Pak. J. Bot., 35(5): 659-669, 2003.

MARINE NATURAL PRODUCTS OF *CAULERPA* (SIPHONOCLADOPHYCEAE)

R. ALIYA AND MUSTAFA SHAMEEL

Department of Botany, University of Karachi, Karachi-75270, Pakistan.

Abstract

Five siphonocladophycean green seaweeds, *Caulerpa chemnitzia* (Esp.) Lamour., *C. faridii* Nizam., *C. manorensis* Nizam., *C. racemosa* (Forssk.) J. Ag., and *C. taxifolia* (Vahl) C. Ag., collected from the coastal waters near Karachi were analyzed phycochemically for their fatty acid, sterol and diterpene constituents using GLC, EI-, FD-, GC-, HR- & IR-MS and ^{1}H- & ^{13}C-NMR spectroscopy. Altogether 36 acids *i.e.* 18 saturated and 18 unsaturated fatty acids were identified as methyl esters. Five different sterols have been identified, isolated, purified and chemically elucidated. Cholesterol was found to be the most abundant sterol, and with the exception of *C. faridii* it was found in all the other four species. Two diterpenoids have also been isolated from 3 species of *Caulerpa*, which are acyclic straight chain compounds and are being reported for the first time from any species of *Caulerpa*. The five species of *Caulerpa* differed from one another phycochemically in many different aspects.

Introduction

Thirteen species of *Caulerpa* have so far been identified from the coast of Pakistan (Shameel, 1978, 1987; Shameel & Tanaka, 1992). A few studies have been carried out on the biochemical constitution and general phycochemistry of *Caulerpa* from the coast of Karachi (Qasim, 1986; Shameel & Khan, 1989, 1991; Zahid, 1989; Shameel, 1990, 1993; Usmanghani & Shameel, 1996; Aliya *et al.*, 1993). From other parts of the world also works have been done on the phycochemistry of various species of *Caulerpa* (Blackman & Wells, 1976; De Napoli *et al.*, 1982; Nielsen *et al.*, 1982; Paul & Fenical, 1985; Prasada-Rao & Venkata-Rao, 1986; Anjaneyulu *et al.*, 1991; Khotimchenko, 1995; Terrados & Lopez-Jimenez, 1996; Xu & Zeng, 1998; Handley & Blackman, 2000; Soliman *et al.*, 2000; Mabrouk *et al.*, 2001). No detailed study has yet been carried out on the comparative phycochemistry of various *Caulerpa* species of our coast. In this report an attempt has been made to present a comparative composition of fatty acids, sterols and diterpenes of five commonly occurring species of this genus *i.e. C. chemnitzia* (Esp.) Lamour., *C. faridii* Nizam., *C. manorensis* Nizam., *C. racemosa* (Forssk.) J. Ag., and *C. taxifolia* (Vahl) C. Ag.

Materials and Methods

Fresh and clean thalli of *Caulerpa chemnitzia, C. faridii, C. manorensis, C. racemosa* and *C. taxifolia* were collected as drift as well as epilithon from mid and lower littoral rocks and sandy pools of Manora, Buleji and Nathiagali near Karachi. They were thoroughly washed with tap water to remove clinging epiplytes and animal castings, the washed specimens were then air-dried in shade.

Detection of fatty acids: The dried thalli of each of the five species (350-1500 g) were soaked in methanol at room temperature for about a month (Fig. 1). The methylated fractions obtained were first analyzed by gas-liquid chromatography (GLC) and then by gas chromatography-mass spectrometry (GC-MS). The fatty acids were identified by matching their spectra with those of the National Bureau of Standards (NBS) mass spectral library (Helles & Milne, 1978). The esterification and GLC & GC-MS techniques were the same as described previously (Shameel, 1990).

Isolation of sterols: The same procedure as that of fatty acids was applied for the sterols but they were eluted at a higher polarity. The order of polarity is given in Fig. 1. Five different sterols have been isolated with the exception of cholesterol, which was already obtained in pure form, all others were purified on large silica plates by running in different polarities of solvents such as: 24-methyl-cholesta-7, 22-dien-3β-ol in *n*-hexane:diethyl ether (4:6, v/v), 24-methyl-cholesterol in *n*-hexane:diethyl ether (1:1, v/v), 4,24-dimethyl-cholesta-5, 22-dien-3β-ol in *n*-hexane:diethyl ether (3:7, v/v) and β-sitosterol in *n*-hexane:diethyl ether (6:4, v/v). The sterols so purified were analyzed by nuclear magnetic resonance (^{1}H- and ^{13}C-NMR), electron impact (EI-), field desorption (FD-), mass spectrometric (-MS) techniques.

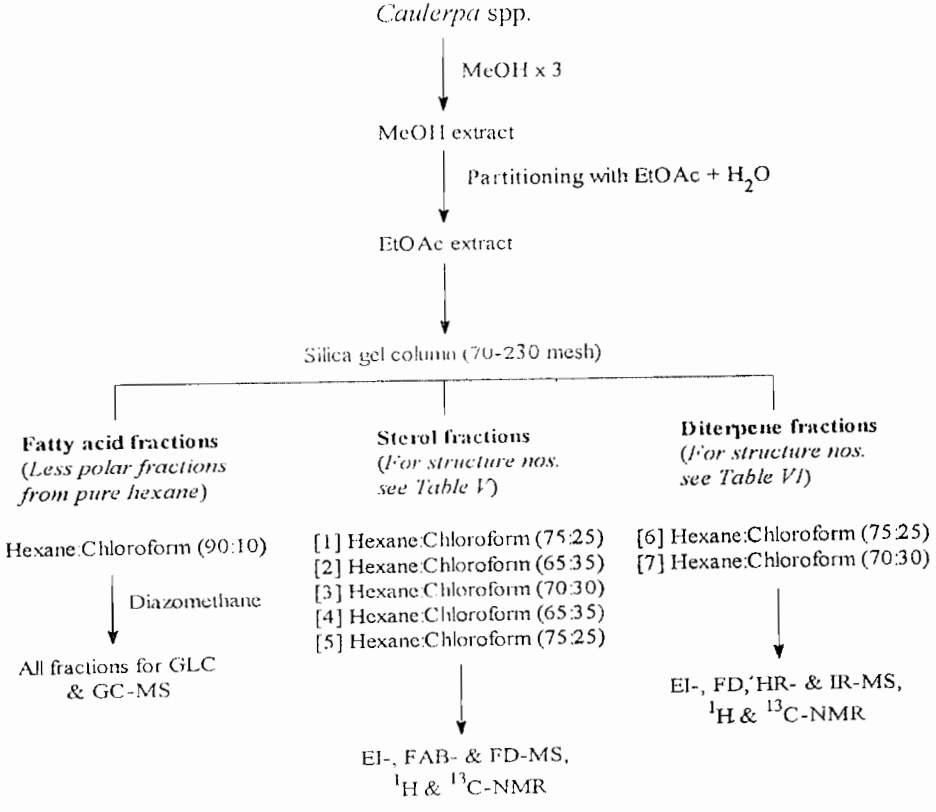


Fig. 1. Scheme for the separation, isolation and analyses of fatty acids, sterols and diterpenes from *Caulerpa* spp.

Extraction of diterpenes: The isolation of diterpenes was also carried out as described in Fig. 1. The 3, 7, 11, 15-tetramethyl-hexadec-2-en-1-ol and 3, 7, 11, 15-tetramethyl-hexadec-3-en-1, 2-diol were purified on TLC cards (20x20 cm) in pure chloroform. The structures were solved by ^{1}H- & ^{13}C-NMR and El-, FD-, high resolution (HR-) and infrared (IR-) MS techniques.

Methodology and instrumentation: The details of column chromatography (CC), purification patterns and instrumentation techniques were the same as described earlier (Aliya & Shameel, 1993).

Results and Discussion

The analysis of fatty acid methyl esters revealed the presence of 36 different fatty acids in the five investigated species of *Caulerpa*. They indicated a wide variety of saturated (Table 1) and unsaturated fatty acids (Tables 2 & 3). It was observed that unsaturated fatty acids were present in a greater quantity (56.26-74.30 %) than the saturated acids (25.62-47.61 %), the only exception is *C. racemosa* where the saturated fatty acids (53.32 %) were slightly larger in amount than the unsaturated ones (46.65 %). This type of distribution of fatty acids has also previously been reported in *Caulerpa* spp., as well as other green seaweeds (Khotimchenko, 1995; Soliman *et al.*, 2000; Mabrouk *et al.*, 2001). The species of *Codium* also contained unsaturated fatty acids in larger amount than the saturated fatty acids (Aliya & Shameel, 1993; Herbreteau *et al.*, 1997; Hainaux *et al.*, 1998; Xu *et al.*, 1998).

Among saturated fatty acids palmitic and stearic acids were found in all the five investigated species, while myristoleic was the only unsaturated acid present in all of them. Palmitic acid has also been detected in largest quantity in most of the previously investigated green seaweeds (Usmanghani *et al.*, 1985; Khotimchenko, 1995; Xu *et al.*, 1998; Mabrouk *et al.*, 2001). The presence of lignoceric, pentacosanoic and heptacosanoic among saturated acids and tricosenoic and pentacosenoic among unsaturated acids in investigated species of *Caulerpa* is the first report, because fatty acids of such a high carbon number have not yet been detected in any previously studied seaweed of Karachi coast.

Among individual species, 7-ethyl-3methyl-2, 6-undecadienoate (Fig. 2:[1]) was found in largest amount in *C. chemnitzia,* oleate in *C. faridii*; myristoleate in *C. manorensis*, palmitate in *C. racemosa* and gadoleate in *C. taxifolia*; the relative percentage of the last mentioned acid appeared to be the greatest in comparison with all the detected saturated as well as unsaturated fatty acids. Although palmitate has been the major component in the species of *Caulerpa* previously described from Karachi coast (Qasim, 1986; Shameel & Khan, 1998), yet it does not seem to be the case in this study. With the exception of palmitic acid, all those acids found in the highest amount belong to the category of unsaturated fatty acids.

Dimethoxy propanoate, heptacosanoate, undecylenate and lauroleate were only found in *C. chemnitzia* and no other species of *Caulerpa*. Likewise, arachidate and tricosenoate were only detected in *C. faridii*, caprylate and laurate in *C. manorensis* and undecylate in *C. racemosa*, no acid was found in *C. taxifolia* alone. Oleate was present in all species except *C. chemnitzia*. Similarly, lignocerate was absent only in *C. faridii*, 7-ethyl-3-methyl-2,6-undecadienoate and hexadecadienoate only in *C. manorensis* and pentacosanoate, heptadecylenate and nonadecylenate only in *C. taxifolia*; *C. manorensis*

Table 1. Relative percentages of saturated fatty acids analysed as methyl esters present in the methanol extracts of *Caulerpa* species.

Acid type	Systematic name	Molecular formula	Mol. wt.	*C. chemnitzia*	*C. faridii*	*C. manorensis*	*C. racemosa*	*C. taxifolia*
$C_{3:0}$	2,3-Dimethoxy propanoate	$C_6H_{12}O_4$	148	6.43	—	—	—	—
$C_{8:0}$	*n*-Octanoate	$C_9H_{18}O_2$	158	—	—	1.24	—	—
$C_{9:0}$	*n*-Nonanoate	$C_{10}H_{20}O_2$	172	—	—	4.50	10.17	—
$C_{11:0}$	*n*-Undecanoate	$C_{12}H_{24}O_2$	200	—	—	—	5.49	—
$C_{12:0}$	*n*-Dodecanoate	$C_{13}H_{26}O_2$	214	—	—	2.49	—	—
$C_{14:0}$	*n*-Tetradecanoate	$C_{15}H_{30}O_2$	242	—	—	1.43	2.94	5.37
$C_{15:0}$	*n*-Pentadecanoate	$C_{16}H_{32}O_2$	256	3.76	6.28	7.34	—	—
$C_{16:0}$	*n*-Hexadecanoate	$C_{17}H_{34}O_2$	270	2.87	11.34	4.42	13.66	15.55
$C_{17:0}$	*n*-Heptadecanoate	$C_{18}H_{36}O_2$	284	0.64	—	8.58	—	—
$C_{18:0}$	*n*-Octadecanoate	$C_{19}H_{38}O_2$	298	1.54	1.37	2.53	3.01	2.23
$C_{19:0}$	*n*-Nonadecanoate	$C_{20}H_{40}O_2$	312	—	4.01	4.41	2.03	—
$C_{20:0}$	*n*-Eicosanoate	$C_{21}H_{42}O_2$	326	—	1.59	—	—	—
$C_{21:0}$	*n*-Heneicosanoate	$C_{22}H_{44}O_2$	340	—	3.45	—	2.61	—
$C_{22:0}$	*n*-Docosanoate	$C_{23}H_{46}O_2$	354	—	—	0.86	7.36	—
$C_{23:0}$	*n*-Tricosanoate	$C_{24}H_{48}O_2$	368	1.76	—	2.90	—	—
$C_{24:0}$	*n*-Tetracosanoate	$C_{25}H_{50}O_2$	382	1.10	—	1.44	2.45	5.11
$C_{25.0}$	*n*-Pentacosanoate	$C_{26}H_{52}O_2$	396	6.42	0.79	5.58	3.60	—
$C_{27:0}$	*n*-Heptacosanoate	$C_{28}H_{56}O_2$	424	1.12	—	—	—	—
		Total:		**25.64**	**28.83**	**47.72**	**53.32**	**28.26**

Table 2. Relative percentages of monoenoic fatty acids analysed as methyl esters present in the methanol extracts of *Caulerpa* species.

Acid type	Systematic name	Molecular formula	Mol. wt.	*C. chemnitzia*	*C. faridii*	*C. manorensis*	*C. racemosa*	*C. taxifolia*
$C_{11:1}$	10-Undecenoate	$C_{12}H_{22}O_2$	198	6.74	—	—	—	—
$C_{12:1}$	9-Dodecenoate	$C_{13}H_{24}O_2$	212	3.67	—	—	—	—
$C_{13:1}$	Tridecenoate	$C_{14}H_{26}O_2$	226	10.68	—	1.06	—	—
$C_{14:1}$	9-Tetradecenoate	$C_{15}H_{28}O_2$	240	5.40	14.21	15.21	6.86	20.46
$C_{15:1}$	Pentadecenoate	$C_{16}H_{30}O_2$	254	—	2.72	2.37	—	—
$C_{16:1}$	9-Hexadecenoate	$C_{17}H_{32}O_2$	268	5.03	—	5.94	—	5.11
$C_{17:1}$	Heptadecenoate	$C_{18}H_{34}O_2$	282	2.24	2.85	1.66	1.62	—
$C_{18:1}$	9-Octadecenoate	$C_{19}H_{36}O_2$	296	—	16.48	5.05	8.17	2.07
$C_{19:1}$	Nonadecenoate	$C_{20}H_{38}O_2$	310	5.75	9.48	4.41	3.25	—
$C_{20:1}$	9-Eicosenoate	$C_{21}H_{40}O_2$	324	3.48	1.37	—	—	25.92
$C_{21:1}$	Heneicosenoate	$C_{22}H_{44}O_2$	338	—	1.93	1.88	—	—
$C_{23:1}$	Tricosenoate	$C_{24}H_{46}O_2$	366	—	0.91	—	—	—
$C_{25:1}$	Pentacosenoate	$C_{26}H_{50}O_2$	394	2.01	1.71	—	—	—
		Total:		**45.00**	**51.66**	**37.58**	**19.90**	**53.56**

Table 3. Relative percentages of di- & trienoic fatty acids extracted as methyl esters from methanol extracts of *Caulerpa* species.

Acid type	Systematic name	Molecular formula	Mol. wt.	*C. chemnitzia*	*C. faridii*	*C. manorensis*	*C. racemosa*	*C. taxifolia*
A. Dienoic fatty acid methyl esters:								
$C_{11:2}$	7-Ethyl-3-methyl-2,6-undecadienoate	$C_{15}H_{26}O_2$ [1]*	238	20.92	3.68	5.22	—	8.47
$C_{16:2}$	Hexadecadienoate	$C_{17}H_{30}O_2$	266	8.39	13.09	4.51	—	5.91
$C_{18:2}$	Octadecadienoate	$C_{19}H_{34}O_2$	294	—	—	—	8.96	1.60
		Total:		**29.31**	**16.77**	**9.73**	**8.96**	**15.98**
B. Trienoic fatty acid methyl esters:								
$C_{12:3}$	3,7,11-Trimethyl-2,6,10-dodecatrienoate	$C_{16}H_{26}O_2$ [2]*	250	—	—	4.95	5.69	2.16
$C_{17:3}$	Heptadecatrienoate	$C_{18}H_{30}O_2$	278	—	2.70	—	12.10	—
		Total:		**0.00**	**2.70**	**4.95**	**17.79**	**2.16**

* See Figure 2

Table 4. Natural products isolated from *Caulerpa* species (+ = present, — = not present).

Systematic name	Common name	Molecular formula*	Mol. wt.	*C. chemnitzia*	*C. faridii*	*C. manorensis*	*C. racemosa*	*C. taxifolia*
A. Sterols								
Cholest-5-en-3β-ol	Cholesterol	$C_{27}H_{46}O$ [3]	386	+	—	+	+	+
24-Methyl-cholesta-7,22-dien-3β-ol	—	$C_{28}H_{46}O$ [4]	398	—	+	—	—	—
24-Methylcho-lest-5-en-3β-ol	24-Methyl cholesterol	$C_{28}H_{48}O$ [5]	400	—	+	—	—	—
4,24-Dimethyl-cholesta-5,22-dien-3β-ol	—	$C_{29}H_{48}O$ [6]	412	—	+	—	—	—
24-Ethylcholesta-5-en-3β-ol	β-Sitosterol	$C_{29}H_{50}O$ [7]	414	—	+	—	—	—
B. Diterpenes								
3,7,11,15-Tetramethyl-hexadec-2-en-1-ol	—	$C_{20}H_{40}O$ [8]	296	—	—	—	+	+
3,7,11,15-Tetramethyl-hexadec-3-en-1,2-diol	—	$C_{20}H_{40}O_2$ [9]	312	+	—	—	—	—

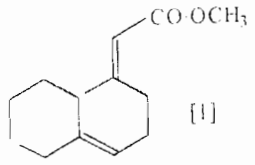
* See Figure 2

did not lack any such acid. All the investigated species of *Caulerpa* showed a different fatty acid composition, *C. manorensis* exhibited the greatest diversity within 24 fatty acids, while *C. taxifolia* appeared to be the least diverse species with only 12 fatty acids. The latter has previously shown a greater diversity in its fatty acids than *C. faridii* and *C. racemosa* (Shameel & Khan, 1989).

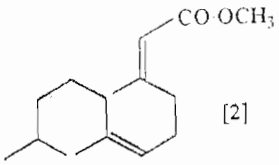
Five different sterols have been isolated from the investigated species of *Caulerpa* (Table 4). Cholesterol (Fig. 2:[3]) was the most abundant sterol and with the exception of *C. faridii* was found in all the other four species. Cholesterol is a very common sterol usually found in green seaweeds, it was also detected in *C. prolifera* (De Napoli, 1982; Soliman *et al.*, 2000). Duraiswamy (1986) separated the shiny colourless needles (melting at 137°C) of β-sitosterol admixed with 3-5 % cholesterol from 18 Indian species of *Caulerpa*. As no detailed work has been carried out on the sterol composition of *Caulerpa* species of Karachi coast, we do not have any previous data to compare with. *Caulerpa faridii* was the only species which exhibited the presence of four different sterols, all of them were not found in any of the other investigated species. Although *C. faridii* did not show the presence of cholesterol, but it possessed four different types of 24-derivatives of cholesterol (Fig. 2:[4-7]). It appears that cholesterol is the characteristic sterol, most probably the chemotaxonomic marker, of the genus *Caulerpa*; as clerosterol is commonly found in its allied genus *Codium* (Aliya & Shameel, 1993; Shen *et al.*, 1995). Both of them belong to the class Siphonocladophyceae (Shameel, 2001).

Two diterpenoids have also been identified, both are acyclic straight chain diterpenes (Table 4). *Caulerpa faridii* and *C. manorensis* did not show the presence of any diterpene. It is interesting to note that one of these 2 species *i.e. C. faridii* is endemic to the coast of Karachi, as it has not yet been found anywhere else. *Caulerpa racemosa* and *C. taxifolia* both showed the presence of the same diterpene 3, 7, 11, 15-tetramethyl-hexadec-2-en-1-ol (Fig. 2:[8]), while *C. chemnitzia* possessed 3, 7, 11, 15-tetramethyl-hexadec-3-en-1, 2-diol (Fig. 2:[9]). Both these diterpenes are being reported for the first time from any species of *Caulerpa*. It is interesting to note that caulerpin and caulerpicin, which have been reported from various species of *Caulerpa* (Nielson *et al.*, 1982; Raub *et al.*, 1987; Anjaneyulu *et al.*, 1991; Xu & Zeng, 1998; Soliman *et al.*, 2000) could not be detected in any of the five investigated species of *Caulerpa*. A variety of diterpenoids have been reported from other species of *Caulerpa* (Capon *et al.*, 1983; Paul & Fenical, 1985; Handley & Blackman, 2000). Sesquiterpenes have also been isolated from different species of *Caulerpa* (Capon *et al.*, 1981).

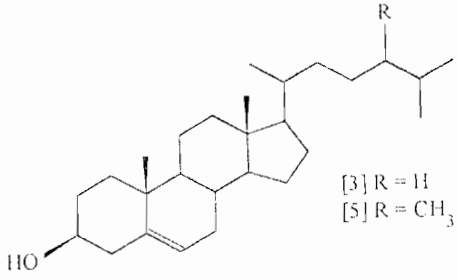
The five investigated species of *Caulerpa* behaved differently in their fatty acid, sterol and diterpene compositions. The above mentioned differences observed among various species may be due to seasonal variation as was observed in *C. prolifera*. It was found that thallus resistance to chilling injury of this alga depends on the maintenance of membrane functions, which was due to varying degree of unsaturation of membrane fatty acids (Terrados & Lopez-Jimenez, 1996). The specific differences may be due to their genetic constitution as well as different ecological conditions in which they are growing. Therefore, a detailed study of the influences of various ecofactors and environmental parameters are necessary, before any conclusion may be drawn in this connection on the basis of existing phycochemical investigations.



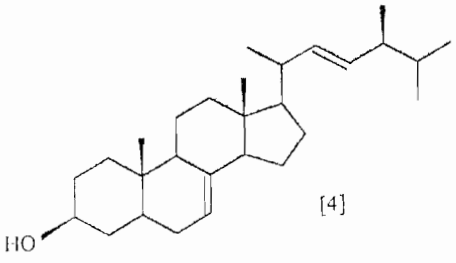
[1]



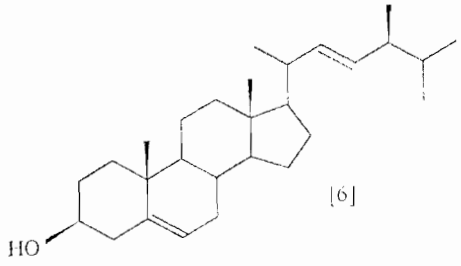
[2]



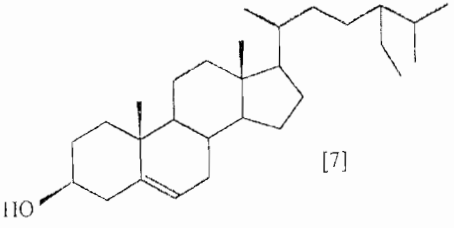
[3] R = H
[5] R = CH_3



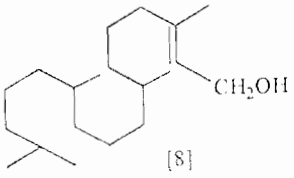
[4]



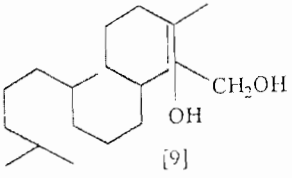
[6]



[7]



[8]



[9]

Fig. 2. Natural products isolated from *Caulerpa* spp.: [1] = 7-ethyl-3-methyl-2,6-undecadienoic methyl ester, [2] = 3,7,11-trimethyl-2,6,10-dodecatrienoic methyl ester, [3] = cholesterol, [4] = 24-methyl-cholesta-7,22-dien-3β-ol, [5] = 24-methyl cholesterol, [6] = 4,24-dimethyl-cholesta-5,22-dien-3β-ol, [7] = β-sitosterol, [8] = 3,7,11,15-tetramethyl-hexadec-2-en-1-ol, [9] = 3,7,11,15-tetramethyl-hexadec-3-en-1,2-diol.

Acknowledgement

We are extremely grateful to Prof. Dr. Viqar Uddin Ahmad for providing facilities for phycochemical studies in his laboratory at HEJ Research Institute of Chemistry, University of Karachi, where this work was accomplished.

References

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

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: 11-16.

Anjaneyulu, A.S.R., C.V.S. Prakash and U.V. Mallavadhani. 1991. Two caulerpin analogues and a sesquiterpene from *Caulerpa racemosa*. *Phycochem.*, 30: 3041-3042.

Blackman, A.J. and R.J. Wells. 1976. Caulerpol a diterpene alcohol, related to vitamin A from *Caulerpa brownii* (Algae). *Tetrahed. Lett.*, 31: 2729-2732.

Capon, R.J., E.L. Ghisalberti and P.R. Jefferies. 1981. New sesquiterpenes from *Caulerpa flexilis* var. *muelleri*. *Aust. J. Chem.*, 34: 1775-1778.

Capon, R.J., E.L. Ghisalberti and P.R. Jefferies. 1983. Metabolites of the green algae, *Caulerpa* species. *Phytochem.*, 22: 1465-1467.

De Napoli, L., E. Fattorusso, S. Magno and L. Mayol. 1982. Three squalene derivatives from *Caulerpa prolifera*. *Phytochem.*, 21: 782-784.

Duraiswamy, R. 1986. A chemotaxonomic approach to the genus *Caulerpa*. In: *Marine Biology and Mariculture*. (Ed.): V. Krishnamurthy, India, p. 129-135.

Hainaux, F., S. Rohan, M. Simon, G. Bourbigot and Y. De Roeck-Holtzhauer. 1998. Determination of the fatty acid composition of a spongy alga of the genus *Codium*. *Ol., Corps Gras, Lipides*, 5: 237-238.

Handley, J.T. and A.J. Blackman. 2000. Monocyclic diterpenes from the marine alga *Caulerpa trifaria* (Chlorophyta). *Aust. J. Chem.*, 53: 67-71.

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

Herbreteau, F., L.J.M. Coiffard, A. Derrien and Y. De Roeck-Holtzhauer. 1997. The fatty acid composition of five species of macroalgae. *Bot. Mar.*, 40: 25-27.

Khotimchenko, S.V. 1995. Fatty acid compositon of green algae of the genus *Caulerpa*. *Bot. Mar.*, 38: 509-512.

Mabrouk, S.S., A.M. Hashem and N.M.A. El-Shayeb. 2001. Polyunsaturated fatty acids of green and brown algae from the Egyptian Mediterranean coast. *Adv. Food Sci.*, 23: 20-24.

Nielsen, P.G., J.S. Carle and C. Christophersen. 1982. Final structure of caulerpicin, a toxin mixture from the green alga *Caulerpa racemosa*. *Phytochem.*, 21: 1643-1645.

Paul, V.J. and W. Fenical. 1985. Diterpenoid metabolites from Pacific marine algae of the order Caulerpales (Chlorophyta). *Phytochem.*, 24: 2239-2243.

Prasada-Rao, N.U.S.A.V. and L. Venkata-Rao. 1986. Structural features of the sulphated polysaccharide from a green seaweed, *Caulerpa taxifolia*. *Phytochem.*, 25: 1645-1647.

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

Raub, M.F., F.J. Cardellina II and J.G. Schwede. 1987. The green algal pigment caulerpin as a plant growth regulator. *Phytochem.*, 26: 619-620.

Shameel, M. 1978. Additions to the knowledge about *Caulerpa* Lamouroux (Bryopsidophyceae) from the coast of Karachi. *Bot. Mar.*, 21: 277-282.

Shameel, M. 1987. A preliminary survey of seaweeds from the coast of Lasbela, Pakistan. *Bot. Mar.*, 30: 511-515.

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

Shameel, M. 1993. Phycochemical studies on the fatty acid 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 and Q.B. Kazmi. Pak. Nat. Comm. 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.

Shameel, M. and R. Khan. 1989. Phycochemical studies on fatty acid composition in *Caulerpa* (Bryopsidophyceae). *Pak. J. Bot.*, 21: 343-346.

Shameel, M. and R. Khan. 1991. Fatty acid composition of nine green seaweeds. *Bot. Mar.*, 34: 501-504.

Shameel, M. and J. Tanaka. 1992. A preliminary checklist of marine algae from the coast and inshore waters of Pakistan. In: *Cryptogamic Flora of Pakistan*. Vol. 1. (Eds.): T. Nakaike and S. Malik. Nat. Sci. Mus. Tokyo, p. 1-64.

Sheu, J.-H., C.-C. Liaw and C.-Y. Duh. 1995. Oxygenated clerosterols isolated from the marine alga *Codium arabicum*. *J. Nat. Prod.*, 58: 1521-1526.

Soliman, F.M., M.M. Fathy and M.Y. Moussa. 2000. Phytochmical and biological investigation of the green alga: *Caulerpa prolifera* Lamx. *Bull. Fac. Pharm. (Cairo Univ.)*, 38: 99-107.

Terrados, J., J.A. Lopez-Jimenez. 1996. Fatty acid composition and chilling resistance in the green alga *Caulerpa prolifera* (Forsskal) Lamouroux (Chlorophyta, Caulerpales). *Biochem. Mol. Biol. Int.*, 39: 863-869.

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

Usmanghani, K. and M. Shameel. 1996. Fatty acid composition of seaweeds of Pakistan. *Pak. J. Pharm. Sci.*, 9(2): 53-68.

Xu, X.-Q., V.H. Tran, G. Kraft and J. Beardall. 1998. Fatty acids of six *Codium* species from southeast Australia. *Phytochem.*, 48: 1335-1339.

Xu, S. and L. Zeng. 1998. Chemical constituents of *Caulerpa sertularicides*. *Zhongcaoyao*, 29: 80-82.

Zahid, P.B. 1989. On the biochemical composition of ten species of the genus *Caulerpa* (Caulerpales-Chlorophyta) collected from Karachi coast. *Pak. J. Sci. Ind. Res.*, 32: 98-100.