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ANTIMICROBIAL ACTIVITY AND PHYTOTOXICITY OF STEROLS FROM *CHARA WALLICHII* A. BR. (CHAROPHYTA)

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The antibacterial, antifungal and phytotoxicity tests were performed on the methanolic extract, ethylacetate fraction and four sterols isolated from the green alga *Chara wallichii* A. Br., collected from flood water of River Indus, near Petaro, Sindh, Pakistan. The compounds named as cholesterol (1), clerosterol (2), stigmasterol (3) and β-sitosterol (4) have been isolated for the first time from this alga and their antimicrobial activities as well as phytotoxicity studies are being reported.

Key words: *Chara wallichii*, Charophyta, Algae, Antifungal activity, Antibacterial activity, Phytotoxicity, Sterols.

Introduction

Charophytes are major components of brackish and freshwater ecosystems, and they reveal certain medicinal properties. *Chara globularis* is reported to contain compounds with insecticidal properties (Jacobsen and Pedersen 1983). Certain cytokinins have also been isolated from this alga (Zhang *et al* 1989). Abscisic acid (ABA) has been detected in *C. foetida* (Tietz *et al* 1989). Some information is available on the natural products of charophytes (Heilbron 1942; Patterson 1972; Alary-Bernard *et al* 1980; Sakano *et al* 1983; Sato and Furuya 1985; Patterson *et al* 1991; Khaliq-uz-Zaman *et al* 1998). The present study describes the isolation of sterols from a charophyte, *Chara wallichii* A. Br. Bioactivity of the extracts and the isolated sterols was examined for a better insight of medicinal significance of this alga.

Materials and Methods

Fresh specimens of *Chara wallichii* A. Br. were collected from flood water of River Indus, Petaro, Sindh, Pakistan during October 1997. The dioecious algae with red coloured gametes were found growing along with grasses. A voucher specimen No. KUH-SW 1026 has been deposited in the herbarium of Seaweed Biology and Phycochemistry Lab., MAHQ Biological Research Center, University of Karachi. The fresh algal material (1 kg) was soaked in distilled methanol for 3 weeks at room temperature and repeatedly extracted 4 times. The combined extracts were evaporated under reduced pressure and the resulting gummy residue (38.6 g) was partitioned between ethyl acetate and distilled water. The ethyl acetate extract (8.2 g) after removal of the solvent under reduced pressure was subjected to column chromatography on silica gel (70-230 mesh). By using *n*-hexane and *n*-hexane: choloroform as a solvent system, about 200 fractions were collected, 25 ml each.

From the above mentioned fractions a number of sterols were collected which were purified by flash chromatography and detected by TLC (DC-Microcards SI F5 x 10 cm, silica gel). The pure compounds were subjected to spectral characterization (MS & NMR) and as a result four sterols namely cholesterol (1), clerosterol (2), stigmasterol (3) and β-sitosterol (4) were identified (Fig 1).

The antibacterial activity was achieved by agar well diffusion method (Bauer *et al* 1966; Barrett and Watt 1979). At first 24 hour old cultures containing approximately 10^4-10^6 CFU were spread on the surface of MHA plates. In the medium a number of wells were dugged with the help of sterile metallic borer. Test samples of different concentrations were added in their respective wells. All the above experimental plates were incubated at 37°C for 24 hours and different zones of inhibition were measured in mm and then compared with standard antibiotics. Different bacteria used for the assay are listed in Table 1.

The antifungal activity was carried out by agar diffusion method (Washington and Sutter 1980; Jack 1991). The test tubes having a sterile SDA were inoculated with test com-

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pounds *i.e.* four sterols, methanolic extract and its ethylacetate fraction; in total there were six test samples. All these test tubes were placed in a slanting position at room temperature and then test fungal cultures were inoculated on the slant and growth inhibition was observed after an incubation period of 7 days. Different fungi used for this assay are listed in Table 2.

For the phytotoxicity bioassay *Lemna minor* L. plants were placed into vials containing 2 ml of medium, with appropriate dilutions of test substances, which were incubated in growth chamber at 29°C for 7 days. The number of fronds were counted and 50 % frond inhibition (FI_{50}) and frond promotion (FP_{50}) values were determined by using a Finney programme on computer. This bioassay is used for inhibitors and promoters of plant growth (Ferrigni *et al* 1982; Einhellig *et al* 1985; Zeringue 1987). *Lemna minor* is an aquatic monocot consisting of three fronds and a filamentous root.

Results and Discussion

The results of antibacterial and antifungal activities and *Lemna* acquinoc tialis Wela bioassay are given in Tables 1, 2 and 3, respectively. The methanolic extract, its ethylacetate soluble fraction and four sterols, i.e. cholesterol (1), clerosterol (2), stigmasterol (3) and β-sitosterol (4) were investigated stepwise (Fig. 1). All the six test samples were tested in order to determine how bioactivity transfers from crude extract to pure compounds.

All the six samples were found to be strongly active against seven bacteria (Table 1) and eight fungi (Table 2). They are inactive against only three bacteria, *Corynebacterium hoffmannii, Klebsiella pneumoniae* and *Shigella flexneriae* and two fungi, *Candida albicans* and *Drechslera rostrata.*

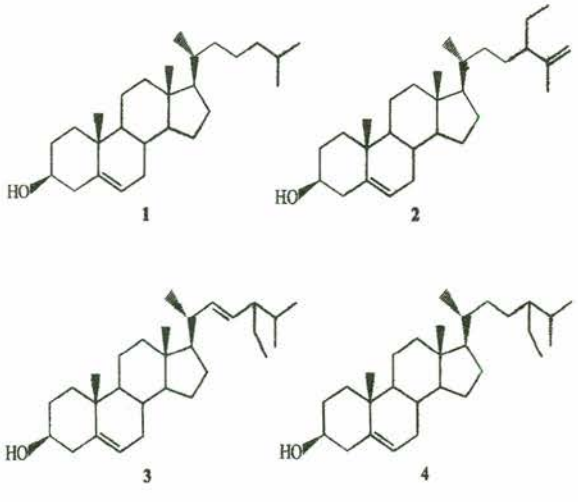

Fig 1. Sterols isolated from *Chara wallichi* A. Br.
1, cholesterol; 2, clerosterol; 3, stigmasterol; 4, β-sitosterol.

Table 1
Antibacterial activity of *Chara wallichii* extracts and isolated sterols

Bacteria	Test Sample *	Zone of inhibition**		Ampicillin	
		100µg 100µl^{-1}	200µg 100µl^{-1}	100µg 100µl^{-1}	200µg 100µl^{-1}
Corynebacterium diptheriae	M	-	6	19	20
	E	-	6		
	CH	-	6		
	CL	-	7		
	ST	-	-		
	SI	-	6		
Escherichia coli	M	-	6	19	20
	E	8	7		
	CH	-	6		
	CL	-	6		
	ST	6	7		
	SI	-	7		
Pseudomonas aeruginosa	M	7	7	17	19
	E	6	8		
	Ch	6	7		
	CL	-	6		
	ST	-	-		
	SI	-	7		
Salmonella typhi	M	-	7	20	22
	E	7	6		
	CH	8	7		
	CL	-	-		
	ST	6	7		
	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 cholerae	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, β-sitosterol; (-), bacterial growth.
** The values indicate zone of inhibition in mm.

Table 2
Antifungal activity of *Chara wallichii* extracts and isolated sterols

Fungi	Test Sample	Inference (Activity)	Griseofulvin (Reference) 400µg ml^{-1}	200µg ml^{-1}
	M	+		
	E	++		
Allescheria	CH	+	++	+
boydii	CL	-		
	ST	++		
	SI	-		
	M	++		
	E	+		
Aspergillus	CH	+	+	+
niger	CL	+		
	ST	-		
	SI	-		
	M	++		
	E	++		
Curvularia	CH	++	+	+
lunata	CL	++		
	ST	+		
	SI	+		
	M	++		
	E	+++		
Microsporum	CH	++	+++	++
canis	CL	+		
	ST	+++		
	SI	++		
	M	++		
	E	++		
Nigrospora	CH	++	++	+
oryzae	CL	++		
	ST	+		
	SI	-		
	M	+++		
	E	++		
Pleurotus	CH	+	++	+
austreatus	CL	+		
	ST	+++		
	SI	++		
	M	+++		
	E	+++		
Stachybotrys	CH	+	+++	++
atra	CL	+++		
	ST	++		
	SI	++		
	M	++		
	E	+++		
Trichophyton	CH	++	++	+++
mentagrophytes	CL	+		
	ST	+++		
	SI	++		

M, MeOH extract (400 µg ml^{-1}); E, EtOAc-soluble part (400µg ml^{-1}); CH, cholesterol (200µg ml^{-1}); CL, clerosterol (200µg ml^{-1}); ST, stigmasterol (200µg ml^{-1}); SI, β-sitosterol (200µg ml^{-1}); (-), no activity; (+), low activity; (++), moderate activity; (+++), strong activity, in terms of developed colonies.

Inhibition was observed in all the cases of *Lemna minor* bioassay (Table 3), so it can be said that the crude extracts as well as all the pure compounds manifest toxicity. The extracts isolated from *Asparagus dumosus* and *Chara corallina* also exhibited no activity against *K. pneumoniae* and *C. albicans* (Ahmad *et al* 1996; Khaliq-uz-Zaman *et al* 1998). Cholesterol and stigmasterol extracted from *Porphyra vietnamensis* also displayed a strong antifungal activity against several fungi (Shameel and Aftab 1993).

The present study reveals excellent antimicrobial activity and phytotoxicity in the alga *Chara wallichii*. Similar results were also obtained on *C. corallina* in a previous study (Khaliq-uz-

Table 3
Phytotoxicity effect of *Chara wallichii* in *Lemna minor*

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

Standard compound used, Paraquat; No. of vials/conc, 10; Incubation period, 7 days; Incubation temperature, 27-29°C.

Zaman *et al* 1998). *Chara globularis* has been reported to contain compounds with insecticidal properties (Jacobsen and Pedersen 1983). These studies can be promoted for further investigations which may be useful for drug therapy.

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