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PHYCOCHEMISTRY AND BIOACTIVITY OF *TETRASPORA* (VOLVOCOPHYTA) FROM SINDH

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Abstract

Two unicellular green algae, *Tetraspora cylindrica* (Wahlenberg) Agardh and *T. gelatinosa* (Vaucher) Desvaux were collected from thermal effluents and industrial wastewater near Hyderabad, Sindh during December 1995 and February 1996. Their methanol extracts revealed the presence of a variety of saturated and unsaturated fatty acids, a steroidal fatty acid, β-sitosterol and *trans*-phytol. In *T. cylindrica* 9 saturated and 11 unsaturated fatty acids were present in equal amounts (44.87 & 44.39 %), hencicosanoic acid (C21:0) was in largest quantity (17.02 %), pentadienoic (C5:2) and undecatrienoic (C11:3) acids were in smallest amount (0.115 %). In *T. gelatinosa* no saturated, no mono- and di-unsaturated fatty acids could be detected, pentadecatetraenoic (C15: 4) acid occurred in largest quantity (30.08 %). The extract of *T. cylindrica* showed antibiotic activity against three of the ten bacterial species, six of the ten fungal pathogens and all the six fungi by food poisoning method. Both algae displayed appreciable amount of bioactivity regarding phytotoxicity with no activity in brine shrimp bioassay and insecticidal test.

Introduction

Algae are very important component of aquatic ecosystem. They inhabit the pelagic as well as benthic environments of hydrosphere in a variety of forms. This variation is more pronounced in green algae, which taxonomically belong to different phyla, such as Prochlorophyta, Volvocophyta, Euglenophyta, Chlorophyta, and Charophyta (Shameel, 2001). Apart from taxonomy, green algae have been investigated from various viewpoints. From time to time a large number of green seaweeds growing at the seashore of Karachi and its adjacent coastal areas have been investigated phycochemically (Usmanghani *et al.*, 1985, 1986; Qasim, 1986; Shameel, 1987, 1990, 1993; Aliya *et al.*, 1991, 1993, 1995; Ahmad *et al*, 1992, 1993; Aliya & Shameel, 1993, 1999). Their bioactivities also showed some interesting results (Usmanghani *et al.*, 1984; Usmanghani & Shameel, 1986; Naqvi *et al.*, 1992; Amjad & Shameel, 1993; Aliya *et al.,* 1994, Atta-ur-Rahman *et al.*, 1997; Rizvi *et al.*, 2000). There are also a few studies made on the brackishwater green algae (Khaliq-uz-Zaman *et al.*, 1998, 2001). There does not appear to be any report on phycochemistry and bioactivity of the freshwater counterparts of green seaweeds.

Two unicellular green algae *viz.*, *Tetraspora cylindrica* (Wahlenberg) Agardh and *T. gelatinosa* (Vaucher) Desvaux collected from Hydrabad, an area not much far away from seashore were used to study their phycochemistry and bioactivity.

Materials and Methods

Collection of material: *Tetraspora cylindrica* was collected from thermal effluents being attached with stones and aquatic grasses in the month of January and February 1996 from Jamshoro. Later on the attached colonies became free floating, appearing as brown coloured gelatinous masses. Therefore, they were again collected in March 1996 in this condition. *Tetraspora gelatinosa* commonly appears in the cold months of the year. It was collected from the industrial wastewater of Coca Cola beverage industry of Hyderabad in the month of December 1995. Pale spherical colonies were found attached with grasses, papers, stones and on other rough objects. Some of them were free floating and certain specimens were also found growing at the bottom of the water.

Detection of fatty acids: The dried algal material was soaked in methanol for one month at room temperature. It was then filtered to remove undissolved material, evaporated in rotary under reduced pressure and eluted in a dark coloured, thick and syrupy residue (Fig. 1). The extract so obtained was dissolved in a little quantity of methanol and poured on silica gel (about 10 g). The solvent was allowed to evaporate in the open atmosphere. Medium sized column was packed with fresh silica using *n*-hexane as solvent, which was allowed to run for one day. After packing the column, slurry was loaded and a layer of silica gel was placed above it, which prevented the disturbance of slurry. Procedure for the detection of fatty acids have been presented in Fig. 2.

The mixtures of products were dissolved in EtOAc and distilled water. Lower layer contained water soluble part and upper layer (EtOAc layer) contained organic compounds; the two layers were rendered apart by separating funnel. The EtOAc layer was dried overnight and fractions were treated with diazomethane. The reaction mixture was kept overnight at room temperature and then evaporated under reduced pressure. The aliquots were directly injected into GC-MS for analysis. It 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 to 250 ºC with a rate of increase of 8 ºC per min. The carrier gas (He) flow rate was 32 mL/min., injector temperature was 250 ºC. The fatty acid methyl esters so obtained were identified by matching their spectra with those in the NBS mass spectral library (Helles & Milne, 1978).

Isolation of sterols and terpenes: The fractions were subjected to column chromatography on silica gel using a mixture of solvent systems; where two compounds were eluted *i.e*. β-sitosterol in 8.5 hexane: 1.5 $CHCl_3$ and diterpenoid (*trans*-phytol) in hexane: $CHCl_3$. Ceric sulphate spray was used for visualization. They were chemically elucidated by HR-MS, ^{1}H- and ^{13}C-NMR spectroscopy.

Bactericidal activity: The wells were dug in the nutrient agar medium with the help of sterile metallic borers with their centers at least 24 mm apart. About 2-8 h old bacterial inoculum containing approx. 10^4 – 10^6 colony forming units (CFU)/mL were spread on the surface of nutrient agar with the help of a sterile cotton swab, which is rotated firmly against the upper inside well of the tube to express excess fluid. Entire agar surface of the plate was streaked with the swab three times turning the plate at 60º between each streaking. Recommended concentration of the sample (2 mg/mL) of dimethyl sulphoxide (DMSO) was then added to respective wells. Other wells supplemented with DMSO and

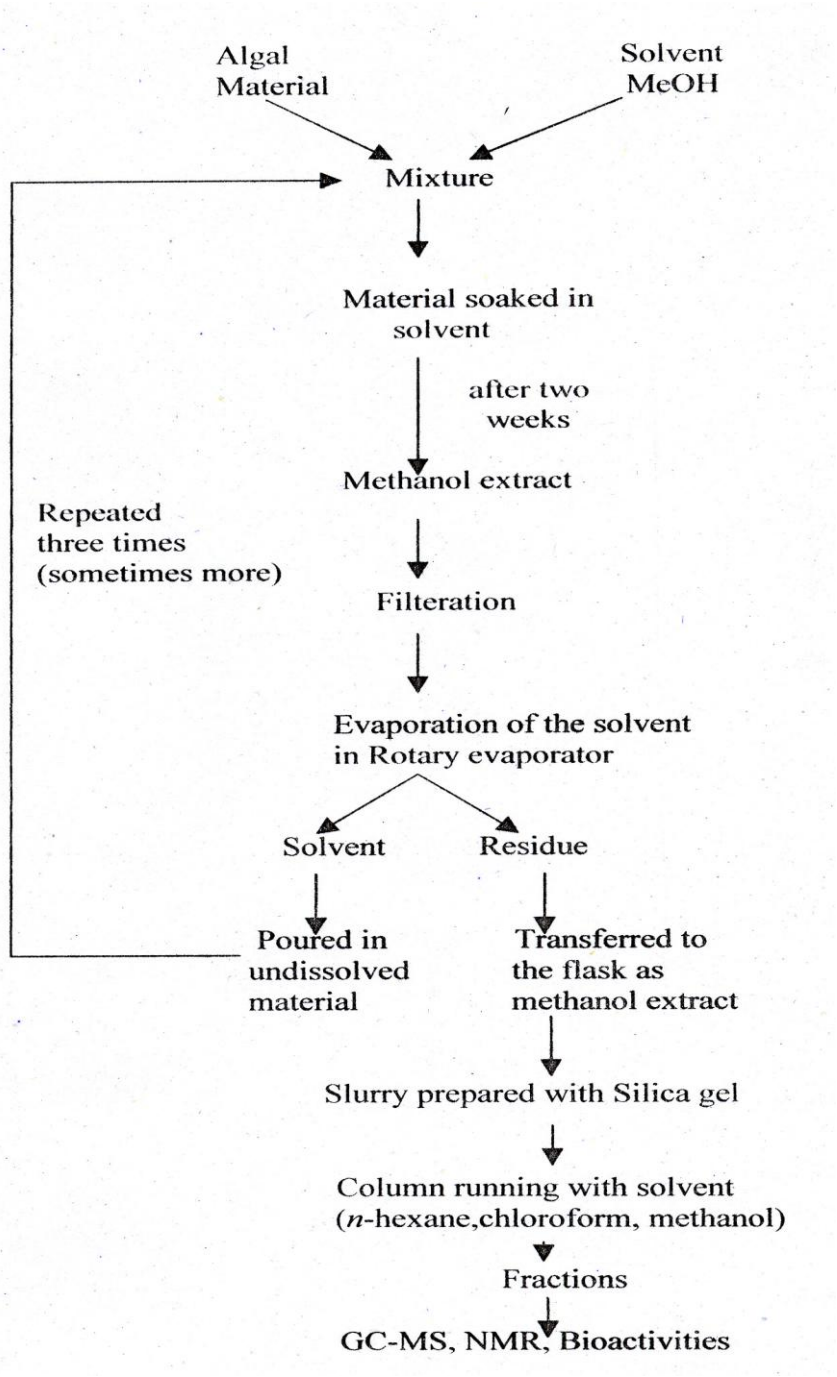

Fig. 1. Scheme for the extraction of methanol extract.

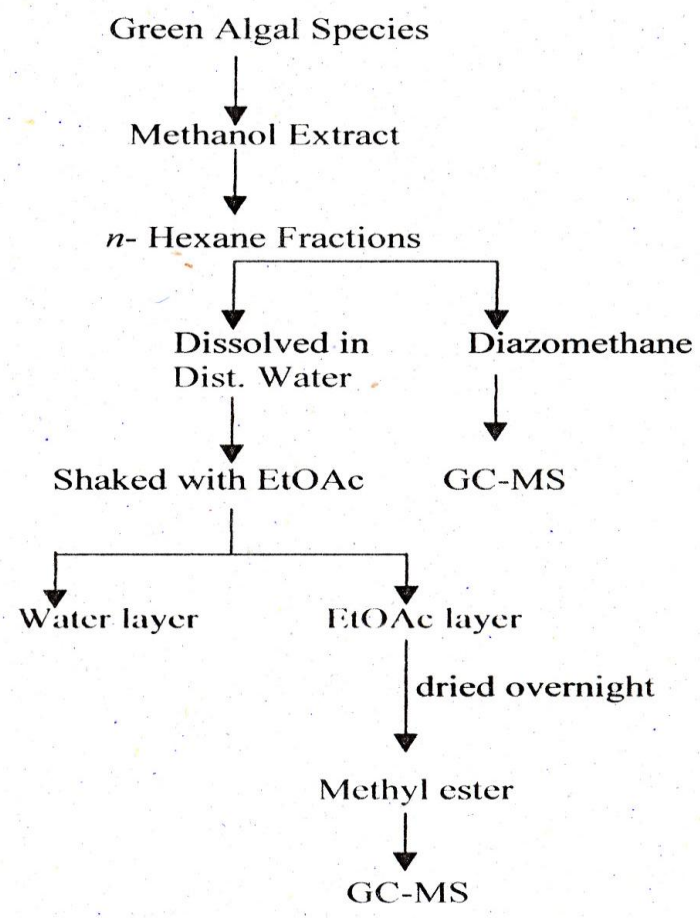

Fig. 2. Scheme for the detection of fatty acids.

reference antibacterial drugs served as negative and positive controls respectively. The plates were incubated immediately at 37 ºC for 14-19 h or more. Activity was determined by measuring diameter of zones showing complete inhibition (in mm) and growth inhibition was calculated with reference to positive control (Amtul, 1997).

Antifungal bioassay: It was investigated with agar well and PDA medium. Broth of boiled potatoes, sugar and agar (20 g each) were dissolved in 1L distilled water and autoclaved. Each plate contained 20 mL medium with diluted crude extract of the sample. Each Petri plate having 5 holes contained different concentrated extracts and one disc of the fungus. At least 3-5 such Petri dishes were prepared for each experimental sample. Initially four sterilized test tubes were taken, 0.9 mL methanol was added in each test tube (with sterilized pipette). About 0.1 mL extract was transferred in test tube No. I [1/10 dil], from which 0.1 mL was transferred to the test tube II [1/100 dilution]. From this test tube 0.1 mL was added to the test tube III [1/1000 dilution]. A poured plate was taken and 5 holes of 0.5 mm diameter were made along the periphery. One drop of each treatment was added in a hole in anticlockwise direction. An inoculum dish (5 mm diam.)

of a test fungus was placed in the center of each of the three replicate plates. It was incubated at room temperature and the growth of the test fungus was recorded towards each hole and compared with the control hole.

Food poisoning activity: In this activity Czapek's Dox agar medium was used in which $NaNO_3$ (3.0 g), K_2HPO_4 (1.0 g), $MgSO_4$ (0.5 g), KCl (0.5 g), $FeSO_4.7H_2O$ (0.1 g), sucrose (30.0 g), agar (20 g) was dissolved in 1 L distilled water and autoclaved at 121 ºC temperature for 15 psi. About 20 mL of this medium was poured into each sterile Petri dish and different fungal species were added for inhibition.

Phytotoxic activity: The E-medium for *Lemna aequinoctialis* Welw. was prepared by mixing various inorganic constituents in 1 L distilled water. The pH was adjusted between 5.5 - 6.0 by adding KOH pellets. It was then autoclaved at 121ºC for 15 minutes. About 12 mg of extract was dissolved in 1.5 mL of solvent (methanol/ethanol) serving as stock solution. Thirty sterilized vials were inoculated with 100 µL, 10 µL and 5 µL of solution pipetted from the stock solution for 500, 50 and 25 ppm. Solvent was allowed to evaporate overnight under sterilized conditions. About 2 mL of E-medium was added and then a single plant of *L. aequinoctialis*, each containing a rosette of three fronds, was placed in each vial. Experimental vials were supplemented with algal extract, and reference plant growth inhibitors and promoters were used serving as negative and positive controls respectively. Each vial was placed in Petri dish filled with about 2 cm of water; the container was sealed with novix and a glass plate. The plates were examined twice during incubation. Dish with vial was placed in growth cabinet for seven days. The numbers of fronds per vial were counted and recorded on the 7th day. Interpretation of result was analyzed as growth regulation in percentage, calculations were made with reference to the control. The details may be found in Farhana (1997).

Brine shrimp bioassay: It was conducted by taking the half filled hatching tray (a rectangular dish (22 x 32 cm) with brine solution (sea salt 38 g/L of d/w), then 500 mg eggs of brine shrimp (*Artemia salina*) were sprinkled and after covering with a lid it incubated at 27 ºC for 2 day for hatching. The brine shrimp larvae were collected through a light source and Pasteur pipette. The sample (20 mg) was dissolved in 2 mL of respective solvent and then 500 µL, 50 µL and 5 µL of this solution was transferred to vials corresponding to 1000, 100 and 10 µg/mL respectively and was evaporated overnight. After two days of hatching, larvae were placed in each vial using a Pasteur pipette. The volume was raised to 5 mL with syringe, by adding seawater and incubated at 27 ºC for 24 h under illumination. After 24 h the number of survivors were counted and recorded. The data were analyzed with Finney computer program to determine LD_{50} values at 95 % confidence intervals. The details of the procedure are given by Mansoor (1997).

Insecticidal activity: The insects (*Tribolium castaneum*, common grain pest) were exposed to the test compound by direct contact toxicity method using filter paper impregnated with test sample (1571.33 $\mu g/cm^2$). Ten adult insects of different types and of same age were transferred to Petri dishes. A check batch of negative control was treated with solvent for determination of solvent effect. Another batch supplemented with reference insecticides *i.e.* Mortein Coopex (Reckilt Benckiser Pak. Ltd.) was used. All of them were kept without food for 24 h (Farhana, 2000). Mortality counts were made after 24 h's exposure period.

Results and Discussion

***Tetraspora cylindrical* (Wahlenberg) Agardh:** Colonies macroscopic, attached, gelatinous, more or less tubular (Fig. 3a), convulated, light green in colour; cells embedded in a single layer of mucilage which is very soft, fragile (Fig. 3b); individual cells or cell-groups surrounded by special sheath of mucilage; pair of pseudocilia emerging from outer surface of each cell extending up to surface of the mucilage (Fig. 3c).

***Tetraspora gelatinosa* (Vaucher) Desvaux:** Colonies macroscopic, indefinitely expanded, attached, gelatinous, cylindrical (Fig. 4), later becoming irregularly lobed; cells spherical, in group of 4 cells, lying in the gelatinous colonial mucilage, chloroplast cup-shaped with pyrenoids, cells 6-9 µm in diameter.

Detection of fatty acids: The GC-MS of the methylated fatty acids (FA) of *T. cylindrica* revealed the presence of 9 saturated and 15 unsaturated fatty acid methyl esters (Tables 1 & 2), while five fatty acids remained unidentified. The GC-MS of FAs of *T. gelatinosa* indicated the presence of only three unsaturated and one steroidal fatty acid methyl esters (Table 3, Fig. 5[1]).

Extraction of sterol & terpene: The fraction eluted with *n*-hexane: chloroform (2: 8 in *T. cylindrica*, and 9.5: 0.5 in *T. gelatinosa* v/v) afforded β-sitosterol (Fig. 5[3]). After ascertaining the purity of the compound the isolated sterol was subjected to spectral analysis (EIMS and ^{1}H-NMR). The diterpene fraction was eluted from the silica gel glass plates in solvent $CHCl_3$: MeOH (9.5:0.5, v/v), purity was then checked on TLC card and after spraying with Ce $(SO_4)_2$ purplish spot was found. After ascertaining the purity of the compound the isolated terpene was subjected to spectral analysis (EIMS and ^{1}H-NMR) and was identified as *trans*-phytol (Fig. 5[4]).

Bioactivity: The methanol extract of *T. cylindrica* showed antibiotic activity against only three of the ten tested bacterial species (Table 4), six of the ten fungal species by agar well diffusion method and all the six fungal organisms by food poisoning method. Its phytotoxicity against *Lemna aequinoctialis* was found to be significant at 500 µg/mL concentration. Its cytotoxic activity against brine shrimp and insecticidal activity were detected to be non-significant. The methanol extract of *T. gelatinosa* showed antibiotic activity against six of the ten tested fungal species (Table 4). Its phytotoxic activity against *Lemna aequinoctialis* was found to be significant at 500 µg/mL concentration. Its cytotoxic activity against brine shrimp and insecticidal test were observed to be non-significant.

The two species of *Tetraspora* have been taxonomically investigated. The methanol extract of *T. cylindrica* revealed the presence of 9 saturated and 15 unsaturated fatty acids (Table 4), both were present in equal amount (44.87 & 44.39 %). Heneicosanoic acid (C21:0) was found to be in largest quantity (17.02 %) as compared to all other fatty acids. Among unsaturated acids pentadienoic (C5:2) and undecatrienoic (C11:3) acids occurred in smallest quantity (0.115%) and heneicosenoic acid (C21:1) in highest amount (13.35%). The unsaturated acids contained 8 monounsaturated, 4 diunsaturated and 3 triunsaturated fatty acids, while no polyunsaturated fatty acid having more than three double bonds could be detected. Analysis of sterol fraction afforded β-sitosterol and *trans*-phytol in pure form. Literature survey reveals that no work has so far been done on the phycochemistry of any species of *Tetraspora*, therefore no comparison may be made with the previous studies.

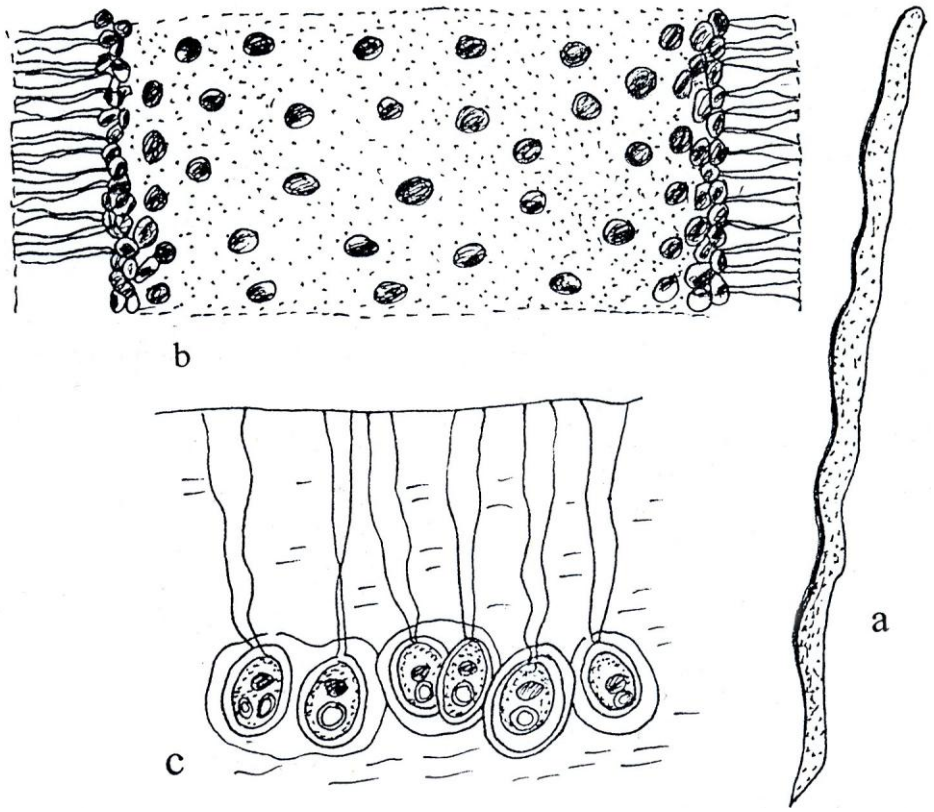

Fig. 3. *Tetraspora cylindrica* (Wahlenberg) Agardh: a. colony (x 200), b. enlarged part of colony (x 600), c. individual cells with pseudocilia (p) (x 1200).

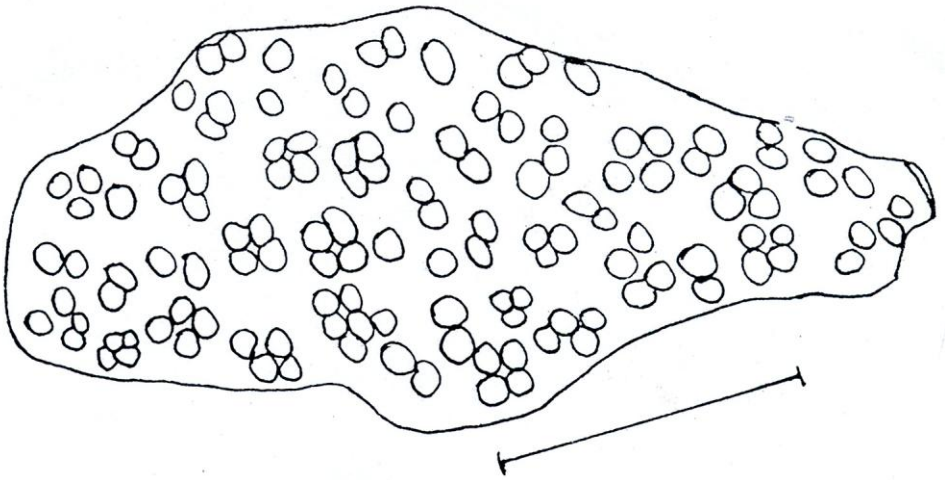

Fig. 4. *Tetraspora gelatinosa* (Vaucher) Desvaux: colony (scale: 132 μm).

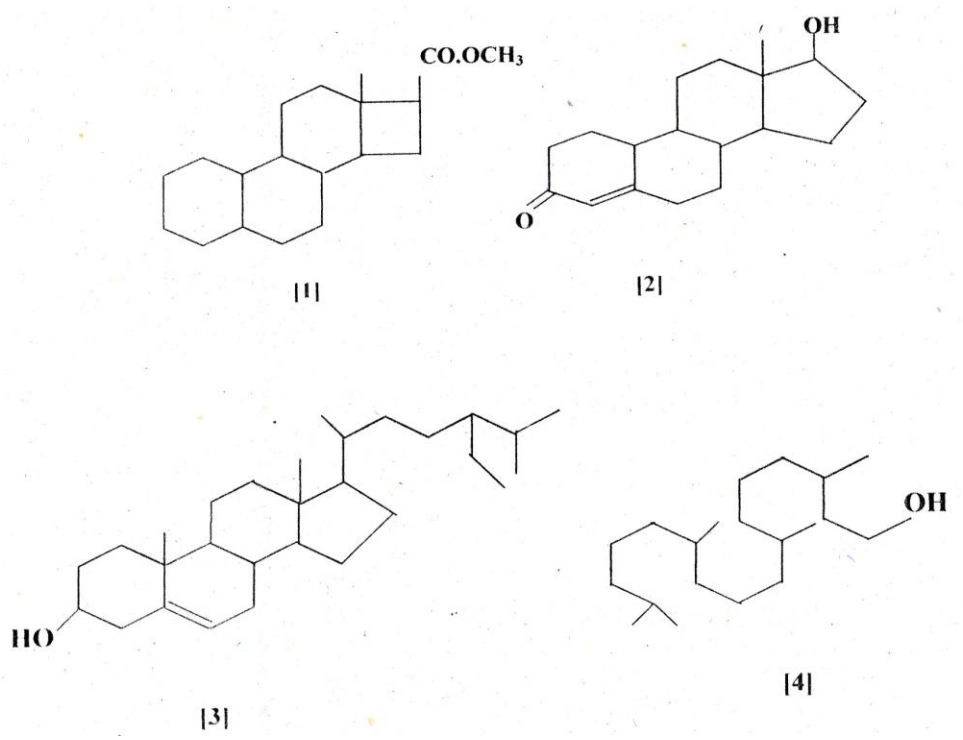

Fig. 5. Structure of the isolated natural products: **[1]**=D-*nor*androstane-16-carboxylate, **[2]**=17β-hydroxyandrost-4-en-3-one (testosterone), **[3]**=β-sitosterol, **[4]**=trans-phytol.

The methanol extract of *T. cylindrica* exhibited an appreciable amount of bioactivity against a variety of organisms (Table 4). It was previously observed that the bloom of *Tetraspora* may decrease the population of trichopteran larvae (Yasuno *et al.,* 1985). Polysaccharides extracted from *Tetraspora* may also be used to treat viral infections (Muto *et al.,* 1988), indicating strong bioactivity of the metabolites present in this alga.

The methanol extract of *T. gelatinosa* revealed the presence of only three unsaturated fatty acid methyl esters and one steroidal fatty acid (Table 4). Pentadecatetraenoic acid (C15:4) occurred in highest amount (30.08 %). It is very interesting to note that no saturated as wall as mono- and di-unsaturated fatty acid could be detected. The steroidal fatty acid was identified as D-*nor*androstane-16-carboxylic acid (5α, 16β; Fig. 5[1]). Androstane (17β-hydroxyandrost-4-en-3-one) is the parent hydrocarbon of the male hormone testerone (Fig. 5[2]). It is very interesting that this derivative of an animal hormone occurred as metabolite in an alga.

Comparison of the two investigated species of *Tetraspora* showed that none of the 9 saturated, 8 monounsaturated and 4 diunsaturated fatty acids isolated from *T. cylindrica* could be detected in *T. gelatinosa* (Table 4). Similarly any of the polyunsaturated and steroidal fatty acids detected in *T. gelatinosa* could not be found in *T. cylindrica*. In this way the two species remarkably differed from each other in their fatty acid composition. In other aspects of phycochemistry and bioactivity the two species exhibited great resemblance, *e.g.* β-sitosterol and *trans*-phytol were isolated from both of them. They

Table 4. Comparison of phycochemistry and bioactivity of the two investigated species of *Tetraspora*.

Compounds/Organisms		*T. cylindrical*	*T. gelatinosa*
I. Saturated fatty acids:			
C14:0	*n*-Tetradecanoic acid	3.133	-
C15:0	*n*-Pentadecanoic acid	9.596	-
C16:0	*n*-Hexadecanoic acid	4.24	-
C17:0	*n*-Heptadecanoic acid	0.867	-
C18:0	*n*-Octadecanoic acid	2.55	-
C19:0	*n*-Nonadecanoic acid	3.359	-
C21:0	*n*-Heneicosanoic acid	17.02	-
C23:0	*n*-Tricosanoic acid	3.757	-
C27:0	*n*-Heptacosanoic acid	0.346	-
II. Monounsaturated fatty acids:			
C12:1	9-Dodecenoic acid	0.37	-
C14:1	9-Tetradecenoic acid	0.93	-
C17:1	Heptadecenoic acid	3.68	-
C18:1	8-Octadecenoic acid	0.150	-
C19:1	10-Nonadecenoic acid	1.85	-
C20:1	9-Eicosenoic acid	2.60	-
C21:1	Heneicosenoic acid	13.352	-
C22:1	11-Docosenoic acid	1.849	-
III. Diunsaturated fatty acids:			
C5:2	Pentadienoic acid	0.115	-
C16:2	Hexadecadienoic acid	2.024	-
C17:2	Heptadecadienoic acid	7.167	-
C18:2	5,8-Octadecadienoic acid	1.156	-
IV. Triunsaturated fatty acids:			
C11:3	Undecatrienoic acid	0.115	-
C15:3	3,7,11-Trimethyl-2,6,10 dodecatrienoic acid	9.028	17.612
C17:3	5,8,11-Heptadecanoic acid	1.62	23.8
V. Polyunsaturated fatty acid:			
C15:4	Pentadecatetraenoic acid	-	30.084
VI. Steroidal fatty acid:			
C19:C	D-*Nor*androstane-16-carboxylic acid	-	28.084
VII. Natural products:			
β-Sitosterol		+	+
Trans-phytol		+	+

Table 4. (Cont'd.)

Compounds/Organisms	*T. cylindrical*	*T. gelatinosa*
VIII. Anibacterial activity:		
Bacillus cereus	-	x
Corynebacterium diphtheriae	†6	x
Escherichia coli	**-**	**x**
Klebsiella pneumoniae	†7.5	x
Proteus mirabilis	**-**	**x**
Pseudomonas aeruginosa	-	x
Salmonella typhi	-	x
Shigella boydii	†8	x
Staphylococcus aureus	-	x
Streptococcus pyogenes	-	x
IX. Antifungal activity:		
a By agar well diffusion method:		
Alternaria alternata	-	x
Curvularia lunata	2.8	x
Drechslera australiensis	-	x
Fusarium solani	-	x
Fusarium sporotrichoids	4.1	x
Fusarium proliferatum	-	x
Macrophomina phaseolina	4.0	x
Rhizoctonis solani	4.0	x
Sclerotium rolfsii	3.8	x
Trichoderma harzianum	3.7	x
b By food poisoning method:		
Curvularia lunata	**3.6**	**x**
Fusarium solani	6.0	x
Macrophomina phaseolina	6.8	x
Rhizoctonia solani	4.4	x
Sclerotium rolfsii	7.5	x
Trichoderma harzianum	7.9	x
X. Phytotoxicity:		
500 µg/mL concentration	14.28	100
XI. Brine shrimp bioassay:		
1000 µg/mL LD_{50}	-	-
XII. Insecticidal activity:		
Tribolium castaneum	-	-

+ = Present, - = absent /no activity, x = not tested.

showed antifungal activity (by agar well diffusion method) against same six out of the ten investigated fungal organisms. Antibacterial and antifungal (by food poisoning method) activities could not be investigated in *T. gelatinosa* due to shortage of material, therefore no comparison may be made in this connection. However, both of them displayed appreciable amount of bioactivity regarding phytotoxicity but exhibited no bioactivity in brine shrimp bioassay and insecticidal activity. *Tetraspora* has previously been observed to show strong bioactivities (Yasuno *et al.,* 1985; Muto *et al.,* 1988).

References

Ahmad, V.U., R. Aliya, S. Perveen and M. Shameel. 1992. A sterol glycoside from marine green alga *Codium iyengarii*. *Phytochem.*, 31: 1429-1431.

Ahmad, V.U., R. Aliya, S. Perveen and M. Shameel. 1993. Sterols from marine green alga *Codium decorticatum. Phytochem*., 33: 1189-1192.

Aliya, R. and M. Shameel. 1993. Phycochemical examination of three species of *Codium* (Bryopsidophyceae). *Bot. Mar.*, 36:371-376.

Aliya, R. and M. Shameel. 1999. Phycochemical evaluation of four coenocytic green seaweeds from the coast of Karachi. *Pak. J. Mar. Biol.*, 5: 65-76.

Aliya, R., M. Shameel, K. Usmanghani and V.U. Ahmad. 1991. Analysis of fatty acids from *Codium iyengarii* (Bryopsidophyceae). *Pak. J. Pharm. Sci.*, 4:103-111.

Aliya, R., M. Shameel, K- Usmanghani and V.U. Ahmad. 1993. Fatty acid composition of five species of *Caulerpa* (Bryopsidophyceae) from north Arabian Sea. *Mar. Res*., 2: 25-34.

Aliya, R., M. Shameel, S. Perveen, M.S. Ali, K. Usmanghani and V.U. Ahmad. 1994. Acyclic diterpene alcohols isolated from four algae of Bryopsidophyceae and their toxicity. *Pak. J. Mar. Sci.*, 3: 15-24.

Aliya, R., M. Shameel, K. Usmanghani, S. Sabiha and V.U. Ahmad. 1995. Fatty acid composition of two siphonaceous green algae from the coast of Karachi. *Pak. J. Pharm. Sci.*, 8: 47-54.

Amjad, M.T. and M. Shameel. 1993. Comparative haemagglutinic activity in the species of *Caulerpa* and *Ulva* (Chlorophyta) of Karachi coast. *Pak. J. Mar. Sci.*, 2: 113-117.

Amtul, Z. 1997. Anti-bacterial techniques in bioassay guided fractions. *In*: Z.Amtul (ed.): *Workshop on Use of Bioassay Techniques for New Drug Development*. HEJ Res. Inst. Chem., Kar. Univ., p. 5-16.

Atta-ur-Rahman, M.A. Khan, M. Shabbir, M. Abid, M.I. Chaudhary, A. Nasreen, M.A. Maqbool, M. Shameel and R. Sualeh. 1997. Nematocidal study of marine organisms. *Pak. J. Nematol.,* 15: 95-100.

Farhana, K. 1997. The *Lemna*-bioassay for inhibitors and promoters of plant growth. *In*: Z.Amtul (ed.): *Workshop on Use of Bioassay Techniques for New Drug Development*. HEJ Res. Inst. Chem., Kar. Univ., p. 5-17.

Farhana, K. 2000. Screening of insecticides by impregnation method on storage pests. *In*: *Manual of Bioassay Techniques*. Worksh. Tech. Prod. Develop. Med. Plants, HEJ Res.Inst. Chem; Pakistan & Inter-Isl. Network Trop. Med., Malaysia, p. 15-17.

Helles, S.R. and G.W.A. Milne. 1978. *EPA/NIH Mass Spectral Data Base*. 4 Vols., NIBS, US Govt. Print. Office, Washington, 3975 pp.

Khaliq-uz-Zaman, S.M., S. Shameel, M. Shameel, S.M. Leghari and V.U. Ahamad. 1998. Bioactive compounds in *Chara corallina* Var. *wallichi* (A. Br.) R.D. Wood (Charophyta). *Pak. J. Bot.,* 30: 19-31.

Khaliq-uz-Zaman, S.M., K. Simin and M. Shameel. 2001. Antimicrobial activity and phytotoxicity of sterols from *Chara wellichi* A. Br. (Charophyta). *Pak. J. Sci. Ind. Res*., 44: 301-304.

Mansoor, S. 1997. Brine-shrimp to screen in vivo lethality of bioactive compounds. In: Z. Amtul (ed.): *Workshop on Use of Bioassay Techniques for New Drug Development*. HEJ Res. Inst. Chem., Kar. Univ., p. 3-4.

Muto, S.K. Niimura, M. Oohara, Y. Oguchi, K. Matsunaga, K. Hirose, J. Kakuchi, N. Sugita, T. Furusho. 1988. Polysaccharides from marine algae and antiviral drugs containing the same as active ingredient. *Eur. Pat. Appl*., EP 295, 956: 13 pp.

Naqvi, S.B.S., D. Sheikh, K. Usmanghani, M. Shameel and R. Sheikh. 1992. Screening of marine algae of Karachi for haemagglutinin activity. *Pak. J. Pharm. Sci.*, 5: 129-138.

Qasim, R. 1986. Studies on fatty acid comosition of eighteen species of seaweeds from the Karachi coast. *J. Chem. Soc. Pak.*, 8: 223-230.

Rizvi, M.A., S. Farooqui and M. Shameel. 2000. Bioactivity and elemental composition of certain seaweeds from Karachi coast. *Pak. J. Mar. Biol.*, 6: 207-218.

Shameel, M. 1987. Studies on the fatty acids from seaweeds of Karachi. *In*: 1. Alahi 7 F. Hussain (Eds.): *Modern Trends of Plant Science Research in Pakistan*. *Proceed. Nat. Conf. Plant Sci.*, 3: 183-186.

Shameel, M. 1990. Phycochemical studies on fatty acids from certain seaweeds. *Bot. Mar.*, 33: 429-432.

Shameel, M. 1993. Phycochemical studies on fatty acids composition of twelve littoral green seaweeds of Karachi coast. *In*: *Proceedings of the National Seminar on Study and Management in Coastal Zones in Pakistan*. (Eds.): N.M. Tirmizi & Q.B. Kazmi. Pak. Nat. Commis. UNESCO, Karachi, p. 17-25.

Shameel, M. 2001. An approach to the classification of algae in the new millennium. *Pak. J. Mar. Biol.*, 7: 233-250.

Usmanghani, K. and M. Shameel. 1986. Studies on the antimicrobial activity of certain seaweeds from Karachi coast. In: R. Ahmed & A. San Pietro (eds.): *Prospects for Biosaline Research*. Proc. US-Pak. Biosal. Res. Worksh., Karachi, p. 519-526.

Usmanghani, K., M. Shameel, M. Sualeh, K. Khan and Z.A. Mahmood. 1984. Antibacterial and antifungal activities of marine algae from Karachi seashore of Pakistan. *Fitotrap.*, 55:73-77.

Usmanghani, K., M. Shameel and M. Alam. 1985. Fatty acids of the seaweed *Ulva fasciata* (Chlorophyta). *Sci. Pharm.*, 53: 247-251.

Usmanghani, K., M. Shameel and M. Alam. 1986. Studies on fatty acids of a green alga *Ulva fasciata* from Karachi coast. *In*: *Prospects for Biosaline Research*. (Eds.): R. Ahmad & A. San Pietro. Proc. US-Pak. Biosal. Res. Worksh., Karachi, p. 527-531.

Yasuno, M., Y. Sugaya and T. Iurakuma. 1985. Effects of insecticides on the benthic community in a model stream. *Environ. Poll.*, Ser. A, 38: 31-43.

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