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FATTY ACIDS AND BIOLOGICAL ACTIVITIES OF CRUDE EXTRACTS OF FRESHWATER ALGAE FROM SINDH

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Abstract

Seven blue-green and 3 green algae were collected from various freshwater habitats of Sindh, (Pakistan), during January 1997-December 1999. Their methanol extracts revealed 17 saturated, 2 monoynoic, 12 monoenoic, 5 diunsaturated, 5 triunsaturated and 6 polyunsaturated fatty acids (FAs), which were identified by GLC and GC-MS. Palmitoleic acid was the most commonly occurring FA, while C15:0, C16:0, C14:1 and C18:1 were the next commonly occurring acids. The unsaturated acids were found in larger proportion (46.50-70.46%) than saturated FAs (16.82-39.20%). The blue-green algae did not differ much from green algae of Sindh in their FA-compositions. Their methanol extracts exhibited poor antibacterial but strong antifungal activities. They showed a significant phytotoxic activity but non-significant cytotoxic and insecticidal activities. The extract of *Lyngbya hieronymusii* enhanced antitumour activity from 20 to 45% with increase in the concentration of extract. Algae belonging to three phyla (Cyanophycota, Chlorophycota and Charophycota) revealed differences in their FA-compositions as well as their bioactivities.

Introduction

From time to time a large number of green seaweeds growing at the seashore of Karachi and the adjacent coastal areas of Pakistan have been investigated phycochemically (Usmanghani *et al*., 1985; Qasim, 1986; Shameel, 1987, 1990, 1993; Aliya *et al*., 1991; Ahmad *et al*., 1993; Aliya, & Shameel, 1993, 1998, 1999, 2003) and their bioactivities were studied (Usmanghani, 1984; Amjad & Shameel, 1993; Aliya *et al*., 1994; Atta-ur-Rahman *et al*., 1997; Rizvi & Shameel, 2003, 2005). But hardly any such study was conducted on freshwater green algae of this area. This paucity of knowledge initiated a research program to compare the observations made on green seaweeds with their freshwater counterparts of Pakistan (Naila *et al*., 2005; Shahnaz *et al*., 2006; Ghazala *et al*., 2007). The present investigation is a continuation of this program in which fatty acids of 7 species of blue-green and 3 of green algae have been studied, and a preliminary screening of their biological activity potential was carried out by different tests.

Materials and Methods

Collection of material: Seven blue-green and 3 green algae were collected from various habitats of Sindh, Pakistan between January 1997 and December 1999 (Table 1). They were thoroughly washed to remove extraneous material and dried in shade. Their voucher specimens were preserved in 5% formalin solution and deposited in Seaweed Biology & Phycochemistry Laboratory, MAHQ Biological Research Centre, Univeristy of Karachi. Identification of the material was made by one of us (MS).

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Table 1. Details for the collection of investigated algal material (classified according to Shameel 2008).

No.	Algal taxa	Locality	Place	Date
	Cyanophycota			
	Chroocophyceae			
	Chroococcales			
	Chroococcaceae			
1.	*Aphanothece pallida* (Kützing) Rabenhorst	Riverin ponds	Hyderabad	Oct. 1997
2.	*Aphanothece stagnina* (Sprengel) A. Braun	Rice fields	Tando Muhammad Khan	Sep. 1997
	Nostocophyceae			
	Nostocales			
	Nostocaceae			
3.	*Nostoc ellipsosporum* (Desmazières) Rabenhorst *ex* Bornet *et* Flahault	Rice fields	Hyderabad	Aug.-Nov. 1998
	Oscillatoraceae			
4.	*Arthrospira platensis* (Nordstedt) Gomont	Riverin ponds	Hyderabad	Oct. 1997
5.	*Lyngbya hieronymusii* Lemmermann	Rice fields	Tando Muhammad Khan	Sep.- Nov. 1999
6.	*Lyngbya mertensiana* Meneghini *ex* Gomont		Jamshoro	Oct.-Dec. 1999
	Rivularaceae			
7.	*Gloeotrichia natans* (Hedwig) Rabenhorst *ex* Bornet *et* Flahault	Rice fields	Hyderabad	Aug.-Dec. 1998-1999
	Chlorophycota			
	Ulvophyceae			
	Ulvales			
	Ulvaceae			
8.	*Enteromorpha intestinalis* (Linnaeus) Nees	Sonharo Lake	Pateji, Badin	July-Oct. 1998
	Siphonocladophyceae			
	Cladophorales			
	Cladophoraceae			
9.	*Pithophora oedogonia* (Montagne) Wittrock	Rice fields	Tando Muhammad Khan	Jan. 1997
	Charophycota			
	Charophyceae			
	Charales			
	Characeae			
10.	*Nitella flexilis* (Linnaeus) C.A. Agardh	Kinjhar Lake	Thatta	Aug.-Sep. 1998

Detection of fatty acids: The algae under investigation weighing 1 kg dry weight (each) were percolated with *n*-hexane:chloroform (1:1 v/v) in an aspirator for two weeks. The extract so obtained was reduced under vacuum and partitioned between EtOAc and water (1:1 v/v), which yielded 20-25 g (each) of residue. An aliquot of the extract was saponified with 10% KOH in 50% methanol and refluxed at 100^{o}C for 6 h. The resulting mixture was evaporated under reduced pressure in rotary evaporator and partitioned between aqueous and ethyl acetate (EtOAc) phases. The EtOAc fraction was acidified with 6N HCl (pH 4-5), dried over anhydrous Na_2SO_4 and concentrated under vaccum. It was then subjected to methylation, 1.5-2.0 mL ethereal diazomethane was added to this mixture and was left in a fuming chamber at room temperature for over-night until dissolved. The aliquotes were then directly injected to a Hewlet Packard GC with 11/73 DEC computer data system. Its details have already been given earlier (Naila *et al*., 2005; Shahnaz *et al*., 2006; Ghazala *et al*., 2007). The relative retention times of the analyzed fatty acids by GC are also given.

Bioactivity tests: A part of the residue of each algal species obtained for the detection of fatty acids was dissolved in methanol and used for the tests of biological activities. The results were compared with simultaneously running control experiments for each test. For this purpose the stamdard antibiotic drugs used for antibacterial activity were ampicillin, amoxicillin and cephalexin, and for antifungal activity ketoconazole and miconazole were employed. The methodologies for antibacterial and antifungal activities by agar well diffusion method, phytotoxicity against *Lemna acquinoctialis* Welw., brine shrimp bioassay against larvae of *Artemia salina* Leach, insecticidal activity against the pest *Tribolium castaneum* and antitumour activity against potato tubers were the same as have been described earlier in detail (Naila *et al*., 2005; Shahnaz *et al*., 2006; Ghazala *et al*., 2007).

Results and Discussion

During this research programme 10 commonly occurring species of freshwater algae were collected from various districs of Sindh Province of Pakistan (Table 1). Their crude extracts have been investigated for the fatty acid composition as well as from the point of view of their bioactivity. Taxonomically all of them were found to be known species, they belonged to 3 phyla, 5 classes, 5 orders, 7 families and 8 genera according to the recent classification (Shameel, 2008). Although a few of them have been previously investigated (Naila *et al*., 2005; Shahnaz *et al*., 2006; Ghazala *et al*., 2007), but most of the species were studied for the first time phycochemically as well as from the viewpoint of their bioactivity during this research work.

Fatty acids: Altogether 49 different fatty acids (FAs) have been detected (Table 2), including 17 saturated (SFAs) and 32 unsaturated fatty acids (UFAs). The UFAs comprised of 14 monounsaturated (MUFAs), 5 diunsaturated (UFAs), 5 triunsaturated (TUFAs) and 6 polyunsaturated fatty acids (PUFAs). The MUFAs also included two monoynoic acids (C141 and C151) with a triple bond. This indicates that UFAs of the investigated species exhibited greater diversity than that of SFAs. Similar observation has also been made in a previous study on freshwater algae from Pakistan (Ghazala & Shameel, 2005). Monoynoic fatty acids (such as C131 and C161) have previously been detected in the seaweed *Codium iyengarii* Børgesen (Aliya *et al*., 1991), they are of rare occurrence in freshwater and marine algae. In most of the investigated species, UFAs were found in a larger proportion (46.50-70.46 %) than the SFAs (16.82-39.20%).

Table 2. Relative percentages of the fatty acids detected in methanol extracts of freshwater algae.

Acid type	Approximate relative percentages in algae									
	*1	2	3	4	5	6	7	8	9	10
I. Saturated fatty acids (SFAs):										
C11:0	13.42	-	-	-	-	-	-	-	-	0.16
C13:0	-	-	-	-	-	0.48	-	-	-	-
C14:0	0.77	-	-	-	-	0.44	-	-	-	13.96
C15:0	5.55	8.47	-	-	1.878	2.54	1.99	-	-	2.45
C16:0	7.00	52.51	-	-	10.90	6.65	21.63	-	-	16.25
C17:0	5.84	-	-	-	10.63	5.73	5.16	-	-	-
C18:0	1.45	-	-	-	2.60	6.13	-	-	-	1.16
C19:0	0.69	-	-	-	-	-	-	-	4.76	-
C20:0	0.92	-	-	-	2.74	-	2.56	-	-	-
C23:0	-	-	-	-	-	-	3.22	-	-	-
C24:0	-	-	-	-	-	-	3.23	-	4.76	-
C26:0	-	-	-	-	-	-	-	-	3.17	-
C27:0	-	-	-	-	-	-	1.38	-	-	-
C30:0	-	-	-	-	4.94	-	-	-	4.12	-
C31:0	-	-	-	-	14.71	-	-	-	-	-
C32:0	-	-	-	-	2.70	-	-	-	-	-
C33:0	-	-	-	-	2.59	-	-	-	-	-
Total	**35.67**	**60.98**	**-**	**-**	**53.72**	**22.01**	**39.19**	**-**	**16.82**	**34.00**
II. Monounsaturated fatty acids (MUFAs):										
C8:1	-	-	-	-	-	-	-	-	-	0.48
C13:1	-	-	-	-	1.89	-	1.22	-	6.34	18.57
C14:1	-	13.54	-	-	-	-	1.38	-	6.66	0.48
C14:1	15.308	-	-	-	1.89	4.081	1.84	-	3.17	5.83
C15:1	24.727	-	-	-	-	-	-	-	9.52	-
C15:1	0.924	-	-	-	5.02	3.399	1.99	-	3.17	-
C16:1	5.549	10.16	-	-	9.67	17.98	4.76	-	2.53	4.32
C17:1	-	-	-	-	1.89	11.57	2.30	-	4.12	-
C18:1	2.12	6.77	-	-	1.44	10.42	2.60	-	-	0.49
C19:1	-	-	-	-	-	3.44	-	-	-	0.48
C20:1	0.69	-	-	-	-	-	-	-	5.07	-
C21:1	-	-	-	-	-	2.50	4.16	-	-	-
C22:1	-	-	-	-	-	3.97	-	-	4.12	-
C24:1	0.42	-	-	-	-	-	1.84	-	3.80	-
Total	**49.75**	**30.47**	**-**	**-**	**21.84**	**57.39**	**22.13**	**-**	**52.68**	**30.67**

Table 2. (Cont'd.).

Acid type	Approximate relative percentages in algae									
	*1	2	3	4	5	6	7	8	9	10
III. Diunsaturated fatty acids (DUFAs):										
C10:2	1.05	-	-	-	-	5.30	-	-	-	-
C14:2	-	-	-	-	1.89	-	1.38	-	-	-
C16:2	-	-	-	-	-	-	-	-	2.22	-
C17:2	-	-	-	-	-	-	-	-	13.96	10.56
C18:2	-	-	-	-	-	-	-	-	-	2.50
Total	**1.05**	**-**	**-**	**-**	**1.89**	**5.30**	**1.38**	**-**	**16.18**	**13.06**
IV. Triunsaturated fatty acids (TUFAs):										
C14:3	-	-	-	-	-	1.51	3.13	-	-	-
C15:3	-	8.47	-	-	0.86	3.66	1.38	-	-	5.71
C16:3	-	-	-	-	1.89	-	-	-	1.58	8.05
C17:3	-	-	-	-	-	6.40	-	-	-	-
C18:3	-	-	-	-	8.33	0.28	-	-	-	-
Total	**-**	**8.47**	**-**	**-**	**11.09**	**11.87**	**4.51**	**-**	**1.58**	**13.76**
V. Polyunsaturated fatty acids (PUFAs):										
C15:4	-	-	-	-	-	-	-	-	-	0.48
C18:5	-	-	-	-	-	3.39	-	-	-	2.98
C20:4	-	-	-	-	-	-	3.23	-	-	-
C21:5	-	-	-	-	-	-	7.84	-	-	-
C22:4	-	-	-	-	-	-	3.68	-	-	0.82
C23:6	-	-	-	-	-	-	1.84	-	-	-
C24:5	-	-	-	-	-	-	1.84	-	-	-
C27:8	0.46	-	-	-	11.39	-	-	-	-	-
Total	**0.46**	**-**	**-**	**-**	**11.39**	**3.39**	**18.46**	**-**	**-**	**4.29**
VI. Unidentified fatty acids										
Total	**12.47**	**-**	**-**	**-**	**-**	**-**	**17.42**	**-**	**16.82**	**4.16**

*1-10 = For names of the algal species are Table 1.

However, in *Aphanothece stagnina* and *Lyngbya hieronymusii* the SFAs were detected in a larger amount (60.98 and 53.72 %) than the UFAs (38.94 and 46.23% respectively). This has a resemblance with the earlier observations made on seaweeds from the present lab. (Qasim, 1986; Shameel, 1987). Similarly in a variety of freshwater green algae (Ghazala *et al*., 2005) and green seaweeds growing at the coast of Karachi (Aliya & Shameel, 1993, 1998, 2003), the UFAs were detected in a greater quantity than the SFAs. In this regard also marine and freshwater green algae behaved similarly.

Palmitoleic acid (C16:1) was the most commonly occurring FA, as it was detected in all the investigated algal extracts. Pentadecylic (C15:0), palmitic (C16:0), myristoleic (C14:1) and oleic (C18:1) acids were the next commonly occurring FAs, as they were found in 6 out of the 10 investigated species, while due to scarcity of the material, 3 species could not be analysed properly. They were followed by pentadecylenic (C15:1) and pentadecatrienoic (C15:3) acids, which could be detected in 5 species. Several acids such as C8:1, C13:0, C15:4, C16:2, C17:3, C18:2, C20:4, C21:5, C23:0, C23:6, C24:5, C26:0, C27:0, C31:0, C32:0 and C33:0 were the least common FAs, as they were found only in any one of the investigated species. The FA found in most dominating quantity varied from species to species *e.g.*, it was C151 (24.727%) in *Aphanothece pallida*, C16:0 (52.51%) in *A. stagnina*, C31:0 (14.718%) in *Lyngbya hieronymusii*, C16:1 (17.986%) in *L. martensiana*, C16:0 (21.630%), in *Gloeotrichia natans*, C17:2 (13.965%) in *Pithophora oedogonia* and C13:1 (18.576%) in *Nitella flexilis*. As a whole C16:0 and C18:1 were present in overwhelming amount. The studies conducted on marine algae from Karachi also showed the common occurrence of palmitic and oleic acids in their dominating quantities (Qasim, 1986; Shameel, 1987, 1990, 1993; Shameel & Khan, 1991). In this way the freshwater algae resembled their marine counterparts.

Gloeotrichia natans exhibited the largest FA-diversity as it contained 24 different FAs, and next diverse were the two species of *Lyngbya* with 20 different FAs (Table 2). *Nitella flexilis* exhibited the presence of 19 FAs, while *Aphanothece pallida* and *Pithophora oedogonia* showed 17 FAs. Only six FAs were found in *Aphanothece stagnina* which showed the smallest diversity. This was the first study on blue-green algae (Cyanophycota) from the present lab., but no remarkable difference could be noted as compared to the previous studies made on freshwater Chlorophycota (Ghazala & Shameel, 2005), marine Chlorophycota (Shameel, 1993; Aliya *et al*., 1991; Ahmad *et al*., 1993; Aliya & Shameel, 1993, 1998, 1999, 2003; Usmanghani, 1984; Amjad & Shameel, 1993; Aliya *et al*., 1994; Atta-ur-Rahman *et al*., 1997; Rizvi & Shameel, 2003, 2005; Naila *et al*., 2005; Shahnaz *et al*., 2006; Ghazala *et al*., 2007), seaweeds in general (Qasim, 1986; Shameel, 1987, 1990), and brackish water algae (Khaliq-uz-Zaman *et al*., 1998, 2001; Shameel, 1998). The FA-composition of the investigated freshwater algae varied not only from phylum to phylum, order to order or family to family but also from species to species and no generalization may be made in this connection. All the investigated species exhibited great variation in their FA-composition. Even the two species of *Lyngbya* and 2 species of *Aphanothece* differed from one another to a great extent. This indicates that different species of the same genus may behave variably in their FA-composition. Such specific differences have also been observed among green seaweeds of the genera *Caulerpa* Lamouroux and *Codium* Stackhouse from the coast of Karachi (Aliya & Shameel, 1993, 1998).

The SFAs ranged from C11 to C33, the MUFAs displayed a range from C8 to C24, the DUFAs showed a range from C10 to C18, TUFAs from C14 to C18, while PUFAs exhibited the shortest range from C15 to C27. The SFAs showed the largest and TUFAs the smallest range of FAs. Largest number of PUFAs were found in *Gloeotrichia natans* (5 FAs) followed by *Nitella flexilis* (3 FAs), and they were mainly C15, C18, C20, C21, C22, C23, C24 and C29 acids. While in other studies on freshwater green algae it was observed that their FA-pattern is characterized as lacking in C20 acids but containing large amounts of C16- and C18-PUFAs (Menzel & Wild, 1989). Studies on other freshwater green algae showed the presence of palmitic, linoleic and linolenic acids (El-Sayed, 1983; Stefanov *et al*., 1996). The present results agree with these observations.

Myristoleic acid (C14:1) appeared to be of common occurrence in the investigated algae. It may be a component of some larger natural products. Recently two novel carotenoid C14:1 *trans*-Δ^2 esters, such as siphonaxanthin C14-1 *trans*-Δ^2 ester and 6′-hydroxy siphonaxanthin C14-1 *trans*-Δ^2 ester have been isolated from a green alga *Pterosperma cristatum*, collected from Japanese waters. An inseparable mixture of nitrogenous glycerolipids have been isolated from the green alga *Ulva fasciata* Delile collected from the Indian Coast (Blunt *et al*., 2004). The FAs are not only the building material of algal lipids but may also constitute some important macromolecules.

Antibacterial activity: Methanol extracts of the algal species were tested against 5 Gram positive and 6 Gram negative bacteria (Table 3). *Nostoc ellipsosporum*, *Arthrospira platensis* and *Nitella flexilis* showed antibacterial activity against 3 bacteria, *Enteromorpha intestinalis* against two and *Aphanothece stagnina* against only one bacterial species. *Corynebacterium diphtheriae* among Gram positive and *Shigella boydii* from Gram negative category were the most sensitive bacteria as they were affected by 4 algal extracts, the growth of *Bacillus aureus* and *Klebsiella pneumoniae* was affected by only 2 algal extracts. Other bacterial species did not show any retardation in their growth and hence appeared to be very resistant. In general, the species of Gram positive and negative bacteria behaved similarly, and no difference could be found out in their sensitivity. *Shigella boydii* also proved to be the most sensitive bacterium against methanol extract of several freshwater green algae (Ghazala & Shameel, 2005), while the growth of *Corynebacterium diphtheriae* was badly affected by MeOH-extract and its EtOAc-soluble part of *Chara corallina* and *C. wallichii* A. Braun (Khaliq-uz-Zaman *et al*., 1998, 2001) and a variety of green, brown and red seaweeds (Rizvi & Shameel, 2005; Ali *et al*., 2000, 2002).

Antifungal activity: The crude extracts of 8 algal species were tested against 3 facultative parasites, 6 plant parasites and one saprophyte by agar well diffusion method (Table 4). The two species of *Aphanothece* resembled one another to a great extent in their antifungal activity. Only one facultative parasite was affected by algal extracts, while two of them did not show any effect. This indicates that they are resistant against algal extracts. Four plant parasites were affected by the crude extracts of algae and two remained unaffected. Growth of the single treated saprophyte was inhibited by algal extracts. Out of 3 species of *Fusarium* tested for this purpose, only one was affected while two resisted the algal extracts. Plant parasitic fungi appeared to be highly susceptible against compounds extracted from freshwater algae, as 4 of the 6 tested parasites were affected by the algal extracts. Plant parasitic fungi were also found to be susceptible against methanol extracts of several seaweeds (Rizvi & Shameel, 2005). It was very interesting to note that all the algal extracts exhibited the similar effects against each of the fungal species, that is why no conclusion may be drawn regarding the question that which algal species is most active and which fungus is most sensitive against such activity. Quite similar results were obtained in a previous study, while investigating freshwater green algae collected from Pakistan (Ghazala & Shameel, 2005). It appears that freshwater algae behave similarly in their antifungal activity.

Table 3. Antibacterial activity (in mm) exhibited by the methanol extracts of algae.

Bacterial species	Algal species									
	*1	2	3	4	5	6	7	8	9	10
Gram positive:										
Alteromonas hydrophila	-	-	-	-	-	-	-	-	-	-
Bacillus cereus	-	-	6.5	-	-	-	-	-	-	6
Corynebacterium diphtheriae	-	-	†7	†7	-	-	-	†7.5	-	†6.5
Staphylococcus aureus	-	-	-	-	-	-	-	-	-	-
Streptococcus pyogenes	-	-	-	-	-	-	-	-	-	-
Gram negative:	-	-	-	-	-	-	-	-	-	-
Escherichia coli	-	-	-	-	-	-	-	-	-	-
Klebsiella pneumoniae	-	†7.5	-	†9	-	-	-	-	-	-
Proteus mirabilis	-	-	-	-	-	-	-	-	-	-
Pseudomonas aeruginosa	-	-	-	-	-	-	-	-	-	-
Salmonella typhi	-	-	-	-	-	-	-	-	-	-
Shigella boydii	-	-	†7	†7.5	-	-	-	†7	-	†8

***1-10** = For names of the algal species see Table 1, - = No activity, † = Decrease in bacterial population/ unit area only.

Table 4. Antifungal activity (in cm) shown by the methanol extracts freshwater algae by agar well diffusion method.

Bacterial species	Algal species									
	*1	2	3	4	5	6	7	8	9	10
Facultative parasites:										
Alternaria alternata	-	-	-	-	-	-	-	-	-	-
Curvularia lunata	3.6	2.6	3.6	3.4	-	-	3.2	3.2	3.3	3.0
Drechslera australiensis	-	-	-	-	-	-	-	-	-	-
Plant parasites:										
Fusarium solani	-	-	-	-	-	-	-	-	-	-
Fusarium sporotrichoids	3.0	3.4	3.6	3.4	-	-	1.5	3.3	3.2	3.5
Fusarium proliferatum	-	-	-	-	-	-	-	-	-	-
Macrophomina phaseolina	4.0	3.5	3.1	3.7	-	-	4.4	2.5	3.2	3.7
Rhizoctonisa solani	3.6	3.8	3.4	3.8	-	-	4.2	3.1	3.9	3.6
Sclerotium rolfsii	2.8	3.7	4.5	4.2	-	-	3.3	3.4	3.3	3.4
Saprophyte:										
Trichoderma harzianum	4.3	3.7	4.0	3.7	-	-	4.0	3.0	3.0	3.0

***1-10** = For names of the algal species see Table 1, - = No activity.

Table 5. Different bioactivities displayed by methanol extracts of freshwater algae.

Units	*1	2	3	4	5	6	7	8	9	10
				Phytotoxic activity against *Lemna acquinoctialis*						
% Inhibition	100	100	33.33	-	6.66	-	100	100	100	-
				Briane shrimp bioassay						
LD_{50} (µg/mL)	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
				Insecticidal activity against *Tribolium castaneum*						
1571.70 µL/Cm_2	-	-	-	-	-	-	-	-	-	-
				Antitumour activity against potato tuber						
10 µg/ µL	-	-	-	-	20	-	-	-	-	-
50 µg/ µL	-	-	-	-	20	-	-	-	-	-
100 µg/ µL	-	-	-	-	20	-	-	-	-	-

*1-10 = For name of the algal species see Table 1, - = No activity

Most of the fungal organisms gave almost same results, without much variation (Table 4). Taking into consideration the average values obtained from the total retardation of the fungal species as affected by an algal extract, there is a very slight difference (3.08 to 3.70 cm). The largest value (3.70 cm) was exhibited by *Arthrospora platensis* and *Nostoc ellipsosporum* and the smallest (3.08 cm) by the extract of *Enteromorpha intestinalis*, but this difference is negligible. Similarly no differences were traceable among the members of Cyanophycota, Chlorophycota and Charophycota. In a similar study against MeOH extract of *Chara corallina* (Khaliq-uz-Zaman *et al*., 1998), no activity was observed against *Drechslera rostrata*, similarly in the present study as well as in a previous study on freshwater green algae (Ghazala & Shameel, 2005), *D. australiensis* remained unaffected indicating that the genus *Drechslera* is resistant against the bioactive constituents of freshwater algae.

Other bioactivities: The methanol extracts obtained from 7 species of freshwater algae were tested against *Lemna acquinoctialis* Welw., for phytotoxic activity (Table 5). Most of the investigated algal species showed 100% phytotoxic activity, only *Lyngbya hieronymusii* and *Nostoc ellipsosporum* have shown lesser activity (6.66-33.33%). In another study methanol extracts obtained from 10 freshwater green algae gave the similar results against *Lemna* spp., (Ghazala & Shameel, 2005). A variety of green, brown and red seaweeds of Karachi Coast gave similar results of phytotoxic activity against two species of *Lemna* plant (Rizvi & Shameel, 2005; Ali *et al*., 2000). Both the species of *Aphanothece* behaved similarly. All the ten investigated methanol extracts of algal species displayed non-significant results of cytotoxic activity through brine shrimp bioassay. Similar results were also obtained for the investigated green algae of Sindh (Ghazala & Shameel, 2005), indicating that the freshwater algae behaved similarly in this regard. The results obtained from brine shrimp lethality bioassay of several marine benthic algae of Karachi Coast (Ali *et al*., 2000) are also not very promising. This indicates that the natural products having cytotoxic properties are probably lacking in these algae.

None of the 10 algal extracts tested for their bioactivity against the insect (pest) *Tribolium castaneum*, showed any activity (Table 5). Similarly the methanol extracts of 8 freshwater green algae of Sindh did not exhibit any activity against this insect (Ghazala & Shameel, 2005). However, *Chara globularis* Thuillier is reported to contain compounds with insecticidal properties (Jacobsen & Pedersen, 1983). Eleven of the 21 extracts of green, brown and red seaweeds of Karachi Coast displayed insecticidal activity against various common grain pests including *T. castaneum* (Rizvi & Shameel, 2005). In this way the freshwater and marine algae differed from one another. Antitumour activity was studied only in the methanol extract of *Lyngbya hieronymussii*, it was found to enhance the activity from 20 to 45% with increase in the concentration of the extract. This activity was also revealed in a previous study by the methanol extracts of a brackish water green alga, *Chara contraria* A. Braun *ex* Kutzing (Ghazala & Shameel, 2005).

Conclusion

The freshwater green algae of Sindh resemble green seaweeds of this area in their FA- composition in certain regards, *e.g.* monoynoic FAs are of rare occurrence in them, the UFAs are found in larger proportion than SFAs, palmitic and oleic acids occur in dominating quantities. They are characterized in having palmitoleic acid as the most commonly present FA, SFAs showing the largest and TUFAs the smallest range of FAs, and containing large amount of C18-, C22- and C27- PUFAs. The blue-green algae exhibit no remarkable difference than green algae. The FA- composition varies not only from phylum to phylum, order to order or family to family but also from species to species. The FAs are not only the building material of algal lipids but may also constitute some important macromolecules. Freshwater algae behave similarly in their antifungal activity as compared to seaweeds and resemble them to a great extent in other forms of bioactivities. The growths of Gram positive and negative bacteria are similarly hampared against algal extracts. Plant parasitic fungi appear to be highly susceptible than facultative parasites and saprophytes. Slight differnces are traceable among the members of Cyanophycota, Chlorophycota and Charophycota regarding their bioactivities.

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