella hyalina* (de Cand.) Ag. Healthy, 10-14 cm tall, uncorticated, male and female plants with 5-6 branches, free from epiphytes and animal castings were thoroughly washed and air-dried in shade. The dried material (450 g) was soaked in distilled methanol (MeOH) at room temperature for 3 weeks and repeatedly extracted 4 times. The combined extracts were evaporated under reduced pressure on a rotary evaporator and the gummy residue (38.56 g) obtained was partitioned between ethyl acetate (EtOAc) and distilled water. The EtOAc layer after evaporation (8.17 g) was subjected to column chromatography (CC) on silica gel (70-230 mesh) using *n*-hexane and *n*-hexane: $CHCl_3$ solvent system, from where about 200 fractions, 250 mL each, were collected.

Analysis of fatty acids: Five oily fractions (A, B, C, D & E) obtained were analysed for fatty acids. Fractions A and B were eluted from EtOAc column in pure hexane, fractions C and D in *n*-hexane:$CHCl_3$ (9:1, v/v), and fraction E in *n*-hexane:$CHCl_3$ (8.1:1.5, v/v). Each fraction was acidified with 6N HCl (pH 5.6) and then extracted several times with Et_2O. The total Et_2O fraction was dried over anhydrous Na_2SO_4 and on evaporation of Et_2O a 5 g residue was obtained. A fraction weighing 0.5 mg was dissolved in MeOH in which 0.5 mL of ethereal diazomethane solution was added. The reaction mixture was kept overnight at room temperature (28°C), then it was evaporated under reduced pressure. The aliquots were directly injected into GC-MS for analysis which was performed on a Hewlett Packard GC with a 11/73 DEC computer data system and a 1.2 m x 4 mm packed glass-capillary column coated with gas chrome Q (100-120 mesh, OV 101 1%). The column temperature was programmed for 70° - 250°C with a rate of increase of 8°C per min. The carrier gas (He) flow rate was 32 mL/min., injector temperature was 250°C. Fatty acid methyl esters were identified by matching their spectra with those in the NBS mass spectral library (Helles & Milne, 1978).

Extraction of sterols: From rest of the above mentioned fractions sterols were extracted. Sterol mixture was separated by TLC (DC-Microcards SI F 5x10 cm, silica gel with fluorescent indicator 254 nm, layer thickness 0.2 mm, Riedel-de-Haen) and purified by flash chromatography. The Pure compounds were subjected to spectral characterisation. The IR spectra were recorded with JASCO A-302 spectrophotometer. The EI-MS was performed on a Finnigan MAT 112 double focussing mass spectrometer connected to a PDP 11/34 (DEC) computer system. The ^{1}H-NMR spectra were obtained in $CDCl_3$ as a solvent and TMS as the internal reference on Bruker AM 300 spectrometer equipped with an Aspect 300 data system. The ^{13}C-NMR spectra were measured on the same instrument at 75.43 MHz.

Bioactivity studies: Antibacterial activity was determined by agar-well diffusion method in which 24 h old culture containing approximately 10^4-10^6 cfu was spread on the surface of Meller Hinton Agar (MHA) plates. Wells were dug in the medium with the help of a 5 mm diam. sterile metallic borer. Test samples of different concentrations

were added in their respective wells. Experimental plates were incubated at 37 C for 24 h and zones of inhibition were compared with ampicillin used as standard antibiotic. The antifungal activity was also studied by using agar diffusion method, in which griseofulvin was used as standard antibiotic. Test tubes having sterile SDA were inoculated with test compound and kept in slanting position at room temperature. Test fungal cultures were inoculated on the slant and growth inhibition was observed after incubation period of 7 days. For phytotoxicity bioassay, single plants of *Lemna minor* were placed into vials containing 2 mL of medium, with appropriate dilutions of test substance incubated in growth chamber at 29°C for 7 days. Number of fronds were counted and 50% frond inhibition (FI_{50}) and frond promotion (FP_{50}) values determined using a Finney program on IBM computer.

Results and Discussion

Using GC-MS technique a total number of 23 fatty acids (FAs) were detected as methyl esters in the MeOH extract of *Chara corallina* var. *wallichii* (Table 1), indicating the presence of a wide variety of saturated (SFAs, 36.5%) and unsaturated fatty acids (UFAs, 63.5%). Such similar reports have been made in Ulvalves, Cladophorales, Siphonocladales, Bryopsidales, Codiales and Caulerpales (Aknin *et al.*, 1992a; Aliya *et al.*, 1995). Pentadecylic (C15:0) and palmitic (C16:0) acids appeared to be the major SFAs, the latter being in largest quantity (17.7%), which is also a character of the Ulvales and several siphonaceous chlorophytes. They show palmitic acid in largest amounts too (Shameel & Khan, 1991; Aknin *et al.*, 1992a; Vaskovsky *et al.*, 1996). However, the detected SFAs ranged from C7, C10-C18, and in this way it differs from marine chlorophytes which possess SFAs up to C29:0 (Aliya *et al.*, 1995) and resemble with higher plants which produce FAs with relatively short carbon chains (Pohl & Zurheide, 1979).

The 16 UFAs detected include 6 monoenoic, 5 dienoic and 5 trienoic acids, of which one is a substituted acid. With the exception of pentacosenoic acid (C25:1), they ranged from C10 to C18, while marine chlorophytes display a long range of C9 - C29 of UFAs (Aliya *et al.*, 1995). In this way again it resembles with land plants, which usually synthesize FAs with a chain of up to C18 (Pohl & Zurheide, 1979). Oleic acid (C18:1) is the most predominant UFA in several green seaweeds (Shameel & Khan, 1991; Aknin *et al.*, 1992a), which was detected in very low amount (1.8%). Among UFAs, the dienoic acids were present in largest proportion (35.2% of all FAs), which is almost equal to that of SFAs (36.5%). Hexadecadienoic acid (C16:2) occurred in greatest amount (23.7%) among all FAs. It was found from 3.8 to 13.1% in several chlorophytes (Aliya *et al.*, 1995). The terrestrial plants usually produce good amounts of 16:1, 16:3, 18:1 and 18:3 FAs (Pohl & Zurheide, 1979), which in the present study were detected in poor ratios (0.9-1.8%).

Green seaweeds are rich in polyunsaturated fatty acids, PUFAs (Aknin *et al.*, 1992a; Vaskovsky *et al.*, 1996), but only a few terrestrial plants are able to synthesize the 18:4 or C20 and C22 PUFAs (Pohl & Zurheide, 1979). It is interesting to note that *C. corallina* did not show the presence of any of these PUFAs and no acid either with more than three double bonds or with 18 C atoms could be detected. Freshwater green

Table 1. Fatty acids analysed as methyl esters from *Chara corallina* var. *wallichii*.

Acid type	Systematic name	Common name	Molecular formula	Mol. wt.	R:R.T.	Rel. % age
A. Saturated fatty acid methyl esters:						**36.45**
C7:0	*n*-Heptanoate	Heptylate	$C_8H_{16}O_2$	144	1.00	0.21
C12:0	*n*-Dodecanoate	Laurate	$C_{13}H_{26}O_2$	214	1.01	3.53
C13:0	*n*-Tridecanoate	Tridecylate	$C_{14}H_{28}O_2$	228	1.02	1.77
C15:0	*n*-Pentadecanoate	Pentadecylate	$C_{16}H_{32}O_2$	256	1.03	10.61
C16:0	*n*-Hexadecanoate	Palmitate	$C_{17}H_{34}O_2$	270	1.04	17.69
C17:0	*n*-Heptadecanoate	Margarate	$C_{18}H_{36}O_2$	284	1.05	1.76
C18:0	*n*-Octadecanoate	Stearate	$C_{19}H_{38}O_2$	298	1.09	0.88
B. Monoenoic fatty acid methyl esters:						**13.24**
C10:1	9-Decenoate	Decenoate	$C_{11}H_{20}O_2$	184	1.05	1.76
C13:1	9-Tridecenoate	Decylacrylate	$C_{14}H_{26}O_2$	226	1.08	4.42
C16:1	9-Hexadecenoate	Palmitoleate	$C_{17}H_{32}O_2$	268	1.09	1.77
C17:1	9-Heptadecenoate	Heptadecenoate	$C_{18}H_{34}O_2$	282	1.40	2.65
C18:1	9-Octadecenoate	Oleate	$C_{19}H_{36}O_2$	296	1.64	1.76
C25:1	Pentacosenoate	Pentacosenoate	$C_{26}H_{50}O_2$	394	1.72	0.88
C. Dienoic fatty acid methyl esters:						**35.17**
C11:2	7-Ethyl-3-methyl-2,6-undecadienoate	Undecadienoate	$C_{15}H_{26}O_2$	238	1.08	0.88
C13:2	Tridecadienoate	Tridecadienoate	$C_{14}H_{24}O_2$	224	1.06	2.65
C16:2	Hexadecadienoate	Hexadecadienoate	$C_{17}H_{30}O_2$	266	1.09	23.69
C17:2	9,12-Heptadecadienoate	Heptadecadienoate	$C_{18}H_{32}O_2$	280	1.31	1.76
C18:2	9,12-Octadecadienoate	Linoleate	$C_{19}H_{34}O_2$	294	1.62	6.19
D. Trienoic fatty acid methyl esters:						**15.06**
C13:3	Tridecatrienoate	Tridecatrienoate	$C_{14}H_{22}O_2$	222	1.06	0.88
C14:3	2,4,5-Tetradecatrienoate	Tetradecatrienoate	$C_{15}H_{24}O_2$	236	1.07	5.30
C16:3	6,10,14-Hexadecatrienoate	Hiragonate	$C_{17}H_{28}O_2$	264	1.08	0.88
C17:3	Heptadecatrienoate	Heptadecatrienoate	$C_{18}H_{30}O_2$	278	1.12	6.24
C18:3	9,12,15-Octadecatrienoate	Linolenoate	$C_{19}H_{32}O_2$	292	1.56	1.76

R.R.T. = relative retention time, in relation with that of methyl heptylate

algae in general, contain few FAs with more than 3 double bonds or more than 18 C atoms (Wood, 1974). The highest PUFA of the freshwater Chlorophyta normally is α-linolenic acid (9,12,15-18:3) as also observed in *C. c. var. wallichii*. The chlorophytes primarily synthesize C16 and C18 FAs, and *C. c. var. wallichii* displayed the presence of C16:0, C16:1, C16:2, C16:3, C18:0, C18:1, C18:2 and C18:3 acids. It would

Table 2. Sterols isolated from *Chara corallina* var. *wallichii*.

Common name	Systematic name	Molecular formula	Mol. wt.	Fraction (v/v) hexane $CHCl_3$	Rel. % age
Cholesterol	Cholest-5-en-3ß-ol	$C_{27}H_{46}O$ [1]	386	8:2	8.7
Clerosterol	24R-Stigmasta-5,25-dien-3ß-ol	$C_{29}H_{48}O$ [2]	412	7:3	7.3
Stigmasterol	24S-Stigmasta-5,22E-dien-3ß-ol	$C_{29}H_{48}O$ [3]	412	9:1	6.4
ß-Sitosterol	24R-Stigmast-5-en-3ß-ol	$C_{29}H_{50}O$ [4]	414	8:2	76.8

The number within bracket under molecular formula refers to the structure in Fig 1.

suggest that *C. c. var. wallichii* resembles with chlorophytes in some characters of FA composition and with higher plants in the others.

From MeOH extract of *Chara corallina* var. *wallichii*, 4 sterols were obtained (Table 2) and their structures were elucidated by IR, EI-MS, ^{1}H- & ^{13}C-NMR spectroscopy (Fig 1). The ß-sitosterol was present in greatest quantity (76.8%), while cholesterol, clerosterol and stigmasterol were found in relatively small amounts (6.4-8.7%). Sitosterol and fucosterol were detected as principal sterols in *Nitella opaca* (Ag. *ex* Bruz.) Ag. (Heilbron, 1942); while clionasterol, an isomer of sitosterol, and 28-isofucosterol were found to be the major sterols of *Chara vulgaris* L. and *Nitella flexilis* (L.) Ag. (Patterson, 1972). In *Chara australis* R. Br., *C. buckelii* G.O.A. and several other charophytes 24-ethylcholesterol (more clionasterol than sitosterol) was observed to be the principal sterol along with 28-isofucosterol (Patterson *et al.*, 1991). No fucosterol or its geometric isomer 28-isofucosterol could be detected in *C. c. var. wallichii*. Clionasterol is the major sterol of several members of Cladophorales, Bryopsidales and Caulerpales, and fucosterol and 28-isofucosterol are the chief sterols of Ulvales and certain other chlorophytes (Aknin *et al.*, 1992b). In contrast, in bryophytes, peteridophytes and higher plants campesterol and sitosterol are the principal components while 28-isofucosterol is present only in small amounts (Nes & McKean, 1977).

Poriferasterol, an isomer of stigmasterol is synthesized by several members of Ulvales, Caulerpales and Codiales (Aknin *et al.*, 1992b), but latter sterol is found mainly in higher plants (Goodwin, 1974). Stigmasterol was found in the present species in small ratio (6.4%). A 24-ethylcholesta-5,22-dienol was also detected in traces (1%) in *C. australis* and *C. buckelii* (Patterson *et al.*, 1991). Cholesterol and clerosterol were detected in *C. c. var. wallichii* in small amounts (7.3-8.7%). Cholesterol is also reported to be present in 7 charophytes upto 25% (Patterson *et al.*, 1991). It is commonly found in chlorophytes and sometimes may be present in higher amounts of

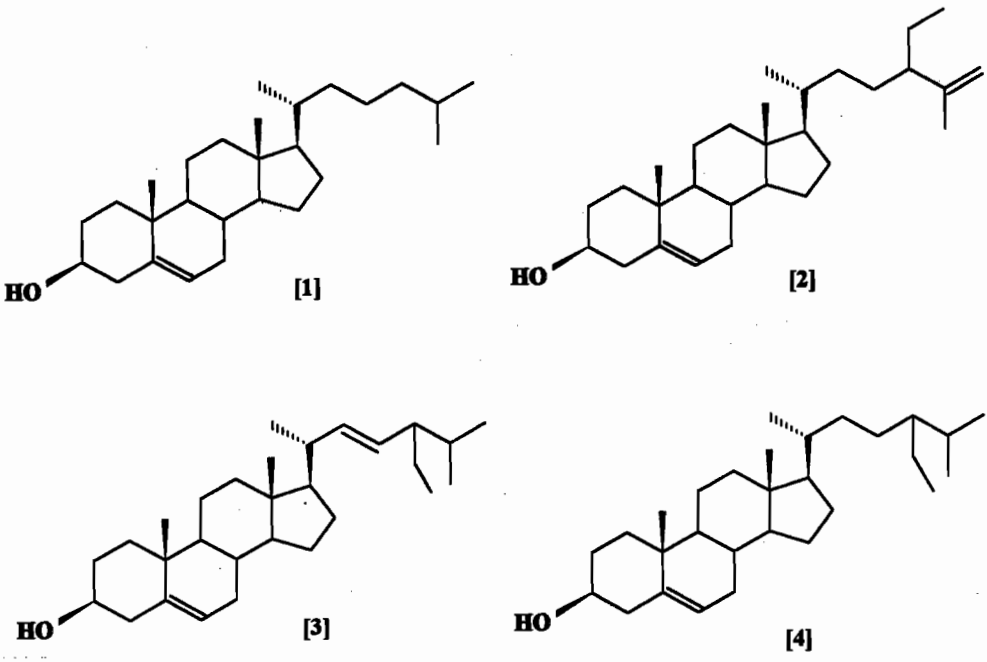

Fig.1. Sterols isolated from *Chara corallina* var. *wallichii*: [1] = cholesterol, [2] = clerosterol, [3] = stigmasterol, [4] = ß-sitosterol.

13.1-41.2% (Aknin *et al.*, 1992b). Although, clerosterol occurs in small quantities of 0.2-3.4% among Ulvales, Cladophorales, Bryopsidales and Caulerpales, but in a great amount of 80.9-83.3% in Codiales (Aknin *et al.*, 1992b; Aliya & Shameel, 1993, 1998). It would suggest that in sterol composition *C. c. var. wallichii* resembles with chlorophytes in some characters and with higher plants in the others.

The MeOH extract, EtOAc-soluble part of MeOH extract and 4 isolated sterols from *Chara corallina* var. *wallichii* were tested for antibacterial activity in order to determine how bioactivity transfers from crude extract to pure compounds. Both MeOH extract and its EtOAc-soluble part were active against *Corynebacterium diphtheriae, Escherichia coli, Pseudomonas aeruginosa, Salmonella typhiae, Staphylococcus aureus, Streptococcus pyrogenes* and *Vibrio choleriae*, but were not active against *Corynebacterium hoffmannii, Klebsiella pneumoniae* and *Shigella flexneriae* (Table 3). The aqueous extract of *Chara globularis* Thuill., which contains Charamin is reported to show antibiotic activity against a natural population of bacteria from pond water (Anthoni *et al.*, 1987).

All the four sterols exhibited activity against *Escherichia coli* and *Streptococcus pyrogenes* only, while against other bacteria some of these sterols were active and others inactive. The ß-sitosterol was active against 4 bacteria and other three sterols against 5 bacteria, but all of them exhibited activity against different bacterial

Table 3. Antibacterial activity of *Chara corallina* var. *wallichii* extracts and isolated sterols.

Bacteria	Test sample	Zone of inhibition		Ampicillin	
		100 μg/100 μL	200 μg/100 μL	100 μg	200 μg
Corynebacterium diphtheriae	M	—	6	19	20
	E	—	6	"	"
	CH	—	6	"	"
	CL	—	7	"	"
	ST	—	—	"	"
	SI	—	6	"	"
Corynebacterium hoffmannii	M	—	—	19	20
	E	—	—	"	"
	CH	—	—	"	"
	CL	—	—	"	"
	ST	—	—	"	"
	SI	—	—	"	"
Escherichia coli	M	—	6	19	20
	E	8	7	"	"
	CH	—	6	"	"
	CL	—	6	"	"
	ST	6	7	"	"
	SI	—	7	"	"
Klebsiella pneumoniae	M	—	—	17	20
	E	—	—	"	"
	CH	—	—	"	"
	CL	—	—	"	"
	ST	—	—	"	"
	SI	—	—	"	"
Pseudomonas aeruginosa	M	7	7	17	19
	E	6	8	"	"
	CH	6	7	"	"
	CL	—	6	"	"
	ST	—	—	"	"
	SI	—	7	"	"
Salmonella typhiae	M	—	7	20	22
	E	7	6	"	"
	CH	8	7	"	"
	CL	—	—	"	"
	ST	6	7	"	"
	SI	—	—	"	"

Table 3 (Cont'd)

Bacteria	Test sample	Zone of inhibition		Ampicillin	
		100 μg/100 μL	200 μg/100 μL	100 μg	200 μg
Shigella flexneriae	M	—	—	16	18
	E	—	—	"	"
	CH	—	—	"	"
	CL	—	—	"	"
	ST	—	—	"	"
	SI	—	—	"	"
Staphylococcus aureus	M	—	6	22	23
	E	—	6	"	"
	CH	—	—	"	"
	CL	—	—	"	"
	ST	—	7	"	"
	SI	—	—	"	"
Streptococcus pyrogenes	M	6	7	17	20
	E	6	7	"	"
	CH	6	7	"	"
	CL	6	7	"	"
	ST	6	7	"	"
	SI	6	7	"	"
Vibrio choleriae	M	—	6	19	20
	E	—	7	"	"
	CH	—	—	"	"
	CL	—	7	"	"
	ST	—	7	"	"
	SI	—	—	"	"

M = MeOH extract, E = EtOAc-soluble part, CH = cholesterol, CL = clerosterol, ST = stigmasterol, SI = ß-sitosteorl; (—) = bacterial growth.
The values indicate zone of inhibition in mm.

organisms. It appears that different sterols vary in their specificity for antibacterial activity. Species belonging to the same genus *e.g.*, *Corynebacterium* showed differences in their sensitivity against extracted sterols. Certain tricyclic diterpenoids isolated from *Stoechospermum marginatum* displayed activity against some of these bacterial species (Shaikh *et al.*, 1990). All the test samples were inactive against *Corynebacterium hoffmannii, Klebsiella pneumoniae* and *Shigella flexneriae*. The MeOH extract, its EtOAc-soluble part and 20-hydroxyecdysone isolated from *Asparagus dumosus* L. also exhibited no activity against *K. pneumoniae* (Ahmad *et al.*, 1996).

Table 4. Antifungal activity of *Chara corallina* var. *wallichii* extracts and isolated sterols.

Fungi	Test sample	Inference (activity)	Griseofulvin (reference) 400 μg/mL	Griseofulvin (reference) 200 μg/mL
Allescheria	M	+	++	+
boydii	E	++	"	"
	CH	+	"	"
	CL	—	"	"
	ST	++	"	"
	SI	—	"	"
Aspergillus	M	++	+	+
niger	E	+	"	"
	CH	+	"	"
	CL	+	"	"
	ST	—	"	"
	SI	—	"	"
Candida	M	—	+	+
albicans	E	—	"	"
	CH	—	"	"
	CL	—	"	"
	ST	—	"	"
	SI	—	"	"
Curvularia	M	++	+	+
lunata	E	++	"	"
	CH	++	"	"
	CL	++	"	"
	ST	+	"	"
	SI	+	"	"
Drechslera	M	—	+	+
rostrata	E	—	"	"
	CH	—	"	"
	CL	—	"	"
	ST	—	"	"
	SI	—	"	"
Microsporum	M	++	+++	++
canis	E	+++	"	"
	CH	++	"	"
	CL	+	"	"
	ST	+++	"	"
	SI	++	"	"

Table 4 (Cont'd)

Fungi	Test sample	Inference (activity)	Griseofulvin (reference) 400 μg/mL	Griseofulvin (reference) 200 μg/mL
Nigrospora	M	++	++	+
oryzae	E	++	"	"
	CH	++	"	"
	CL	++	"	"
	ST	+	"	"
	SI	—	"	"
Pleurotus	M	+++	++	+
austreatus	E	++	"	"
	CH	+	"	"
	CL	+	"	"
	ST	+++	"	"
	SI	++	"	"
Stachybotrys	M	+++	+++	++
atra	E	+++	"	"
	CH	+	"	"
	CL	+++	"	"
	ST	++	"	"
	SI	++	"	"
Trichophyton	M	++	++	+++
mentagrophytes	E	+++	"	"
	CH	++	"	"
	CL	+	"	"
	ST	+++	"	"
	SI	++	"	"

M = MeOH extract (400 μg/mL), E = EtOAc-soluble part (400 μg/mL), CH = cholesterol (200 μg/mL), CL = clerosterol (200 μg/mL), ST = stigmasterol (200 mg/mL), SI = ß-sitosterol (200 mg/mL); (—) = no activity, (+) = low activity, (++) = moderate activity, (+++) = strong activity, in terms of developed colonies.

Six test samples of MeOH extract of *Chara corallina* var. *wallichii* and its EtOAc-soluble part showed antifungal activity against *Allescheria boydii, Aspergillus niger, Curvularia lunata, Microsporum canis, Nigrospora oryzae, Pleuroetus austreatus, Stachybotrys atra* and *Trichophyton mentagrophytes* (Table 4). Both these samples were equally active against *C. lunata* and *S. atra*, while against other fungi one test sample was more active than the other. All the four sterols displayed activity against *Curvularia lunata, Microsporum canis, Pleurotus austreatus, Stachybotrys atra* and *Trichophyton mentagrophytes*, while against other three fungi some of these sterols were active and others not. Cholesterol appeared to be the most active sterol as it

Table 5. Phytotoxicity effect of *Chara corallina* var. *wallichii* in *Lemna minor*

Test sample	Concentration (ppm)	% Inhibition	
		Sample	Standard
MeOH extract	5	26.25	100
	50	40.00	"
	500	60.00	"
EtOAc-soluble part	5	30.85	100
	50	42.55	"
	500	71.27	"
Cholesterol	5	15.06	100
	50	25.24	"
	500	42.26	"
Clerosterol	5	35.65	100
	50	40.25	"
	500	72.15	"
Stigmasterol	5	26.25	100
	50	35.00	"
	500	51.25	"
ß-Sitosterol	5	14.89	100
	50	24.46	"
	500	40.42	"

showed inhibitory effects against 8 fungi, while ß-sitosterol being the least active sterol exhibited activity against only 5 fungal species. Cholesterol and stigmasterol extracted from *Porphyra vietnamensis* also displayed a strong antifungal activity against several fungi (Shameel & Aftab, 1993). A tricyclic diterpenoid isolated from *Stoechospermum marginatum* showed activity against *Trichophyton rubrum* (Shaikh *et al.*, 1990). All the test samples were inactive against *Candida albicans* and *Drechslera rostrata*. The MeOH extract, its EtOAc-soluble part and 20-hydroxyecdysone isolated from *Asparagus dumosus* also showed no activity against *C. albicans* (Ahmad *et al.*, 1996).

Different test samples *i.e.*, MeOH extract, its EtOAc-soluble part, cholesterol, clerosterol, stigmasterol and ß-sitosterol isolated from *Chara corallina* var. *wallichii* exhibited strong inhibition in *Lemna minor* and with increase in concentration from 5.0-500 ppm percent inhibition also increased (Table 5). Clerosterol displayed the highest percentage of inhibition (72.2%) and ß-sitosterol the lowest (40.4%) at 500 ppm concentration. It appears that ß-sitosterol is the least active sterol in antibacterial and antifungal activities as well as phytotoxicity. *Chara globularis* is reported to contain compounds with insecticidal properties (Jacobsen & Pedersen, 1983). The cytokinins, known to be ubiquitous among higher plants, have also been isolated from this alga (Zhang *et al.*, 1989) and abscisic acid (ABA) has been detected in *C. foetida* (Tietz *et al.*, 1989).

The results of the present study would suggest that strong bioactivity and phytotoxic effects of *C. corallina* are presumably responsible for its dominance in the aquatic ecosystem of River Indus in Sindh, Pakistan, with the result that it develops a dense forest in the water channels and brooklets. Herbivores avoid these forests and other water plants are prevented to grow in its vicinity. This alga is highly significant as its extract is strongly effective against different bacteria producing diseases and could be used as a potent ingredient in different medicines. Its extract is also effective against common pathogenic fungi causing great damage to crops, human body and other organisms. Its extract may also be used to eradicate aquatic weeds.

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