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STUDIES ON THE BIOACTIVITY AND PHYCOCHEMISTRY OF *MICROCYSTIS AERUGINOSA* (CYANOPHYCOTA) FROM SINDH

M.N. KHALID[1], MUSTAFA SHAMEEL[1], V.U. AHMAD[2], SALEEM SHAHZAD[3] AND S.M. LEGHARI[4]

[1]*Department of Botany, University of Karachi, Karachi-75270, Pakistan*
[2]*HEJ Research Institute of Chemistry, University of Karachi*
[3]*Department of Agriculture & Agribusiness Management, University of Karachi*
[4]*Institute of Advance Research Studies in Chemical Sciences, University of Sindh, Jamshoro-76080*

Abstract

A toxic alga, *Microcystis aeruginosa* (KÜtzing) KÜtzing was collected from ponds of Mancher Lake, near Thatta, Sindh (Pakistan) during October 1994 and extracted in methanol. The crude extract showed a strong antimicrobial activity against 14 bacterial and 20 fungal species including 7 human-, 5 plant- pathogens and 8 saprophytes, but its cytotoxic activity against brine shrimp larvae was non-significant. A variety of fatty acids (FAs) were detected from the extract by GC-MS, including 7 saturated, 7 mono-, 4 di-, 7 tri- and 2 poly-unsaturated FAs. Oleic acid was present in the higest proportion (30.5 %) followed by hexa- decatetraenoic and pentadecylic acids (9-10 %). Palmitic acid was also present in appreciable quantity (5.9 %). Furthermore cholesterol, stigmasterol, β-sitosterol, phytol and sucrose have also been isolated from this extract and chemically elucidated by a variety of spectroscopic techniques.

Introduction

Phycochemistry is a new term first used by Shameel (1990), which is actually the study of natural products and chemical constituents occurring within algal thallus from a biological point of view. It primarily investigates the distribution of secondary metabolites in different body parts of algae under different seasons and variety of habitat conditions (Shameel, 2005). All over the world phycologists studied the different types of natural products occurring within marine algae. A variety of fatty acids (both saturated and unsaturated), sterols, terpenes and sugars have been isolated from them (Harvey, 1936; Percival & Young 1971, 1972; Patterson, 1972; Stewart, 1974; Patterson *et al.,* 1991; Khotimchenko, 1993; Loban & Harrison, 1997; Jensen, 2003). A very limited amount of phycochemical knowledge is available about freshwater algae, in comparison with the detailed work carried out on seaweeds, which includes not only the isolation of fatty acids but also a complete phycochemical analysis showing the types of sterols, terpenes, glycosides, polyols, halogenated compounds as well as new and novel metabolites.

The reason for the limited amount of work done on freshwater algae in comparison with seaweeds is partly due to their large size which makes a mass scale collection of seaweeds possible. There may be another possible reason that the isolation and purification of various types of compounds is easier in brown and red seaweeds due to less amount of chlorophyll pigment in them. The excessive amount of chlorophyll a and b present in freshwater algae (*e.g.* chlorophycotes and charophycotes) hinders the extraction of other compounds and their purification becomes difficult. The amount of chlorophyll is so much that it can never be completely removed by any solvent. Hence scientists have preferred to work on brown and red seaweeds for phycochemical studies.

Keeping the above ideas in mind, a long-term program was planned to collect algae from various freshwater habitats of Sindh and to investigate them phycochemically and from the point of view of their bioactivity. The idea was to compare the results obtained with their counterparts growing in the marine and estuarine environments. This is the first publication in this regard.

Materials and Methods

Algal material: *Microcystis aeruginosa* (KÜtzing) KÜtzing is very common in Mancher Lake, ponds of Thatta and also slow stream water at the Super High Way near Karachi (40 miles east), becoms abundant during the late summer time and appears in such dense growth that the colour of the water becomes bluish green. The specimens were collected during October 1994 from Nari. Colonies were found in attached condition but some were also free floating. They were in the form of irregularly lobed cylinders with firm mucilage, which were 1 meter or more long attached with the stones forming a long green stream of cold water at Gadaji Fall. The specimens were collected in a variety of forms of occurrences such as epilithic, epiphytic and free floating, and were pooled together. The collected material was dried in sunshade.

Extraction: The dried, chopped and weighed material was soaked in methanol (MeOH) in large glass jars and kept in the solvent for at least a month at room temperature. The extract of the material thus obtained was filtered to remove all solid algal particles and then evaporated on a rotary evaporator under reduced pressure. This yielded a dark green, thick residue which was then weighed. A small part of the residue was used for column chromatography.

Isolation of fatty acids (FAs)

Saponification: For this purpose 150 mL ethanol:water (EtOH:H_2O, 1:1, v/v) containing 10 % KOH was added to a small (weighed) portion of the extract. The mixture was then concentrated under reduced pressure and afterwards H_2O and diethyl ether (Et_2O) were added to it. It now vigorously shaken and the Et_2O layer was separated, which was evaporated and used for FA-analysis after esterification.

Column chromatography: The extracts were directly partitioned with H_2O and *n*-hexane or ethyl acetate (EtOAc). The washing with Et_2O or *n*-hexane was repeated thrice and the Et_2O or *n*-hexane layer was removed. The extract of *n*-hexane or Et_2O was then evaporated under reduced pressure to obtain a thick oily, dark green residue, which was weighed. A suitable sized silica gel column of grade 60 (70-230 mesh) was selected and the material was loaded on it. The fractions (100 mL) were initially eluted in *n*-hexane and then polarity was gradually increased by increasing the amount of Et_2O. The initial fractions, which were thick and oily starting from pure *n*-hexane up to about *n*-hexane:ether (70:30), were analysed for FAs after being methylated.

Esterification and identification: The FA-fractions obtained both by column chromatography and by saponification were esterified with diazomethane. For this purpose 0.5 mg of each fraction was dissolved in MeOH and 0.5 mL of diazomethane was added to it. After that the reaction mixture was kept overnight at room temperature

(28°C) and evaporated under reduced pressure. The fractions are then methylated, which were analysed for FAs methyl esters first by GLC and finally by the GC-MS. The identification of FAs was ascertained by matching their GC-mass spectra with those of the NBS-mass spectral library (Helles & Milne, 1978).

Separation of other natural products

Extraction: The procedure for the extraction of other natural products such as sterols, terpenes and carbohydrates was the same as described above for FAs. After the elution of initial fractions of FAs, the next eluted fractions were checked on TLC cards (5×10 cm) and the ones having similar profiles after being sprayed with $Ce(SO_4)_2$ were pooled together and then purified further for natural products.

Purification: It was carried out in order to remove the chlorophyll molecules attached with any natural product which might have been eluted along with the compound to be isolated. The purification was carried out either by repeated column chromatography (CC) or by thin layer (TLC) cards (20×20 cm). It was done by running the plates or cards in the suitable solvent system and then spraying them by $Ce(SO_4)_2$. The purified strip with the compound was scratched and the compound was filtered with chloroform in *n*-hexane and then the compound with the chloroform and MeOH was evaporated under vacuum to yield the purified compound.

Identification: The purified natural products were finally identified with the help of different spectroscopic methods, such as IR and UV, EI-, FAB-, FD- and HR-MS, NMR (^{1}H- & ^{13}C) and COSY-45 and 2D ^{1}H-^{13}C chemical shift experiments.

Instrumentation

CC and TLC: Thin layer chromatography (TLC) was performed on DC-Micro cards SIF 5×10 cm (silica gel with fluorescent indicator 254 nm on aluminium cards, layer thickness 0.2 nm). While purification of the isolated compounds was carried out on DC-cards SIF 20×20 cm (silica gel with the same properties). Preparative thick layer plates were prepared by pouring dilute silica gel PF 254 on simple glass plates (20×20 cm), which were first air-dried and then activated in an oven at 110°C. Final purity of a compound was usually checked on TLC (thin layer or thick layer) by spraying with 10% solution of $Ce(SO_4)_2$ in 2N H_2SO_4.

GLC and GC-MS: The FA fractions were initially analysed by gas liquid chromatography (GLC) along with methyl ester standards on a Shimadzu GC-9A model gas chromatograph, equipped with a Shimadzu C-R6A chromatopac integrator. The column length was 2 m, whereas inner diameter of the column was 3 mm and outer diameter 5 mm. The column material used was GP 3% SP-2310/2% SP-2300 on 100/120 Chromosorb WAW. The column initial temperature was 150°C, while final temperature was 250°C with a rate of increase of 8° C/min. The detector as well as injector temperature was 300°C, nitrogen flow rate was 30 mL/min. The FA methyl esters were finally analysed by gas chromatography-mass spectrometry (GC-MS), which was performed on a Hewlett Packard GC with a 11/73 DEC computer system and a 1.2 m × 4 mm packed glass capillary column, coated with gas

chrome Q (100-120 mesh, OY 101, 1%). The column temperature was programmed from 70 to 250° C with a rate of increase of 8° C/min. The carrier gas (He) flow rate was 32 mL/min., injector temperature was 250°C.

UV, IR and EI-MS: Ultraviolet (UV) spectra were recorded on a Pye-Unicam SP-800 spectrophotometer. Infra-red (IR) spectra were measured on a JASCO A-302 spectrometer. Electron impact mass spectra (EI-MS) were obtained on a Finnigan MAT-112 and -113 spectrometer coupled with PDP 11/34 computer system.

HR-, FD- and FAB-MS: The high resolution-mass spectrometry (HR-MS) and field desorption-mass spectrometry (FD-MS) were performed on a MAT-312 mass spectrometer. Negative ion fast atom bombardment-mass spectra (FAB-MS) were recorded on Finnigan MAT-312 and Joel JMS H×110 spectrometers coupled with PDP 11/34 and 11/73 computer systems, respectively.

^{1}H-, ^{13}C-NMR, COSY-45 and 2D ^{1}H-^{13}C: Nuclear magnetic resonance spectra (^{1}H- and ^{13}C-NMR) were performed on Bruker AM-300 and -400 spectrometers operating at 300 and 400 MHz for ^{1}H- and 75 and 100 MHz for ^{13}C-nuclei, respectively. The chemical shifts are reported in ppm relative to TMS. Two-dimensional COSY-45 experiments were acquired at 300 MHz with a sweep width of 4000 Hz (2K data points) in ω_2 and 2000 Mhz (256 t_1 values zero-filled to 1K) in ω_1. Heteronuclear 2D ^{1}H-^{13}C chemical shift correlation experiments were carried out at 300 MHz with a sweep width of 12820 H_2 (2K data points) in ω_1 and 1024 H_2 (256 t_1 values zero-filled to 2K) in ω_2. In both the 2D experiments a 2 seconds relation delay was used and 16 transients were performed for each t_1 value.

Bioactivity tests

Antibacterial activity: Methanol extract of the alga was used for this purpose. In bacterial diffusion technique antibacterial agents would diffuse into the medium in a circle around the reservoir (well/disc), inhibiting the growth of the organism wherever the concentration of antibacterial agent is high enough. The activity was determined by the agar well diffusion method (Carron *et al.,* 1987). About 24 hours old culture containing approximately 10^4-10^6 CFU (colony forming unit) was spread on the surface of MHA (Mueller Hinton Agar) plates. Wells were dug in the medium with the help of sterile metallic borer. Algal extracts of different concentrations were added in their respective wells. Experimental plates were incubated at 37°C for 24 hours, and zones of inhibition were measured and compared with standard antibiotics.

Antifungal activity: The agar tube dilution protocol of Brass *et al*., (1979) for *In vitro* fungicidal bioassay is as follows:

Methanol extract of the alga was dissolved in sterile DMSO, serving as stock solution.
Sabouraud agar was prepared by mixing Sabouraud 4% glucose agar and of agar-agar in 500 mL × distilled water.
It was then steamed to dissolve and dispensed a known amount into screw capped tubes.
Tubes containing medium were autoclaved at 121°C for 15 minutes.

The tubes were allowed to cool to 50°C, and nonsolidified Sabouraud agar medium were poisoned with 200 µL of extract pipetted from the stock solution. This gave final concentration of 200 µg/mL of medium and 400 µg/mL of medium for pure and crude extracts respectively.
Tubes were then allowed to solidify in slanted position at room temperature.
Each tube was inoculated with a 4 mm diameter piece of inoculum removed from a 7 day old culture of fungi.
For nonmycelial growth an agar surface streak was employed.
Other media were supplemented with DMSO to serve as control.
The tubes were incubated at 27-29° C for 7-10 days growth on the extract amended medium was determined visually with reference to the control.

Brine shrimp lethality bioassay: The protocol for this test is as follows:

A rectangular dish, 22×32 cm was filled up to half with brine solution (sea salt = 38 g/L of distilled water) then (50 mg) eggs of brine shrimp sprinkled and coved with lid. Incubate for hatching at 27° C for 2 days. Brine shrimp larvae *via* a light source and Pasteur pipette.
Dissolve sample (20 mg) in 2 mL of respective solvent and from this solution transfer 500 µL, 50 µL and 5 µL to vials corresponding to 1000, 100 and 10 µg/mL respectively. Let the solvent evaporate overnight.
After 2 days of hatching place 10 larvae per vial using a Pasteur pipette.
Raise the volume to 5 mL with syringe by adding sea water.
Incubate at 27°C for 24 h under illumination.
After 24 h recount and record the number of survivors.
Analyze the data with Finney computer program to determine LD_{50} (lethal dose) values at 95% confidence intervals. A copy of this program for IBM PC's is available from Dr. McLaughlin.
Additional dilutions at less than 10 µg/mL may be needed for potent materials. Intermediate concentrations may be prepared and tested to narrow the confidence intervals *e.g*., by using 2 mg/L (step 4 above) dilutions at 100, 10 and 1 µg/mL are easily prepared.

Results

Biological activities: Crude methanol 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 ***M. aeruginosa*** revealed very promising results of antimicrobial activities, but its cytotoxic activity against brine shrimp larvae was non-significant (Table 3).

Detection of fatty acids (FAs): Two fractions were obtained from column chromatography and analysed for FAs, fraction A was eluted from the column in *n*-hexane (100%) and fraction B in *n*-hexane:chloroform (95:05). Both the fractions were methylated by diazomethane and then analysed initially by GLC and finally by GC-MS. Identification of individual fatty acids was carried out by matching their mass spectra with NBS mass spectral library (Helles & Milne, 1978). It was observed that 27 different FAs were present in the extract, including 7 saturated and 20 unsaturated acids (Table 4).

Table 1. Antibacterial activity shown by the methanol extract of *Microcystis aeruginosa*.

Bacterial culture	Zone of inhibition (mm)	Reference drugs	Zone of inhibition (mm)
Bacillus cereus	17	Amoxicillin $(H_2O)_3$	19
		Ampicilln $(H_2O)_3$	19
Corynebacterium diphtheriae	19	Amoxicillin $(H_2O)_3$	-
		Ampicillin $(H_2O)_3$	16
Escherichia coli	16	Ainoxicillin $(H_2O)_3$	12
		Ampicillin $(H_2O)_3$	14
Klebsiella pneumoniae	13	Amoxicillin $(H_2O)_3$	-
		Ampicillin $(H_2O)_3$	9
Listeria monocytogenes	14	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	9	Amoxicillin $(H_2O)_3$	10
		Ampicillin $(H_2O)_3$	10
Pseudomonas aeruginosa	10	Amoxicillin $(H_2O)_3$	-
		Ampicillin $(H_2O)_3$	12
Salmonella typhi	23	Amoxicillin $(H_2O)_3$	20
		Ampicillin $(H_2O)_3$	21
Shigella boydii	20	Amoxicillin $(H_2O)_3$	21
		Ampicillin $(H_2O)_3$	21
Staphylococcus aureus	25	Amoxicillin $(H_2O)_3$	2
		Ampicillin $(H_2O)_3$	2
Streptococcus faecalis	14	Amoxicillin $(H_2O)_3$	11
		Ampicillin $(H_2O)_3$	11
Streptococcus pyogenes	24	Amoxicillin $(H_2O)_3$	17
		Ampicillin $(H_2O)_3$	20
Vibrio choleriae	16	Amoxicillin $(H_2O)_3$	11
		Ampicillin $(H_2O)_3$	11

- = Not tested

Extraction of sterols: Three sterols were identified from the fractions eluted from the silica gel column. Compound 1 was eluted in mixture form in *n*-hexane:chloroform (85:15) from the column and purified on preparative thick layer silica gel glass plates in solvent system *n*-hexane:chloroform (80:20). Purity was checked on a TLC card in a solvent system *n*-hexane:chloroform (80:20) by spraying with $Ce(SO_4)_2$. On heating this gave a single pink red spot. After using different spectroscopic methods it was identified as β-sitosterol (Fig. 1). The compound 2 was eluted in mixture form in *n*-hexane:chloroform (80:20) from column and purified on preparative thick layer silica gel glass plates in solvent system *n*-hexane:chloroform (75:25). Purity was checked on a TLC card in a solvent system *n*-hexane:chloroform (75:25) and after spraying with $Ce(SO_4)_2$ a pink red spot was found. After using various spectroscopic methods it was identified as stigmasterol. Compound 3 was eluted in pure form in solvent system *n*-hexane:chloroform (70:30), purity was checked on a TLC card (5×10 cm) in a solvent system *n*-hexane: chloroform (60:40) by spraying with $Ce(SO_4)_2$. On heating this gave a single dark red spot. After using different spectroscopic methods it was identified as cholesterol. Some of the physical properties of the identified sterols are given in Table 5.

Table 2. Antifungal activity exhibited by the methanol extract of *Microcystis aeruginosa*.

Fungal culture	Colony sample	Diam. mm) control	Inhibition %	MIC µg/mL miconazole	Ketoconazole
Human pathogens:					
Allescheria boydii	08	82	90.24	0.05	0.1-4
Candida albicans	09	95	90.52	0.1-2.0	0.1-8.0
Epidermophyton floccosum floccosum	11	108	89.81	0.5-1.0	0.1-8.0
Microsporum canis	13	53	75.47	0.5-10	0.05-12.8
Trichophyton longifusus longifusus	19	35	45.71	2.54	5.20
Trichophyton mentagrophytes mentagrophyte	59	102	42.15	2.59	5-19
Trichophyton semi	07	95	92.63	2.59	5.19
Plant pathogens:					
Fusarium oxysporm	10	89	88.76	-	-
Macrophomina phaseolina	14	98	85.71	-	-
Pythium aphanidermatum	16	58	72.41	-	-
Pythium oedochilum	29	47	38.29	-	-
Rhizoctonia solani	18	66	72.72	-	-
Saprophytes:					
Aspergillus flevus	23	98	76.53	-	-
Drechslera rostrata	18	60	70.00	0.3	0.3
Gliocladium virens	12	102	88.23	-	-
Nigrospora oryzae	12	65	81.53	0.3	0.3
Paecilomyces lilacinus	17	86	80.23	-	-
Stachybotrys atra	27	84	67.85	0.3	0.3
Trichoderma hamatum	16	60	73.33	-	-
Trichoderma harzianum	18	104	82.69	-	-

MIC = Minimum inhibitory concentration of standard drugs, - = not tested.

Table 3. Brine shrimp bioassay of the methanol extract of *Microcystis aeruginosa*.

$LD_{50} \leq 165$ µg /µL	G = --
Upper --	Lower --
G (probability value) --	Lower (toxic concentration) -
Upper (toxic concentration) – incubation temperature = 27°C	Inhibition period = 24 hours

Table 4. Fatty acids detected in the methanol extract of *Microcystis aeruginosa.*

Systematic name	Common name	Molecular formula	Mol. Wt.	Rel. % age
Saturated acids:				22.22
n-Octanoic	Caprylic	$C_8H_{16}O_2$	144	0.50
n-Decanoic	Capric	$C_{10}H_{20}O_2$	172	0.30
n-Dodecanoic	Lauric	$C_{12}H_{24}O_2$	200	1.52
n-Tetradecanoic	Myristic	$C_{14}H_{28}O_2$	228	4.01
n-Pentadecanoic	Pentadecylic	$C_{15}H_{30}O_2$	242	9.01
n-Hexadecanoic	Palmitic	$C_{16}H_{32}O_2$	256	5.87
n-Heptadecanoic	Margaric	$C_{17}H_{34}O_2$	270	1.01
Unsaturated acids:				77.71
Decatrienoic	—	$C_{10}H_{14}O_2$	166	2.50
9-Decenoic	Caproleic	$C_{10}H_{18}O_2$	170	0.30
Dodecadienoic	—	$C_{12}H_{20}O_2$	196	7.66
9-Dodecenoic	Lauroleic	$C_{12}H_{22}O_2$	198	0.40
Tridecatrienoic	—	$C_{13}H_{20}O_2$	208	1.66
Tridecenoic	Decylacrylic	$C_{13}H_{24}O_2$	212	0.39
Tetradecatetraenoic	—	$C_{14}H_{20}O_2$	220	2.50
Tetradecatrienoic	—	$C_{14}H_{22}O_2$	222	1.52
7-Ethyl-3-methyl-2,	—	$C_{14}H_{24}O_2$	224	6.74
6-undecadienoic	Myristoleic	$C_{14}H_{26}O_2$	226	0.50
9-Tetradecenoic	—	$C_{15}H_{24}O_2$	236	0.40
3,7,11-Trimethyl-2, 6,10-dodecatrienoic				
Pentadecadienoic	—	$C_{15}H_{26}O_2$	238	2.48
Pentadecenoic	Pentadecylenic	$C_{15}H_{28}O_2$	240	
Hexadecatetraenoic	—	$C_{16}H_{24}O_2$	248	10.21
6,10,14-Hexadeca- trienoic	Hiragonic	$C_{16}H_{26}O_2$	250	2.54
Hexadecadienoic	—	$C_{16}H_{28}O_2$	252	2.47
9-Hexadecenoic	Palmitoleic	$C_{16}H_{30}O_2$	254	1.22
Heptadecatrienoic	—	$C_{17}H_{28}O_2$	264	3.47
9,12,15-Octadeca- trienoic	Linolenic	$C_{18}H_{30}O_2$	278	0.30
9-Octadecenoic	Oleic acid	$C_{18}H_{34}O_2$	282	30.45

Isolation of diterpenes: Two diterpenes were identified from the fraction eluted from the silica gel column. Compound 1 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:chloroform (60:40). The purity was checked on a TLC card in the above system and purplish spot was found after spraying with $Ce(SO_4)_2$. After using various types of spectroscopy it was identified as 3, 7, 11, 15-tetramethyl-hexadec-2-en-1-ol which is actually *trans*-phytol (Fig. 1). The compound 2 was eluted in mixture form in *n*-hexane:chloroform (65:35) from column and purified on preparative thick layer silica gel glass plates in solvent system *n*-hexane:chloroform (60:40). Purity was checked on TLC card (same system as above) and after spraying with $Ce(SO_4)_2$ a pure purple spot was found. After using various spectroscopic methods it was identified as *cis*-phytol. Some of the physical properties of the identified diterpenes are given in Table 5.

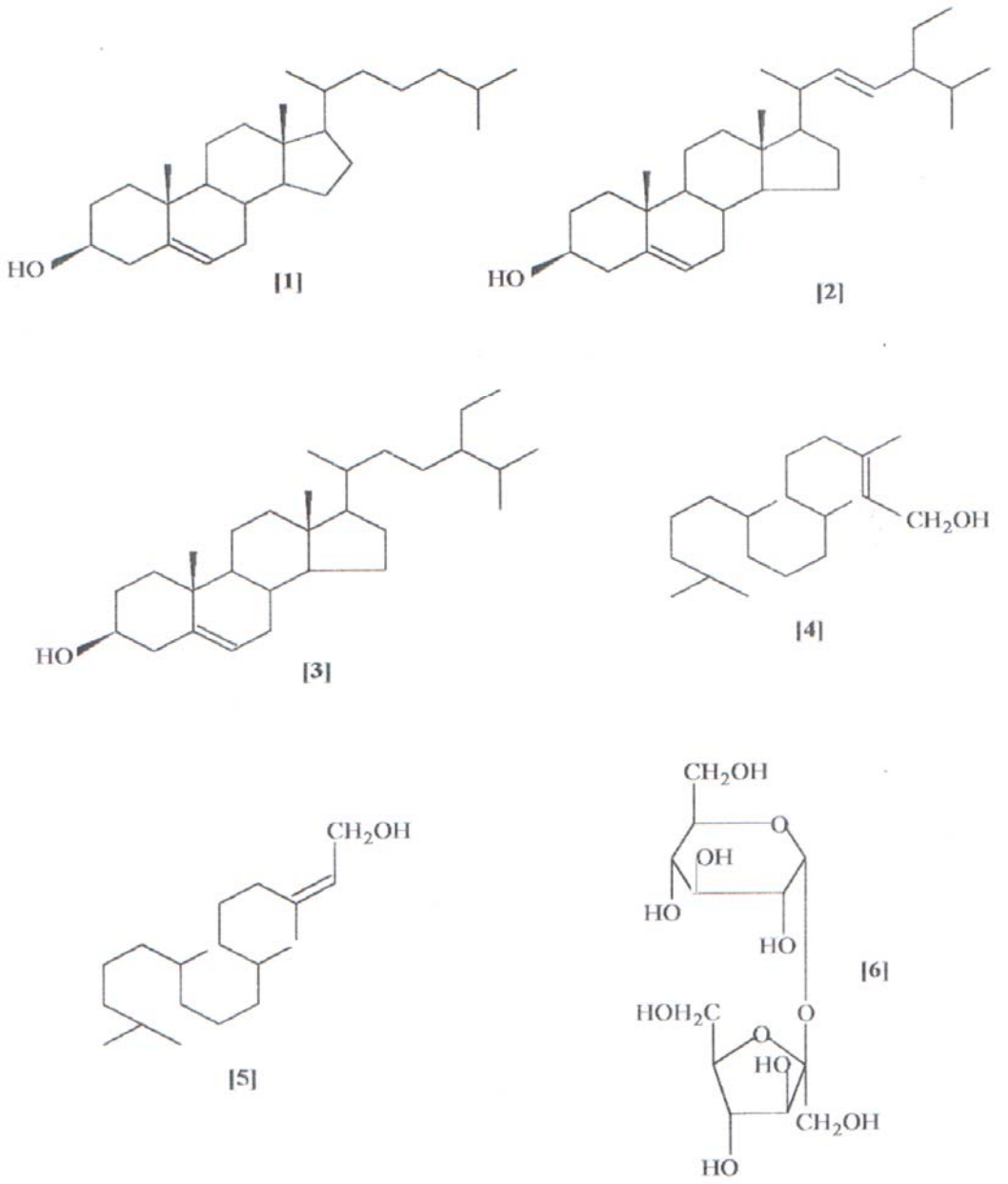

Fig. 1. Natural products isolated from *Microcystis aeruginosa*: **[1]**=Cholesterol, **[2]**=Stigmasterol, **[3]**=β-Sitosterol, **[4]**=*Trans*-phytol, **[5]**=*Cis*-phytol, **[6]**=Sucrose.

Table 5. Natural products obtained from methanol extract of *Microcystis aeruginosa.*

Str. No.	Common name	Molecular formula	Mol. Wt.	Mel. Pt.	$[\alpha]_d$ ($CHCl_3$)
	Sterols:				
1.	Cholesterol	$C_{27}H_{46}O$	386		
2.	Stigmasterol	$C_{19}H_{48}O$	412	169.5°	50°
3.	β-Sitosterol	$C_{29}H_{50}O$	414	134.5°	-40°
	Diterpenes:				
4.	*Trans*-phytol	$C_{20}H_{40}O$	296		
5.	*Cis*-phytol	$C_{20}H_{40}O$	296		
	Carbohydrate:				
6.	Sucrose	$C_{12}H_{22}O_{11}$	342	185-187°	+66.5

Str. No. = Structure number in Fig. 1, **Mol. Wt.** = Molecular weight, **Mel. Pt.** = Melting point.

Separation of a disaccharide: Residue from the pooled fraction eluted with chloroform:methanol (95:5) was crystallized and recrystallized from methanol to afford fine white needles. Purity was checked on a TLC card in a solvent system chloroform:methanol: water (4:6:0.5) by spraying with $Ce(SO_4)_2$. On heating this gave a single dark purple spot. After using different spectroscopic methods it was identified as sucrose. Some of its physical properties are given in Table 5.

Discussion

Microcystis aeruginosa (KÜtzing) KÜtzing is a unicellular, colonial blue-green alga (family Chroococcaceae, order Chroococcales, class Chroocophyceae, phylum Cyanophycota; *fide* Shameel, 2008). It produces in its system a wide range of toxic metabolites of different types, such as hepatotoxins, neurotoxins, cytotoxins, dermatotoxins and irritant toxins (lipopolysaccharides). Its blooms are hazardous due to the production of such secondary metabolites and endotoxins, which could be toxic to freshwater flora and fauna (Wiegand & Pflugmacher, 2005). Due to this reason its methanol extract displayed very promising results of biological activity against different pathogenic organisms (Tables 1-3). Similar results were obtained by the same species collected from estuarine environment (Aftab & Shameel, 2006). Microcystin, a hepatotoxin known to be the cause of animal and human deaths, is produced in freshwater by the blooms of this species. The toxin is produced nonribosomally via a multifunctional enzyme complex, consisting of both peptide synthetase and polyketidesynthase modules coded for by the *mcy* gene cluster (Kaebernick *et al.,* 2000).

In the extract of *M. aeruginosa* 7 saturated, 7 mono-, 4 di-, 7 tri- and 2 poly-unsaturated FAs were detected (Table 4). The unsaturated FAs were present in a larger proportion than saturated ones, therefore it resembled the other blue-green algae of Sindh investigated earlier (Valeem & Shameel, 2005). Oleic acid was present in the highest proportion (30.5 %), it was followed by hexadecatrienoic and pentadecylic acids (9-10 %). Palmitic acid was also present in appreciable quantity (5.9 %). Palmitic and oleic acids were found in large proportions in several seaweeds of Karachi Coast (Qasim, 1986; Shameel, 1990).

Three sterols (cholesterol, stigmasterol and β-sitosterol), two diterpenes (*cis*- & *trans*-phytol) and a disaccharide (sucrose) have been isolated from the extract of *M. aeruginosa* (Table 5). Similar results were obtained by the same species collected from the estuarine environment (Aftab & Shameel, 2006). This indicates that an alga may occur in different environments, but it contains the same metabolites and secondary products. It is interesting to observe that, although *M. aeruginosa* contains β-sitosterol but β-sitosterol-β-D-glucoside and dicyclohexanyl arizane, obtained from crude extract of rice hull, powerfully inhibit the growth of colonial *M. aeruginosa* cells (Park *et al.,* 2008).

Microcystis aeruginosa is known to produce a diverse array of toxic or otherwise bioactive metabolites in the freshwater environment. However, the functional role of the vast majority of these compounds, particularly in terms of the physiology and ecology of the organism that produces them, remains largely unknown. Some studies have suggested that these compounds may have ecological roles as allelochemical, specially including compounds that may inhibit competing sympatric macrophytes, algae and microbes (Berry *et al.,* 2008). These allelochemicals may also play a role in defense against potential predators and grazers, particularly aquatic invertebates and their larvae.

Microcystis has been recognized in recent years as a producer of a high number of secondary metabolites. Among these, peptides that are produced by the nonribosomal peptide synthetase pathway often show bioactivity or are toxic to humans (Welker *et al.,* 2004). It produces the cytotoxic peptide microclamyde, which provides evidence that the cyclic hexapeptide is formed by a ribosomal pathway through the activity of a set of processing enzymes closely resembling these to be involved in patellamide biosynthesis in cyanophycotian symbionts of ascidians (Ziemert *et al.,* 2008).

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