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FATTY ACID COMPOSITION OF SOME BROWN SEAWEEDS (PHAEOPHYTA) FROM THE COAST OF KARACHI

Ehsan Elahi Valeem and Mustafa Shameel

Department of Botany, Govt. Degree Science and Commerce College Gulshan-e-Iqbal, Block VII, Gulshan-e-Iqbal, Karachi-75300 (EEV);
Department of Botany, University of Karachi, Karachi-75270, Pakistan (MS).
E-mail: valeem@go.com.

ABSTRACT: Ten species of filamentous and multicellular thalloid algae (Phaeophyta) belonging to six genera of the class Dictyophyceae were collected from different seawater habitats of Karachi, Pakistan at the northern boundary of the Arabian Sea during September 1997 and July 1998. They were extracted in chloroform:methanol, saponified, subjected to column chromatography (CC, TLC), esterified and analysed for fatty acid (FA) composition initially by gas-liquid-chromatography (GLC) and finally by gas chromatography-mass spectrometry (GC-MS). Algae of the classes Laminariophyceae and Fucophyceae (Phaeophyta) displayed only a few SCFAs, PUFAs and substituted FAs, no VLCFA, C22 UFA, CFA, DCFA and monoynoic FA, large amount of C16:0, very large quantity of C18:1, very small RCCL and FA-diversity, C18 UFAs up to four DBs, C20 UFAs up to three DBs only. They were characterized by the largest amount of C18:1, lowest degree of unsaturation of C20 UFAs, lack of C22 UFAs, the shortest RCCL and the smallest FA-diversity as compared to other phyla.

KEY WORDS: Fatty acids, algae, Dictyophyceae, Phaeophyta, Karachi, Pakistan.

INTRODUCTION

Several studies have been undertaken on the fatty acid (FA) composition of seaweeds growing in the coastal waters of Karachi (Qasim, 1986; Shameel, 1987, 1990, 1993) as well as algae thriving in the freshwater and brackish water habitats of Sindh Province (Ghazala *et al.,* 2005; Naila *et al.,* 2005; Shahnaz *et al.,* 2006). Conventionally, blue-green and green algae are positioned as secluded species (Valeem and Shameel, 2005a,b, 2006a,b, 2007). In continuance, the present study was conducted on the brown algae of Pakistan as they are exclusively found in the marine environment. Table 1 lists different abbreviations used in this paper along with their explanations.

MATERIALS AND METHODS

Collection of the material: The brown seaweeds were collected from various coastal areas of Karachi, Pakistan such as Sandspit, Somar Goth, Buleji, Naugaza Mazar and Cape Monze during September 1997 and July 1998 (Table 2). The intertidal algae were detached from the rocks, pebbles and boulders during low tide, when the rocks were emerged. The drift algae were picked up from the tidal pools while wading during rising tides. A small portion of the fresh material was preserved in 4 % formalin solution in seawater for systematic identification. A few healthy and reproductive specimens were mounted on herbarium sheets as voucher specimens, which were deposited in the Seaweed Biology and Phycochemistry Laboratory (Room No. 18), M. A. H. Qadri Biological Research Centre (BRC), University of Karachi, Pakistan.

Table 1. List of abbreviations or symbols used along with their explanations.

Term	Explanation	Term	Explanation
ALA	α-Linolenic acid	**DUFA**	Diunsaturated fatty acid
C	Carbon	**EPA**	Eicosapentaenoic acid
CC	Column chromatography	**ETOAC**	Ethyl Acetate
^{c}C	Cyclic fatty acid (CFA)	**Et_2O**	Diethyl ether
CFA	Cyclic fatty acid	**EtOH**	Ethanol
CLA	Conjugated linolenic acid	**FA**	Fatty acid
Cn:0	Saturated Fatty acid (SFA)	**GC-MS**	Gas chromatography – mass spectrometry
Cn:1	Monounsaturated fatty acid (MUFA)	**GLC**	Gas-liquid-chromatography
Cn:2	Diunsaturated fatty acid (DUFA)	**h**	Hour
Cn:3	Triunsaturated fatty acid (TUFA)	**MeOH**	Methanol
Cn:4	Polyunsaturated fatty acid	**MUFA**	Monounsaturated fatty acid
Cn:5	Polyunsaturated fatty acid	**PUFA**	Polyunsaturated fatty acid (with 4-8 DBs)
Cn:6	Polyunsaturated fatty acid	**RCCL**	Range of C chain length
Cn:8	Polyunsaturated fatty acid	**SCFA**	Short chain fatty acid (< 9 C)
Cn:1	Monoynoic fatty acid	**SFA**	Saturated fatty acid
CUFA	Cyclic unsaturated fatty acid	**^{s}C**	Substituted fatty acid
^{d}C	Dicarboxylic fatty acid (DCFA)	**TB**	Triple Bond
DB	Double Bond	**TUFA**	Triunsaturated fatty acid
DCFA	Dicarboxylic fatty acid	**TLC**	Thin layer chromatography
DHA	Docosahexaenoic acid	**UFA**	Unsaturated fatty acid
DPA	Docosapentaenoic acid	**VLCFA**	Very long chain fatty acid (>25 C)

Preparation of the material: The algal material in bulk was washed thoroughly with tap water to remove epiphytes, epizoons, animal castings, attached debris and sand particles. The healthy, mature and clean thalli were selected, rinsed with distilled water and dried under shade with sufficient aeration to avoid the break down of long-chain fatty acids under sunlight and high temperature. The dried thalli of seaweeds (0.5-1.5 kg) were chopped into small pieces and milled.

Table 2. Algal collection data systematically arranged according to Shameel (2001).

#	Algal Taxa	Locality	Date
	PHYLUM PHAEOPHYTA		
	CLASS DICTYOPHYCEAE		
	ORDER Ectocarpales		
	Family Ectocarpaceae		
1.	*Hinksia mitchelliae* (Harvey) Silva	Hawkes Bay	September 1997
	ORDER Dictyotales		
	Family Dictyotaceae		
2.	*Dictyopteris australis* (Sonder) Askenasy	Somar Goth	February 1998
3.	*Dictyota dichotoma* (Hudsen) Lamouroux	Cape Monze	February 1998
4.	*Dictyota dumosa* Børgesen	Sandspit	June 1998
5.	*Dictyota hauckiana* Nizamuddin	Buleji	January 1998
6.	*Dictyota indica* Sonder *ex* Kützing	Buleji	January 1998
7.	*Dictyota maxima* Zanardini	Sandspit	July 1998
8.	*Padina tetrastromatica* Hauck	Buleji	January 1998
9.	*Spatoglossum variabile* Figari *et* De Notaris	Naugaza Mazar	February 1998
10.	*Stoechospermum marginatum* (C. Agardh) Kützing	Buleji	January 1998

Extraction: The dried and chopped algal material was kept for cold percolation in chloroform:methanol (1:1, v/v) at room temperature for a period of one month to obtain a total soluble extract. This extract was then evaporated under reduced pressure in rotary evaporator and a dark brown or brownish black, thick residue of 30 to 50 g was obtained depending on the colour and quantity of the algal material. This process was repeated two to three times for each algal species (Fig. 1).

Saponification: The lipid sample (up to 100 mg) was dissolved in a solution of 1 M potassium hydroxide (KOH) in 2 mL 95 % ethanol, it was either refluxed at 100° C for 1 h or left at room temperature for 6 h or long. The resulting mixture was evaporated under reduced pressure in rotary evaporator and was partitioned between aqueous (H_2O) and ethylacetate (EtOAc) or diethyl ether (Et_2O) phases. On cooling, water (5 mL) was added and the solution was extracted with Et_2O (10 mL) to remove any non-saponifiable material with centrifugation, if necessary to break any emulsion, which might have formed. The partitioning procedure between (EtOAc) and (H_2O) or (Et_2O) was repeated for several times. The aqueous layer, the total combined EtOAc or Et_2O fraction was acidified with 6 N HC1 having pH 4-5 and then re-extracted with EtOAc or Et_2O (3 to 5 mL), which then contained the free FAs. They were washed with ester (5 mL) and dried over anhydrous sodium sulphate (Na_2SO_4) before the solvent was removed and then concentrated under vacuum.

Column chromatography: The mixture to be analyzed by CC was applied to the top of the column. The eluent was passed through the column by gravity or air pressure. Equilibrium was established between the solute adsorbed on the adsorbent and the eluting solvent flowing down through the column. The individual components or elutents were collected, as the solvent dripped from the bottom of the column.

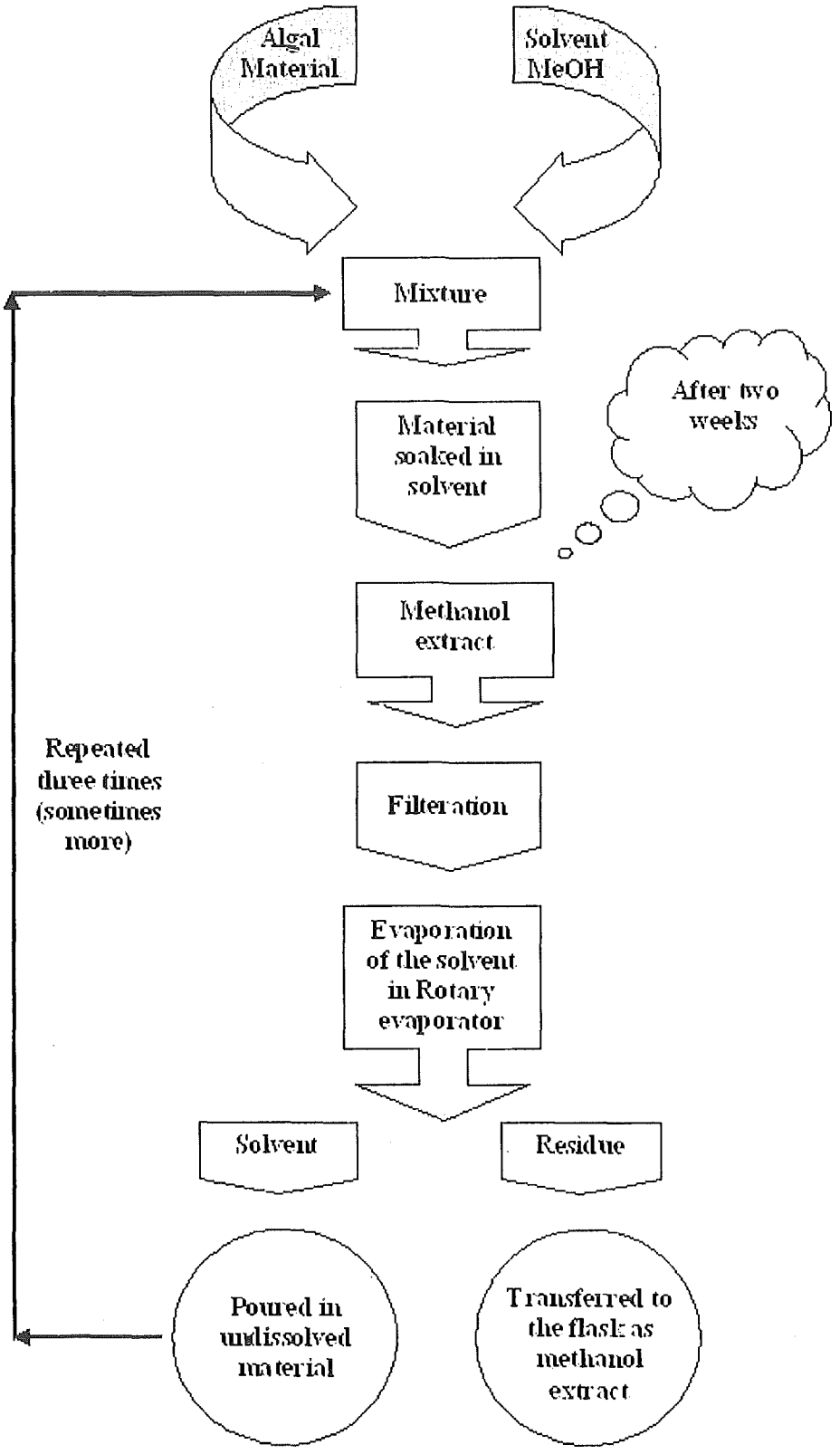

Fig. 1. Scheme for the extraction of methanol extract.

Esterifation: The FA-fraction (0.5 mg) was subjected to methylation, 0.5 mL of ethereal diazomethane was added and the FA-mixture or the reaction mixture was left in fume cupboard at room temperature for overnight until dissolved (Fig. 2). On evaporation the aliquots obtained were directly injected into GLC or GC-MS.

Identification: The methylated FA fractions were analysed first by GLC and finally by GC-MS. The final identification of FAs was carried out with the help of NBS-mass spectral library (Helles and Milne, 1978).

Instrumentation, CC & TLC: The TLC plates (silica gel 254 nm Mikrokarten SIF, 5x10 cm, or 20 x 20 cm, 0.2 nm; Riedel-de-Haen) were used, and for column CC Merck silica gel 60 (230 - 400 mesh size) was employed. It was observed that as the polarity of the solvent system was increased, all the components of the mixture moved faster (and *vice versa* with lowering the polarity). The ideal solvent system simply separates the components.

Preparative TLC: The glass plates were first air-dried and then activated in oven at 110° C. The final purity of the compound was checked on TLC by spraying with 10 % solution of Ce $(SO_4)_2$ in 2 N H_2SO_4. A small amount of the mixture to be analyzed was spotted near bottom of this plate. The TLC plate is then placed in a shallow pool of a solvent in a developing chamber so that only the bottom of the plate was in the liquid. This liquid or the eluent being the mobile phase, slowly rose up the TLC plate by capillary action.

GLC: The unknown FA fractions were analyzed first by GLC along with methyl ester standards on a Shimadzu GC-9A model gas chromatograph, equipped with a Shimadzu C-R6A chromatopac integrator. The column length was 2 mm and outer diameter 5 mm. The column material used was GP 3 % SP-2310 / 2 % SP-2300 on 100/120 chromosorb WAW. The column initial temperature was 150° C, while final temperature was 250° C with a rate of increase of 8° C, nitrogen flow rate was 30 mL/min.

GC-MS: The GC-MS was performed on a Hewlett-Packard GC with 11/73 DEC computer data system (Fig. 3) and a 1.2 m x 4 mm packed glass capillary column coated with gaschrome Q (100 - 120 mesh 0V 101, 1 %). Once the sample solution is introduced into the GC inlet, it is vaporized immediately because of the high temperature, which was programmed between 70-250° C with a rate of increase of 80° C per minute and swept onto the column by the carrier gas (usually Helium). The flow rate of carrier gas was 32 mL/min and the injector temperature 250° C.

RESULTS AND DISCUSSION

In Pakistan, brown algae occur only in the marine environment, ten species showing isomorphic alternation of generation were investigated (Table 2). They were characterized by a short RCCL (C11-C24), very low FA-diversity (6-13 acids), and lack of SCFA, VLCFA, monoynoic, substituted, cyclic FAs and PUFA; only one substituted FA (^{s}C15:3) was detected in one species (Table 3). In this way they completely differed from all previously described categories of green algae (Valeem and Shameel, 2005b, 2006a,b, 2007).

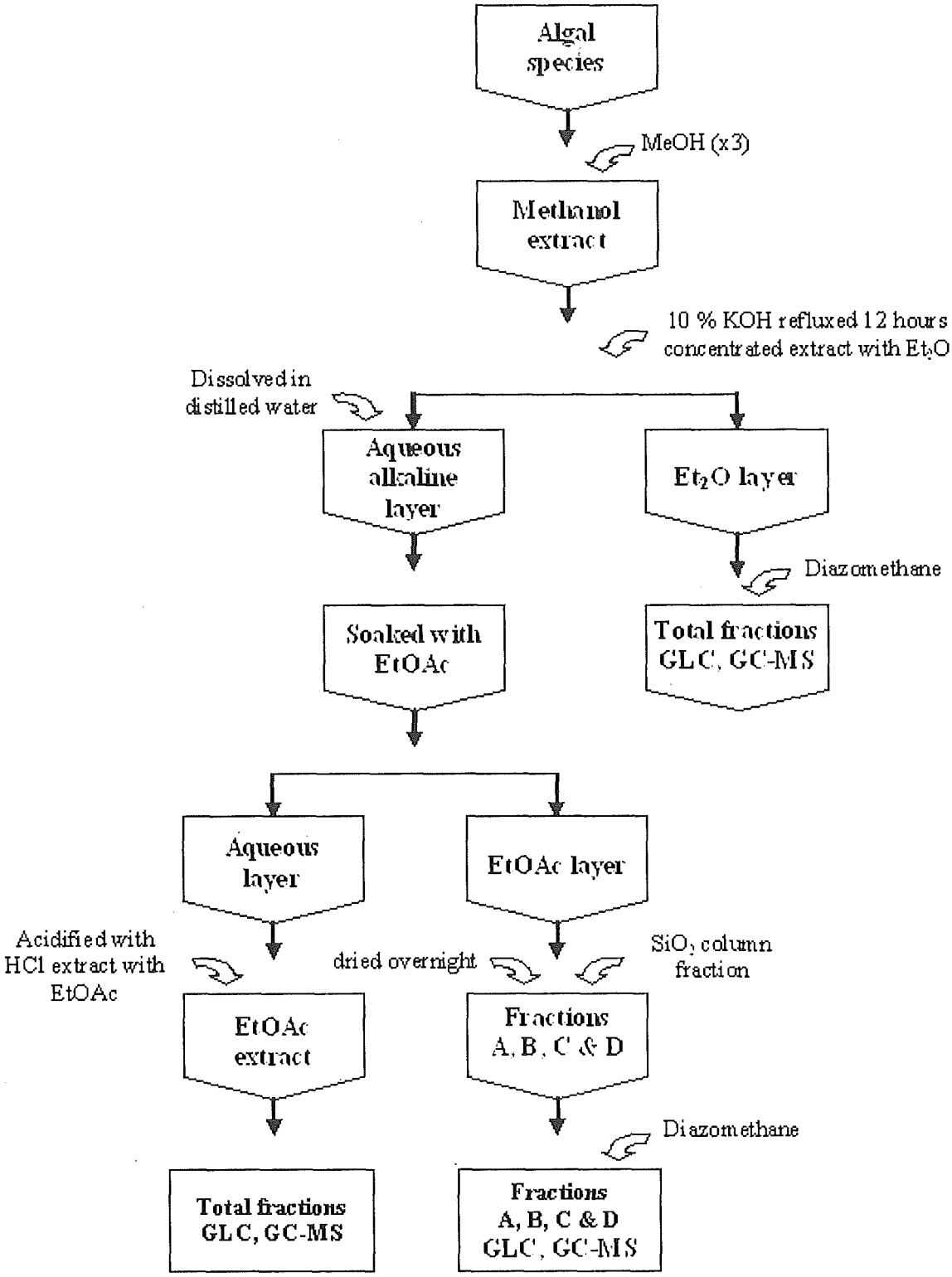

Fig. 2. Scheme for the detection of fatty acids.

Table 3. Fatty acid composition of algal species from Dictyophyceae (Phaeophyta) in relative percentage.

Fatty	Algal Species									
Acids	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
C11:1	-	-	-	-	-	-	-	-	1.62	-
C12:0	-	-	3.51	7.98	-	-	7.33	-	1.65	-
C12:1	-	-	3.90	-	-	-	-	-	-	-
C13:0	4.87	8.35	-	-	-	-	2.85	-	1.05	-
C14:0	2.96	10.53	-	-	6.32	5.99	6.12	6.64	21.08	17.01
C14:1	-	-	-	-	-	-	-	-	2.31	3.37
C14:3	1.58	2.24	-	-	-	-	-	3.79	-	3.68
C15:0	4.83	3.92	10.31	14.30	15.50	2.68	-	-	0.53	4.97
C15:1	-	-	-	-	-	-	-	-	-	6.18
C15:3	-	0.85	-	-	-	-	-	-	-	-
^{s}C15:3	-	4.37	-	-	-	-	-	-	-	-
C16:0	15.36	12.80	19.32	24.28	21.53	16.38	21.61	33.49	36.27	20.02
C16:1	-	7.96	-	-	-	2.85	-	2.22	-	-
C16:3	-	-	-	-	-	-	-	-	-	6.69
C17:0	15.84	19.39	15.11	-	18.45	19.39	17.47	7.04	-	11.98
C17:1	19.56	-	-	-	-	-	-	-	-	2.94
C17:2	-	-	-	3.49	-	2.83	2.18	-	-	-
C18:0	-	11.90	14.64	12.67	-	-	16.76	10.96	6.45	-
C18:1	22.39	-	9.27	22.18	19.92	18.60	12.38	22.45	3.11	4.61
C18:2	-	-	-	-	-	7.33	-	-	25.04	-
C18:3	-	-	-	-	-	-	-	9.37	-	-
C19:0	2.35	11.18	-	-	18.29	-	-	-	-	4.93
C20:0	2.48	2.64	12.82	11.38	-	10.08	13.30	4.05	-	-
C20:2	-	-	11.13	-	-	-	-	-	2.51	6.22
C20:3	-	-	-	-	-	13.87	-	-	-	-
C21:0	2.39	3.86	-	-	-	-	-	-	-	-
C22:0	3.72	-	-	3.73	-	-	-	-	-	7.40
C24:0	1.65	-	-	-	-	-	-	-	-	-
Acids	**13**	**13**	**9**	**8**	**6**	**10**	**9**	**9**	**11**	**13**

1 = *Hincksia mitchelliae*, **2** = *Dictyopteris australis*, **3** = *Dictyota dichotoma*,
4 = *Dictyota dumosa*, **5** = *Dictyota hauckiana*, **6** = *Dictyota indica*,
7 = *Dictyota maxima*, **8** = *Padina tetrastromatica*, **9** = *Spatoglossum variabile*,
10 = *Stoechospermum marginatum*

Palmitic and oleic acids were not only the most commonly occurring FAs as present in nine or all the ten investigated species, but they were found in overwhelming quantities (12.80-36.27 % and 3.11-22.39 % respectively) in them. In this aspect these algae completely resembled green seaweeds (Qasim, 1986; Shameel, 1987, 1990, 1993). The next common acids were: C14:0, C15:0, C17:0 and C20:0; they occurred in 7-8 of the investigated species and were found in fairly high proportion (up to 21.08 %). The C11:1, C12:1, C15:1, C15:3, ^{5}C15:3, C16:3, C18:3, C20:3 and C24:0 acids were least common, they could be detected in any one of the investigated algae. The C14:1, C16:1, C17:1, C18:2, C20:2, C21:0 and C22:0 acids were slightly better in occurrence, they were found in 2-3 species. In this way, they resembled marine algae of other oceans (Pohl and Zurheide, 1979; Khotimchenko, 1993).

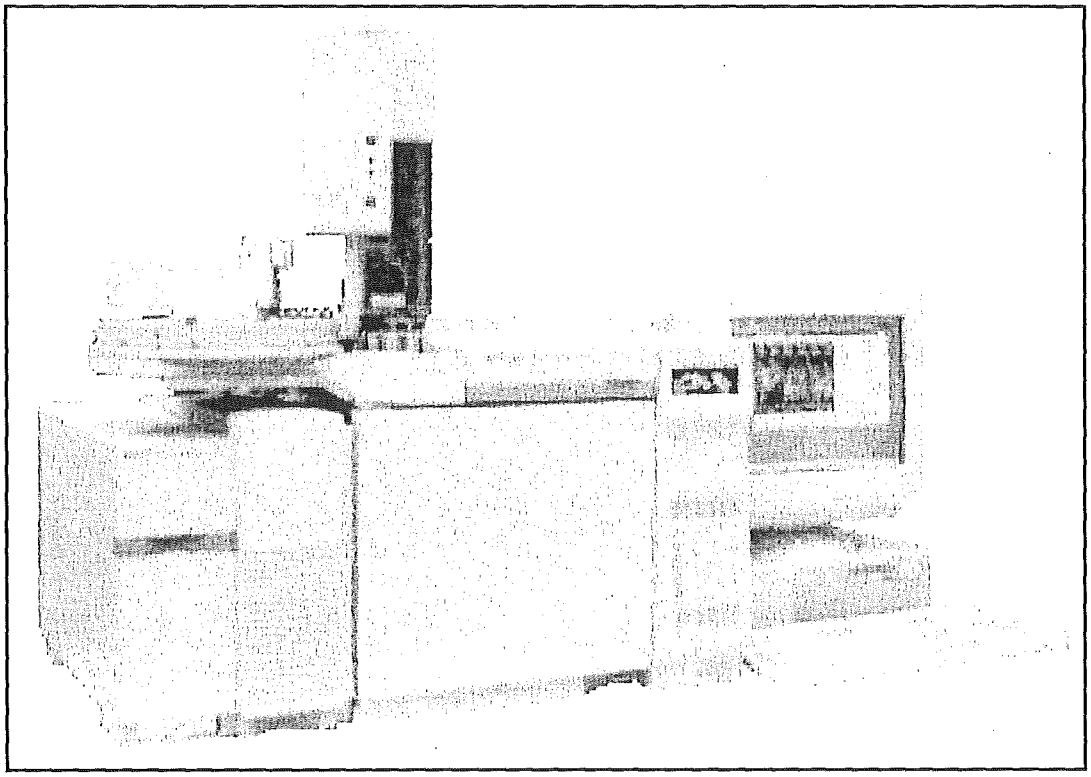

Fig. 3. The GC-MS of Hewlett-Packard with 11/73 DEC computer system.

Hincksia mitchelliae (# 1) was the sole representative of the order Ectocarpales. It was characterized by a small RCCL (C13-C24), large FA-diversity (13), no substituted FA, small amount of C16:0 and large amounts of C17:1 and C18:1 acids (Table III). All the other nine species (# 2-10) belong to the order Dictyotales, which showed a large RCCL (C11-C22), small FA-diversity (6-13), presence of a substituted FA, small C chain length (up to C22 only), large quantity of C16:0 and small amounts of C17:1 and C18:1 acids.

The five species of *Dictyota* (# 3-7) exhibited remarkable specific differences in their FA-compositions. Similar observations have also been made previously on five species of *Dictyota* by the present laboratory (Shameel *et al.,* 1991). The present observations on *Dictyopteris australis* and *Hincksia mitchelliae* agreed with the previous results obtained on these algae (Aslam *et al.,* 1994; Shaikh and Shameel, 1999). The PUFAs with 18 and 20 C atoms, palmitic and oleic acids were observed to be the major components of a large number of species of Dictyophyceae from the Russian Far East (Khotimchenko, 1998).

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