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REVIEW

FATTY ACID COMPOSITION OF SEAWEEDS OF PAKISTAN

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ABSTRACT

An interest in seaweeds was evident in the Orient particularly China and Japan from an early date: the interest, at least in part, remained associated with the economic importance of these plants of the sea. The use of four distinct species of marine algae in the treatment of goitre was described in 300 A.D., and now lot many seaweeds are being constantly consumed in modern day Japan, where goitre incidences are almost non-existent.

In order to generate an interest in the plants of sea the food chain studies on the seaweeds from Pakistan coastal waters, conducted in our laboratories, have exhibited an interesting array of fatty acid composition. The marine benthic algae so far screened for fatty acid components belong to all the three important divisions i.e. Chlorophyta, Phaeophyta and Rhodophyta. The species examined were *Caulerpa manorensis* Nizam., *Ca. racentosa* (Forssk.) J. Ag., *Codium decorticatum* (Woodw.) Howe, *Co. flabellatum* Silva *et* Nizam., *Co. iyengurii* BΦrg. and *Ulva fasciata* Delile among green seaweeds; *Colpomenia sinuosa* (Roth) Derb. *et* Sol., *Dictyota dichotoma* (Huds.) Lamour., *D. indica* Sond. *ex* Kütz, *Iyengaria stellata* (BΦrg.) BΦrg., *Padina tetrastrontatica* Hauck and *Stoechospermum marginatum* (C. Ag.) Kütz. from brown seaweeds; and *Centroceras clavulatum* (C. Ag.) Mont., *Demtonenta abbotliae* Afaq., Nizam. *et*. Shameel, *Gracilaria foliifera* (Forssk.) BΦrg., *Hypnea musciformis* (Wulf.) Lamour. and *Scinaia fascicularis* (BΦrg.) Desikach. *et* Singh among red seaweeds.

Marine plants present a wide and varied field of study, where much remains to be discovered from biological to chemical and from commercial to pharmaceutical viewpoints. An attempt has been made to indicate the beginning and extend of the distribution of fatty acids in the seaweeds of Pakistan, but naturally it is not possible to indicate all of many facets.

Introduction

Seaweeds are one of the interesting and multifaceted groups of herbal drugs and commercial plants. There are approximately 30,000 species of them found in the oceanic world. As a commodity marine algae have been utilized as a delicacy of food items, fodder and fertilizer, and for commercial purposes agar-agar, carrageenan, alginates, furcellaran, funori, fucoidin, laminarin, algin and other phycocolloids are well known algal products. In folk medicine marine benthic algae have been used since prehistoric times. The use of four distinct species of marine algae in the treatment of goiter (*palcoto*) was described in 300 A.D. An interest in seaweeds was evident in the Orient particularly China and Japan from an early date. The interest, at least in part, remained associated with the economic importance of these plants of the sea. A variety of pharmacological properties have been attributed to marine algae, which include the accounts of bioactive molecules such as antibiotic, antitumour, anticoagulant, antiviral, antiulcer, haemolytic, analgesic, antilepimic, cardioinhibitory and other convalescent substances, besides having fungicidic, insecticidic, larvicidie and vermifugic properties.

An interest in the marine algae found in the coastal waters of Pakistan has very recently attracted the attention of research workers and, after the write up of comprehensive listing of seaweeds occurring in our region (Shameel and Tanaka, 1992), these efforts have picked up the speed. This paper is meant to provoke the utilization of marine algae in nutrition, found either as benthos (attached forms) or plankton (free-floating forms), at or near the coast of Pakistan. Although marine algae elaborate different types of chemical constituents such as fatty acids, sterols, terpenes, phycocolloids etc., which vary in their effects on biological functions. As a constituent of membrane as well as cytoplasm of algal cells, the fatty acids are amongst the most frequently encountered natural products. Since seaweeds produce a variety of fatty acids, there appear at least three major factors viz. light conditions, the water temperature and nitrogen contents of the medium, which influence the biosynthesis of the algal fatty acids.

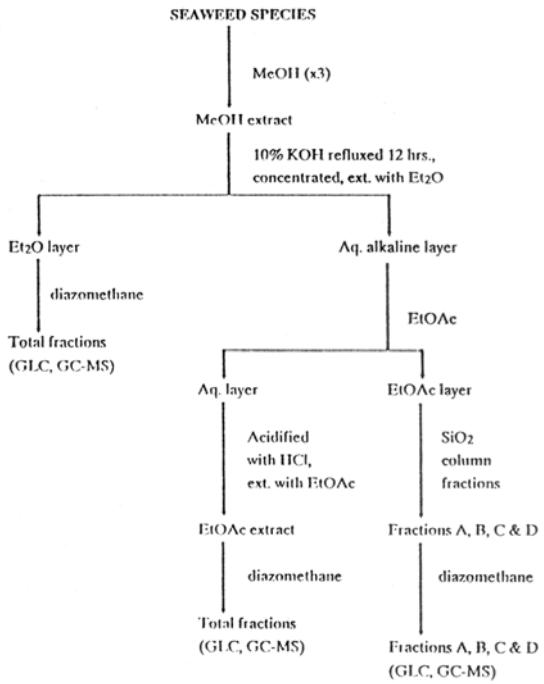
Material and Methods

Algal materials were collected from the coastal areas of Karachi (Pakistan), such as Monora, Buleji and Paradise Point during October-March 1987-1992. A schematic presentation of the extraction, saponification, chromatographic separation and esterification is given in Scheme 1. Extraction was carried out with the mixture $CHCl_3$:MeOH (1:1) by percolation at room temperature for three weeks.

Free fatty acids can be released from glycerolipids with relative ease by hydrolysis (or saponification) of the ester bond under basic conditions. Any lipid soluble non-saponifiable material (e.g. cholesterol, glycerol ethers, hydrocarbons) can be removed if

necessary, when the reaction was complete, by extraction of the basic hydrolysis mixture with diethyl ether prior to acidification to obtain the free fatty acids.

The lipid sample (upto 100 mg) was dissolved in a solution of 1M potassium hydroxide in 95% ethanol (2 mL), it was either refluxed for one hour or left at room temperature. After cooling, water (5 mL) was added and the solution was extracted with diethyl ether (10 mL) to remove any non-saponifiable material with centrifugation, if necessary to break any emulsion, which might have formed (this step can be omitted if unwanted by-products are soluble in aqueous layer). The aqueous layer was then acidified with dilute hydrochloric acid, before it was extracted with diethyl ether (3 x 5 mL). The combined ether layers, which now contain the free fatty acids, were washed with eater (5 mL) and dried over anhydrous sodium sulphate, before the solvent was removed.

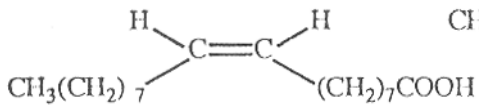


Scheme 1: Separation and isolation of fatty acids.

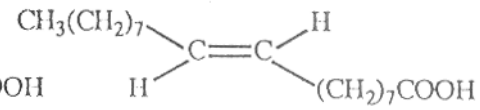
The fatty acid fraction (0.5 mg) was dissolved, and 0.5 mL of ethereal diazomethane was added to it. The reaction mixture was kept overnight at room temperature, and on evaporation the aliquotes so obtained were directly injected into gas-liquid chromatography or GC-MS. The gas chromatography-mass spectrometry of the fatty acid methyl esters was carried out on a Hewlett Packard GC connected to a Jasco mass spectrometer 302 attached to a 11/73 DEC computer data system. The GC was packed with 1-2 m x 4 mm glass capillary column coated with gas chrom 0 (100-120 mesh, OV 101, 1%). The column temperature was programmed from 70°-250°C, with a rate of increase of 5-8°C per minute. The carrier gas (helium) flow rate was 32 mL/min., and the injector temperature was 250°C.

Experimental

The fatty acids occurring in seaweeds are classified into saturated and unsaturated ones. Generally, the saturated fatty acids consist of even number of carbon atoms. Therefore, these acids belong to homologous series $CH_3.(CH_2)n\text{-}COOH$, where n has an even number, however odd number of fatty acids are also reported in the literature and that these rarely exceed 1% of the total fatty acids present. The unsaturated (enoic) fatty acids are usually found in greater yield than the saturated ones. The occurrence of unsaturated centres within fatty acid chains leads to the possibility of positional isomerism of the double bond. The presence of double bond in a fatty acid chain furnishes the possibility of geometrical isomerism. Thus, in fatty acids the carbon atoms involved in a double bond can be arranged *cis*- or *trans*- to each other e.g.:

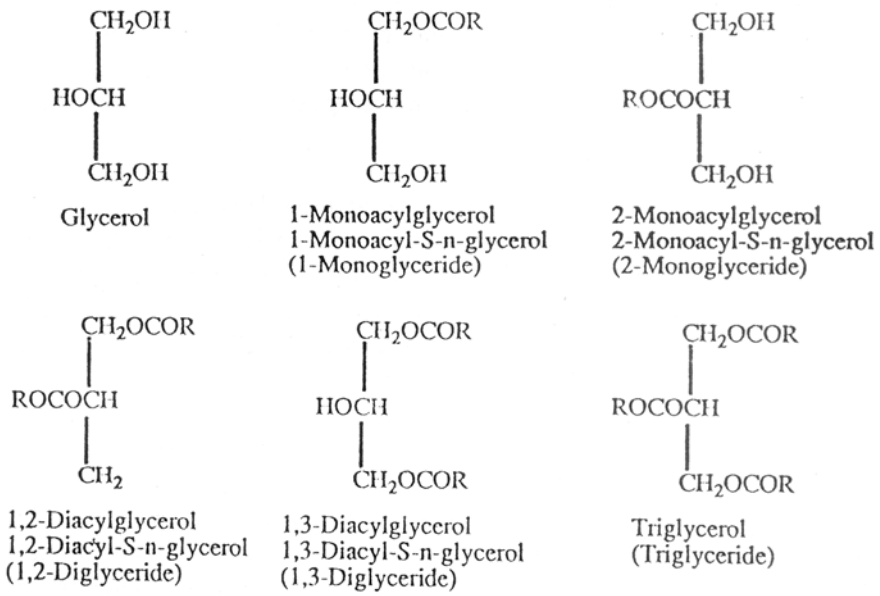


Oleic acid



Elaidic acid

Most of the Most of the fatty acids are found as complex fatty acyl compounds i.e. as esters in marine algae or terrestrial plants. Glycerol is a polyhydric alcohol with which fatty acids are usually esterified. Glycerol is a trihydroxy alcohol (propane-1,2,3- triol), therefore, it can react with one, two or three molecules of monocarboxylic fatty acids to produce monoglycerides, diglycerides and triglycerides respectively. According to IUPAC-IUB Commission on Biochemical Nomenclature of 1967, the following would be the structures and the names of glycerol and its fatty acyl esters:



All three classes of acylglycerol or glycerides (mono-, di- and triglycerols) are found in marine plants, but only the triglycerol is accumulated in large quantity. Since the three classes of acylglycerol are esters of carboxylic acid, they undergo both acid-and base-catalyzed hydrolyses via most commonly acyl- oxygen rather than alkyl-oxygen fission. Alkaline hydrolysis of these esters has traditionally been known as saponification because soap is manufactured by alkaline hydrolysis of triacyl glycerol. Such a hydrolysis basically is similar to many other reactions of carboxyl compounds, in which the negatively charged nucleophile attacks the carboxyl carbon.

The short chain fatty acids (i.e. of chain length up to C_8) are all water soluble, although in solution they are associated and do not exist as single molecules. The long chain acids are much less soluble because of the size of the hydrocarbon chain. Therefore, majority of the fatty acids of seaweeds are only sparingly soluble in water (mg/100 mL water at 20°C). Indeed the most notable characteristic of fatty acids is that they are hydrophobic, hence they are relatively insoluble in aqueous medium but are readily soluble in organic solvents such as hexane, diethylether, ethyl acetate, benzene, chloroform and chloroform-methanol mixtures. The exact method of isolation of fatty acids depends on the nature of marine algae. In general, the mixture of chloroform and methanol is used for the extraction of fatty acids. Ultimately a two phase mixture is obtained with the fatty acids found in the chloroform layer. At this stage it is important to

consider in mind not to use plasticizer or teflon or tygon at all, either as container or tubes, because these plasticizers interfere with GC-MS determination, and a very prominent gas chromatographic peak appears in mass spectra of an unwanted phthalate plasticizer (characterized by m/z 149 base peak and the very few other fragments).

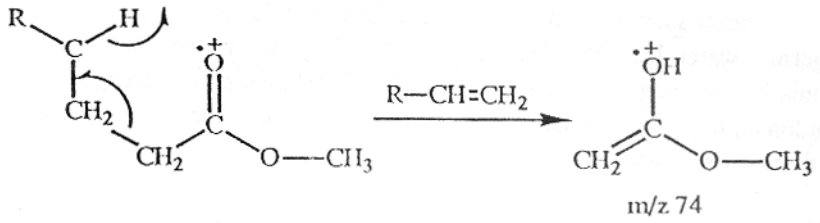
The extract so obtained is then usually subjected to silica gel column chromatography. After application of the lipid mixture of the column for elution with chloroform yields neutral lipids (triglycerides, fatty acids, fatty acid esters, cholesterol and cholesterol ester). If need arises, further purification of neutral lipids can be affected by recolumn chromatography using diethyl ether in hexane. The TLC is also utilized sometimes to augment the determination of various classes of lipids.

Usually, fatty acids do not exist as free carboxylic acids because of their marked affinity for many proteins. One result is their inhibitory action on most enzymes. Where free acids have been reported as major constituents, they are usually artifacts due to cell damage, which allows lipase to act on acyl lipids of the tissue. In general, the main functions of fatty acids and lipids seem to be their participation in cell membrane as storage product to serve as a source of energy and as metabolic precursors, and that in many seaweeds they are components of outer protecting layer.

Fatty acids are found in two different forms in lipid extract of seaweeds: free and esterified. It is a common practice to derivalize fatty acids so as to enhance their volatility and facilitate the chromatographic separation. The carboxyl group of fatty acids is involved in a number of reactions, perhaps the most important technologically being esterification. Thus by treating long chain fatty acids with boiling methanol in the presence of a little concentrated hydrochloric acid the methyl esters can be formed. Methylation of fatty acids is usually carried out with diazomethane, borontrinuoride-methanol or acetylchloride-methanol. The unambiguous separation of fatty acids can be obtained only by gas-liquid chromatography (GLC), and one advantage of this chromatography lies in its ability to use very small amount of material.

Methyl esters of fatty acids prepared by direct esterification or trans-esterification can be easily separated through gas chromatography (GC). Mass spectrometry (MS) has, therefore, made a great contribution in the identification of fatty acids. Electron impact mass spectrometry (EI-MS) with gas chromatography is now routinely applied for characterization of fatty acid methyl esters. The collisional activation decomposition mass spectra (CAD-MS) for locating double bonds in polyenoic fatty acids has also been reported in the literature (Jensen *et al,* 1985). The development of fast atom bombardment (FAB) for effectively disorbing preformed ions such as carboxylate anions and tandem mass spectrometry (NIS/NIS) for collisional activation provides new possibilities for locating double bonds in unsaturated fatty acids (Haddon and McLafferty, 1968; Levsen and Schwartz, 1976; Barber *et al*, 1981).

Fatty acid methyl esters are very stable and their mass spectra are well documented in the literature. The mass spectra of saturated fatty acids exhibit the acylium ion formed by the loss of a methoxy group (M^+-31), while the peak at m/z 74 is the most characteristic ion formed by McLafferty rearrangement as given below:



The other diagnostic features of saturated fatty acids is ion m/z 143, which is very consistent. As regards the monoenoic fatty acids m/z M^+-32, M^+-74 and M^+-116 fragments are quite characteristic, the dienoic acids exhibit peaks at m/z M^+-31 and M^+-74, while in the trienoic fatty acids peaks at m/z M^+-56 and M^+-69 are of considerable value. The other mass fragmentation values in both saturated and unsaturated fatty acids are observed at m/z M^+-15, M^+-29, M^+-32 and M^+-43, while the free fatty acids show fragments at m/z M^+-15, M^+-17, M^+-45, M^+-73, M^+-87 and M^+-101. The book by Waller (1972) on biochemical application of mass spectrometry is an excellent starting point to understand and comprehend the mass characterization of fatty acids found as free or esterifed.

Results and Discussion

Seaweeds produce a great variety of fatty acids as saturated and *cis*-unsaturated components. However, there are significant differences in the composition of fatty acids in between the individual algal divisions (phyla) and from species to species. In brief, the main algal fatty acids are saturated and *cis*-unsaturated ones with mostly 12-20 carbon atoms and 0 to 6 double bonds. Although, the marine benthic algae have been used by herbivorous fishes, crustaceans and other marine animals as their staple diet, in geographic areas like Pakistan having upwelling in its continental shelf seaweeds occur in appreciable quantities (Shamed and Tanaka, 1992), but are not utilized for economic potential. A list of the folklore, medicinal and economical uses of the investigated seaweeds is given in Table 1. In China and Japan, on the contrary, the seaweeds arc consumed in bulk quantities for commercial, food and herbal purposes. Again marine algae are eaten frequently in modern day Japan, where goiter incidence is almost non-existent.

The group of physiologically active fatty acids, which are particularly useful for animals, are the unsaturated fatty acids. As they are not synthesized by animals, have to be taken up from vegetable food. Out of unsaturated fatty acids, linoleic (C18:2) and arachidonic (020:4) acids are of important essential fatty acids. When the animals are kept on diet without essential fatty acids for a longer period of time, they develop an essential fatty acid deficiency syndrome (EFAD) characterized by the increase of unusual fatty acids, which are normally present relatively in small amounts such as palmitoleic (C16:1) and oleic (C18:1) acids. It has been shown that EFAD-rats develop dermal symptom such as scaly skin with impaired barrier properties and high rate of transdermal water loss. Besides that essential fatty acids have an effect on hyperlipidemia by lowering the levels of plasma lipids (cholesterol and triglycerides). Hyperlipidemia, however, is related to the development of atherosclerosis and coronary heart disease (Ross and Harker, 1976).

We have initiated a research programme to evaluate the fatty acid components of marine algae found along the seashore of Karachi and the adjacent coastal areas of Pakistan. The marine benthic algae so far screened for fatty acid components belong to the divisions Chlorophyta (green seaweeds), Phaeophyta (brown seaweeds) and Rhodophyta (red seaweeds). Analyses of total fats and lipids of green, brown and red seaweeds, and their saturated and *cis*-unsaturated fatty acid compositions have been reported from different areas (Hoppe, 1979; Pohl and Zurheide, 1979). Chlorophyta generally contains small amount of saturated fatty acids, Phaeophyta 0.6-3% lipids with highly unsaturated fatty acids while Rhodophyta contains only a small quantity of lipids (approximately 3%) and consists mainly of high unsaturated fatty acids.

Table 1
Folkore, medicinal and economic utility of the investigated seaweeds

Taxonomic position	Seaweed	Utility
Chlorophyta		
Ulvales	*Ulva fasciata*	Potential source of protein for food and fooder, astringent, scrofula and gout
Codiales	*Codium decorticatum* *C. flabellatum* *C. iyengarii*	Antiviral, antifungal, antibacterial and vermifuge activities

Table continued . . .

Taxonomic position	Seaweed	Utility
Caulerpales	*Caulerpa manorensis* *C. racemosa*	Neurotropic
Phaeophyta		
Dictyotales	*Dictyota dichotoma* *D. indica*	Antimicrobial activity (anti-bacterial and antifungal)
	Padina tetrastromatica	Spasmogenic activity and antifertility
	Stoechospermum marginatum	Extract shows spasmolytic activity
Scytosiphonales	*Colpomenia sinuosa*	Extraction of fucoidin
	Iyengaria stellata	Isolation of alginates
Rhodophyta		
Nemaliales	*Dermonema abbottiae*	Extraction of agar
	Scinaia fascicularis	Isolation of carrageenan
Gigartinales	*Hypnea musciformis*	Extract exhibits diuretic activity
	Gracilaria foliifera	Produces firm gel, used in various industries
Ceramiales	*Centroceras clavulatum*	Used as folk medicine in east Asia

I. Green seaweeds

The marine green algae evaluated for fatty acid composition were Caulerpa manorensis Nizam. (1), *Ca. racemosa* (Forssk.) J. Ag. (2), *Codium decorticatum* (Woodw.) Howe (3), *Co. flabellatum* Silva et Nizam. (4), *Co. iyengarii* BΦrg. (5) and *Ulva fasciata* Delile (6). They were compared with the literature values of *Codium elongation* (Turn.) C. Ag. (7) and *Ulva fasciata* (8) (Pohl *et al*, 1968) and are presented in Table 2. Such an assessment revealed that green seaweeds contain many saturated and unsaturated fatty acids of all chain lengths and all degrees of unsaturation. Relatively C16:0, C18:0, C18:1 and C22:0 fatty acids are usual in all the species examined. However, in case of Chlorophta from Pakistan C16:0 fatty acid is rather a feature of significance and forms the highest percentage composition. It appears that bulk of the fatty acid composition of green seaweeds found at the Karachi coastal region is composed of unsaturated fatty acids, and this fact is also verified with the

literature values of *Codium elongatum* and *Ulva fasciata* (Pohl et al., 1968). The result further shows that in marine algae the biosynthesis of fatty acids depends on the environmental conditions and can change rather rapidly, and this fact implied in case of marine algae growing at the coastal areas of Pakistan by the presence of variation and nature of fatty acids.

Marine macroscopic Chlorophyta are characterized by a wide variety of saturated and unsaturated fatty acids. They primarily synthesize C16 and C18 fatty acids, with larger proportions of the C16:3, C16:4 and C18:4 acids. The unsaturated C20 and C22 fatty acids are usually much less abundant i.e., mainly C20:4 and C20:5 but sometimes C22:4, C22:5 and C22:6 acids (Pohl and Zurheide, 1979). Substantial quantities of acids with more than three double bonds and more than 18 carbon atoms are found in marine green algae, while their fresh-water counterparts lack such acids. It appears that this may be an adaptation necessary for survival in the marine environment (Shameel, 1990).

Table 2

Fatty acid values of green seaweeds in relative percentages

Acid	1	2	3	4	5	6	7*	8*
C7:0	-	1.34	1.40	1.35	0.54	-	-	-
C8:0	1.34	-	-	1.61	0.51	-	-	-
C9:0	-	-	-	-	-	-	-	-
C9:1	-	-	-	-	1.62	-	-	-
C10:1	-	-	-	-	2.90	-	-	-
C11:0	-	1.49	-	-	-	-	-	-
C11:1	-	-	1.30	-	-	-	-	--
C12:0	2.70	2.50	-	-	-	-	2.41	1.30
C12:1	-	-	3.30	-	2.82	2.65	-	-
C12:2	-	-	-	1.91	-	-	-	-
C12:3	-	-	2.90	-	-	-	-	-
C13:1	1.15	-	4.50	-	6.10	-	-	-
C13:3	-	-	3.90	-	-	-	-	-
C14:0	1.55	5.94	-	-	1.92	0.74	4.40	0.83
C14:1	16.43	6.86	-	4.62	10.12	-	-	-
C14:2	5.64	-	5.01	-	-	-	-	-
C15:0	-	-	2.25	-	-	0.62	-	-
C15:1	2.56	-	5.14	2.28	-	-	-	-
C15:3	5.34	5.70	-	-	-	-	-	-
C16:0	4.78	13.66	12.60	13.60	13.49	84.85	10.21	10.91

Table continued . . .

Acid	1	2	3	4	5	6	7*	8*
C16:1	10.00	9.92	-	3.00	5.60	-	10.25	3.90
C16:2	4.87	2.05	1.68	-	-	-	3.00	1.30
C16:3	-	-	-	-	-	-	1.61	-
C16:4	-	-	-	-	-	-	-	3.00
C17:0	1.20	-	1.41	2.71	0.96	-	-	-
C17:1	-	-	7.89	1.25	-	-	-	-
C17:3	-	-	1.58	1.80	-	-	-	-
C18:0	2.73	3.30	11.40	1.25	9.19	2.41	2.00	0.50
C18:1	18.00	10.00	12.01	16.00	18.36	7.24	18.33	20.70
C18:2	4.00	11.45	2.94	-	-	-	9.72	7.50
C18:3	-	-	-	-	-	-	16.50	13.50
C18:4	-	-	-	-	-	-	4.90	21.20
C19:0	2.77	2.03	3.75	-	-	-	-	-
C19:1	-	-	-	4.90	7.50	-	-	-
C20:0	-	-	-	5.30	-	-	6.20	-
C20:1	-	-	1.80	1.61	-	-	-	-
C20:4	-	4.96	-	-	-	-	4.80	1.40
C20:5	-	3.92	-	-	-	-	3.60	0.40
C21:0	-	2.61	-	2.54	3.80	-	-	-
C21:1	-	-	-	2.61	-	-	-	-
C22:0	0.93	1.36	1.28	8.32	2.35	1.43	-	-
C22:4	-	-	-	11.04	-	-	0.70	0.46
C22:5	-	-	-	-	-	-	-	3.40
C23:0	1.44	1.44	-	5.44	5.40	-	-	-
C23:1	-	-	1.40	-	1.91	-	-	-
C24:0	3.14	2.45	2.01	-	-	-	-	-
C25:0	6.03	3.60	-	-	-	-	-	-
C27:0	-	-	-	-	3.80	-	-	-
C29:0	-	-	7.40	-	-	-	-	-
C29:3	-	-	1.00	1.90	-	-	-	-
	98.46	98.18	99.84	96.55	98.89	99.94	99.33	98.60

1. *Caulerpa manorensis*, 2. *Caulerpa racemosa*, 3. *Codium decorticatum*, 4. *Codium flabellatum*, 5. *Codium iyengarii*, 6. *Ulva fasciata*, 7. *Codium elongatum*, 8. *Ulva fasciata* (*after Pohl *et al.*, 1968).

II. Brown seaweeds

The marine brown algae investigated were *Colpomenia sinuosa* (Roth) Derb. *et* Sol. (1), *Dictyota dichotoma* (Hods.) Lamour. (2), *D. indica* Sond. ex Kütz. (3), *Iyengaria stellata* (BΦrg.) BΦrg. (4), *Padina tetrastromatica* Hauck (5) and *Stoechospermum marginatum* (C Ag.) Kütz (6). Their fatty acid values are compared with the literature values of *Dictyosiphon foeniculaceus* (Huds.) Grev. (7) (Jamieson and Reid, 1972) and reported in Table 3. Relatively C16:0, C17:0 and C18:1 fatty acids are common in all the species examined. It appears that comparatively saturated fatty acids dominate over the unsaturated ones in the investigated brown algae. However, literature citation on brown algae shows the abundance of unsaturated fatty acids (Pohl and Zurheide, 1979). The fatty acids of brown algae showed the range between C12 and C20 chain lengths with different degrees of saturation and unsaturation. The major saturated fatty acid in case of brown algae is C16:0, whereas the unsaturated fatty acid comprises of C18:1. Based on these results it is concluded that brown seaweeds occupy a position between green and red algae with respect to the composition of their fatty acids. This may be an ecological adaptation, as brown algae occur at an intermediate depth between green and red seaweeds on the continental shelf.

The marine Phaeophyta generally have higher levels of unsaturated C18 and C20 fatty acids 4e. C18:3, C18:4, C20:4 and C20:5, while the proportion of unsaturated C16 fatty acids usually is very low (Pohl and Zurheide, 1979). Such observations could not be confirmed in the few seaweeds examined from Karachi coast. The brown algae have rather less of the C20:5 acid, slightly more of the C20:4 acid, and oleic (C18:1), linoleic (C18:2) and linolenic (C18:3) acids are more abundant. The brown seaweeds of Pakistan appear to exhibit a greater diversity in the composition of saturated fatty acids than green and red ones (Shamed, 1990).

Table 3
Fatty acid values of brown seaweeds in relative percentages

Acid	1	2	3	4	5	6	7*
C12:0	-	0.68	-	-	-	-	0.10
C12:1	-	4.20	-	-	-	-	-
C14:0	12.60	-	6.53	-	6.00	10.93	4.50
C14:1	-	-	-	-	-	3.80	-
C14:3	2.83	-	-	-	3.80	-	-
C15:0	-	6.12	2.92	10.20	-	5.23	-
C15:1	-	-	-	-	-	7.93	-
C16:0	22.07	20.83	16.32	19.10	26.60	18.25	12.40

Table continued . . .

Acid	1	2	3	4	5	6	7*
C16:1	-	-	3.11	4.80	1.90	-	4.70
C16:2	-	-	-	-	-	-	1.10
C16:3	-	-	-	-	-	9.20	0.20
C16:4	-	-	-	-	-	-	0.70
C17:0	11.00	16.29	21.15	10.60	13.40	13.49	-
C17:2	-	-	3.09	-	-	-	-
C18:0	-	15.79	-	5.00	13.60	-	1.20
C18:1	20.07	10.00	12.29	21.00	22.20	18.57	7.20
C18:2	-	-	8.00	-	-	-	7.60
C18:3	-	-	-	6.00	-	-	12.80
C18:4	18.30	-	-	-	-	-	14.50
C19:0	1.05	-	-	-	-	5.55	-
C20:0	-	7.82	11.00	7.30	3.80	-	0.50
C20:2	-	12.00	-	15.10	-	7.00	0.10
C20:3	9.76	-	15.13	-	-	-	0.90
C20:4	-	-	-	-	-	-	15.20
C20:5	-	-	-	-	-	-	14.20
C22:0	9.76	-	-	-	-	-	-
	97.68	99.85	99.54	99.10	91.30	99.95	93.40

1. *Colpomenia sinuosa,* 2. *Dictyota dichotoma,* 3. *Dictyota indica,* 4. *Iyengaria stellata,* 5. *Padina tetrastromatica,* 6. *Stoechospermum marginatum,* 7. *Dictyosiphon foeniculaceus* (*after Jamieson and Reid, 1972).

III. Red seaweeds

The marine red algae studied for fatty acid analysis from Karachi coastal regions were *Centroceras clavulatum* (C. Ag.) Mont. (1), *Demronema abbottiae* Afaq., Nizam. *et* Shameel (2), *Gracilaria foliifera* (Forssk.) BΦrg. (3), Hypnea muscifomris (Wulf.) Lamour. (4) and *Scinaia fascicularis* (BΦrg.) Desikach. *et* Singh (5). Their fatty acid values are in turn compared with the literature values given for *Laurencia pinnatifida* (thuds.) Lamour. (6) (Jamieson and Reid, 1972) and presented in Table 4. Relatively the C16:0, C17:0, C18:0 and C18:1 fatty acids are common in all the species examined. The red algae have one rather common feature, the formation of high amounts of unsaturated fatty acids. The saturated fatty acids present are C16:0 and C18:0, while the unsaturated fatty acids mostly present are C20:1, C20:2 and C20:3 i.e. of C20 series.

The composition of the fatty acids of marine Rhodophyta is very complex and remarkable for the high content of CO and C22 acids with 4, 5 and 6 double bonds. Mostly C20:5 acid is much more abundant in red seaweeds (Pohl and Zurheide, 1979), but it was not detected in the marine red algae of Pakistan. The unsaturated C18 fatty acids are present in lower quantities, and the polyenoic CI6 fatty acids i.e. C16:2, C16:3 and C16:4 are absent or present in very small proportion e.g. C16:2 in Hypnea muscifonnir and C16:3 in *Halymenia prophyroides* BΦrg (Shameel, 1990).

Table 4
Fatty acid values of red seaweeds in relative percentages

	1	2	3	4	5	6*
C12:0	1.06	-	2.90	3.36	3.02	0.30
C12:1	-	-	-	1.26	- -	
C13:1	7.22	13.03	-	5.80	10.08	-
C14:0	4.76	-	7.15	2.32	4.61	4.30
C14:1	-	5.97	-	-	-	-
C14:3	-	-	-	1.69	-	-
C15:0	-	-	-	3.95	3.55	-
C15:1	-	-	-	0.42	-	-
C16:0	15.89	25.65	17.52	27.10	16.64	20.90
C16:1	2.24	-	5.47	2.85	-	4.00
C16:2	-	-	-	0.74	-	0.30
C16:3	-	-	-	-	-	1.00
C16:4	-	-	-	-	-	0.90
C17:0	2.35	7.20	5.96	3.16	4.83	-
C17:1	3.04	-	-	-	-	-
C17:3	-	-	-	5.36	-	-
C18:0	10.47	10.03	15.53	5.92	4.22	3.70
C18:1	9.33	9.95	22.18	7.55	8.00	7.90
C18:2	-	-	-	-	-	4.90
C18:3	-	-	-	2.11	7.85	3.80
C18:4	-	-	-	-	-	3.50
C19:0	6.09	-	-	5.93	7.25	-
C20:0	2.76	5.17	3.70	3.26	-	0.30
C20:1	26.10	2.92	-	-	-	0.30
C20:2	-	-	15.31	15.00	-	0.40
C20:3	-	20.00	2.50	-	27.61	0.50
C20:4	-	-	-	-	-	6.70
C20:5	-	-	-	-	-	35.40

Table continued . . .

	1	2	3	4	5	6*
C22:0	2.72	-	1.52	6.32	-	-
C23:0	2.08	-	-	-	-	-
C25:0	1.92	-	-	-	-	-
	98.03	99.92	99.74	98.00	97.05	99.10

1. *Centroceras clavulatum*, 2. *Dermonema abbottiae*, 3. *Gracilaria foliifera*, 4. *Hypnea musciformis*, 5. *Scinaia fascicularis*, 6. *Laurencia pinnatifida* (*after Jamieson and Reid, 1972).

Conclusions

Seaweeds in general contain a bewildering array of major fatty acids. Only a few marine benthic algae have been investigated in this connection in any detail and in most of them, which have been examined, unknown fatty acids still remain to be identified. Considering the tremendous variety of algae in the marine environment it will not be surprising to uncover fatty acid compositions of other divisions like Bacillariophyta, Chrysophyta, Coccolithophyta, Cyanophyta, Dinophyta, Euglenophyta, Prymnesiophyta, Raphidophyta and Xanthophyta. Undoubtedly, the biotechnological exploitation of marine algae will become more prevalent in the last decade of the departing century. The present prerequisite phycochemical study will provide a better insight to start investigations on the fatty acid compositions of the seaweeds of Pakistan and may possibly augment elucidation of their pharmacology.

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