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Antioxidant Capacity and Lipophilic Content of Seaweeds Collected from the Qingdao Coastline

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Lipophilic extracts from 16 species of seaweeds collected along the Qingdao coastline were screened and evaluated for their antioxidant activities (AA) using the β-carotene-linoleate assay system. The diethyl ether soluble extracts of all selected seaweeds exhibited various degrees of antioxidative efficacy in each screen. The highest antioxidant capacities among the tested samples were observed for *Rhodomela confervoides* and *Symphyocladia latiuscula* and were comparable with that of the well-known antioxidant butylated hydroxytoluene and greater than that of propyl gallate. The lipophilic content of all 16 samples and the chemical composition of 4 selected seaweeds, *R. confervoides* and *S. latiuscula*, which had higher AA, *Laminaria japonica*, which had intermediate AA, and *Plocamium telfairiae*, which had lower AA, were analyzed by gas chromatography and gas chromatography−mass spectrometry, respectively. Fatty acids and alkanes were found. The present data indicated an increase in antioxidative property with increasing content of unsaturated fatty acid. The result of this study suggests that seaweeds can be considered as a potential source for the extraction of lipophilic antioxidants, which might be used as dietary supplements or in production in the food industry. This is the first report on the antioxidant activities of lipophilic extracts from seaweeds.

KEYWORDS: Seaweed; β-carotene-linoleate; natural antioxidants; GC; GC-MS; lipophilic composition; unsaturated fatty acid

INTRODUCTION

Lipid autoxidation is not only one of the major reasons for the deterioration of food products during processing and storage but is also associated with aging, membrane damage, heart disease, stroke, emphysema, and cancer in living organisms (*1*, *2*). The addition of antioxidants to food is effective in retarding the lipid oxidation to extend shelf life; furthermore, the intake of antioxidants can reduce the risk of the above diseases (*3*, *4*).

Several synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and butylated hydroxyquinone (TBHQ) are commercially available and currently used. However, these antioxidants have been restricted for use in foods as they are suspected to be carcinogenic (*5*, *6*). Some toxicological studies have also implicated the use of these synthetic antioxidants in promoting the development of cancerous cells in rats (*7*). These findings, together with consumers' interests in natural food additives, have reinforced the efforts for the development of alternative antioxidants from natural origins.

Fresh seaweeds, both wild and cultivated, are commonly eaten in China, particularly along the coastlines in eastern and southern China. Some of the seaweeds are considered to be a rich source of antioxidants (*8*, *9*), and different types of antioxidants from various species of seaweeds have been reported. These can be exemplified by fucoxanthin in *Hijikia fusiformis* (*10*), phlorotannins in *Sargassum kjellamanianum* (*11*), protean extracts from *Spirulina platensis* (*12*), and different extracts and fractions from some marine algae (*13*−*16*). On the other hand, many fatty acids have the ability to act as antioxidants or prooxidants. Recently, Henry et al. (*17*) reported the antioxidant activities of 29 commercially available C-8−C-24 saturated and unsaturated fatty acids. Most of the unsaturated fatty acids tested showed good antioxidant activities (*17*). A literature survey revealed that most seaweeds are very rich in fatty acids, especially in the lipophilic extracts (*18*). To the best of our knowledge, there are no reports about the antioxidant activity of lipophilic fractions extracted from seaweeds. This prompted us to evaluate their antioxidant activities. The lipophilic constituents from seaweeds might be useful in the food industry to protect against lipid peroxidation because the low polarity of the chemical components could be readily dissolved in the lipid fraction of the food. In this study, 16 species of seaweeds were extracted with diethyl ether in a Soxhlet extractor and the

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antioxidant activities (AA) of the resulting extracts were evaluated using the β-carotene-linoleate assay system. In addition, the lipophilic contents of all tested seaweeds and the chemical composition of four selected samples, namely, *Rhodomela confervoides* and *Symphyocladia latiuscula*, which had higher AA, *Laminaria japonica*, which had intermediate AA, and *Plocamium telfairiae*, which had lower AA, were analyzed by gas chromatography (GC) and gas chromatography−mass spectrometry (GC-MS).

MATERIALS AND METHODS

Seaweed Sampling. The seaweeds used for this study were freshly collected from the Qingdao coastline of Shandong Province, People's Republic of China. Samples collected were transported to the laboratory immediately and were gently rinsed with filtered seawater. They were packed and stored in a refrigerator until the experiments were carried out. The species were identified at the Institute of Oceanology, Chinese Academy of Sciences, Qingdao, where the voucher specimens were deposited.

Chemicals. Linoleic acid and Tween-40 (polyoxyethylene sorbitan monopalmitate) were purchased from Shanghai Chemical Reagents Co. (Shanghai, China). BHT, propyl gallate (PG), and β-carotene were purchased from Sigma Chemical Co. (St. Louis, MO). All organic chemicals used in the experiments were of analytical grade.

Sample Preparation. The dried seaweed sample was ground into small pieces by a Waring blender and extracted with diethyl ether in a Soxhlet extractor for 12 h. The resulting extractive solution was evaporated to dryness in a rotary flash evaporator (Büchi Labortechnik AG, Switzerland) to give the lipophilic extract.

Antioxidant Assay with the β-Carotene-Linoleate Model System. The antioxidant activities of the lipophilic extract from seaweed as well as BHT and PG were evaluated following procedures previously described by Jayaprakasha et al. (*19*). A 0.2 mg sample of β-carotene-linoleate in 0.2 mL of chloroform, 20 mg of linoleic acid, and 200 mg of Tween-40 were mixed. Chloroform was removed at 40 °C under vacuum, and the resulting mixture was diluted with 10 mL of water and mixed well. To this emulsion was added 40 mL of oxygenated water. Four milliliter aliquots of the emulsion were pipetted into different test tubes containing 0.2 mL of diluted lipophilic extract (equivalent to 100 and 200 ppm, respectively), BHT (equivalent to 100 and 200 ppm, respectively) in hexane, and PG (equivalent to 100 and 200 ppm, respectively) in ethanol. BHT and PG were used for comparative purposes. Two controls containing 0.2 mL of hexane and ethanol, respectively, and 4 mL of the above emulsion were prepared. The tubes were placed at 50 °C in a water bath, and the absorbance at 470 nm was taken at zero time ($t = 0$). The measurement of absorbance was continued at intervals of 30 min until the color of β-carotene disappeared in the control tubes ($t = 180$ min). A mixture prepared as above without β-carotene served as the blank. All determinations were carried out in triplicate. The antioxidant activity (AA) of the extracts was evaluated in terms of bleaching of the β-carotene, using the following formula: $AA = 100[1 - (A_0 - A_t)/(A_0^0 - A_t^0)]$; A_0 and A_0^0 are the absorbance values measured at zero time of the incubation for the test sample and the control, respectively, and A_t and A_t^0 are the absorbance values measured in the test sample and the control, respectively, after incubation for 180 min.

GC Analysis. After methylation by the method of the previous paper (*20*), the lipophilic composition was analyzed on an HP 5890A GC equipped with an FID detector and a DB-5 bonded-phase fused-silica capillary column (30 m × 0.25 mm × 0.25 μm). The injector and detector temperatures were 280 °C. The oven temperature was programmed from 80 °C (1 min isothermal) to 260 °C (held for 10 min at final temperature) at a ramp rate of 8 °C/min. Nitrogen was used as the carrier gas at 40 mL/min. Peak areas were computed by an HP 3392A integrator.

GC-MS Analysis. The composition of the methylated lipophilic extract was analyzed on an HP 5890 GC coupled to an HP 5989A MS system. A column (30 m × 0.25 mm × 0.25 μm, HP-5 bonded-phase fused-silica capillary column) was used. Helium was used as the carrier gas at a flow rate of 1.2 mL/min. The injector and detector temperatures were 290 and 300 °C, respectively. The oven temperature was programmed from 80 °C (1 min isothermal) to 290 °C (held for 10 min at final temperature) at a ramp rate of 8 °C/min. Mass spectra were recorded under electron impact ionization at 70 eV of electron energy with a range from 40 to 450 at a rate of 1 scan/s. Ion source and MS quadrupole temperatures were set at 220 and 100 °C, respectively. The constituents of the lipophilic extract were identified by comparing retention times of GC peaks with those of the standard compounds under the same chromatographic conditions and Christie's method (*21*) and by mass spectral data.

Table 1. Content of Total Lipophilic Constituent of 16 Seaweeds

species of seaweed	total lipophilic content (mg/g, dry wt)
Rhodomela confervoides	37.7
Symphyocladia latiuscula	38.7
Grateloupia filicina	23.8
Scytosiphon lomentarius	27.5
Chondrus ocellatus	124.3
Polysiphonia urceolata	46.7
Laminaria japonica	65.1
Porphyra yezoensis	80.7
Ceramium kondoi	51.1
Corallina officinalis	50.2
Chondria tenuissima	50.6
Porphyra haitanensis	168.3
Gloiopeltis furcata	84.0
Undaria pinnatifida	124.1
Chondria crassicaulis	35.1
Plocamium telfairiae	30.5

RESULTS AND DISCUSSION

Content of Total Lipophilic Constituent. Table 1 shows the total lipophlilc contents of 16 species of seaweeds extracted using diethyl ether as solvent. The yields of *Porphyra haitanensis*, *Chondrus ocellatus*, and *Undaria pinnatifida* were >100 mg/g (dry weight), whereas the yields of *Scytosiphon lomentarius* and *Grateloupia filicina* were <30 mg/g (dry weight). The total lipophilic contents of the other seaweeds were in the range of 30.5−84.0 mg/g (dry weight).

Antioxidant Activities. The antioxidant activities of the lipophilic extracts from 16 species of seaweeds as well as BHT and PG as measured by the bleaching of β-carotene are presented in **Figure 1** and **Table 2**. The mechanism of bleaching of β-carotene is a free radical mediated phenomenon resulting from the hydroperoxide formed from linoleic acid. The β-carotene in this model system undergoes rapid discoloration in the absence of an antioxidant. The linoleic acid free radical attacks the highly unsaturated β-carotene molecules. As β-carotene molecules lose their double bonds by oxidation, the compound loses its chromophore and characteristic orange color, which can be monitored spectrophotometrically (*19*); that is, the absorbance values decrease with time as indicated by the rate of peroxidation (**Figure 1**). The presence of antioxidant can hinder the extent of β-carotene bleaching by neutralizing the linoleate free radical and other free radicals formed in this system, and the consequence is the absorbance values show different decreasing patterns. Generally, the stronger the antioxidant activities were, the more slowly the absorbance values decreased with time (**Figure 1**).

According to the formula, $AA = 100[1 - (A_0 - A_t)/(A_0^0 - A_t^0)]$, the antioxidant activities of the lipophilic extracts at various concentrations (100 and 200 ppm) were evaluated in terms of bleaching of the β-carotene (**Table 2**). The values are mean ± standard deviation ($n = 3$). The extracts exhibited a concentration-dependent manner in the inhibitory activities. As

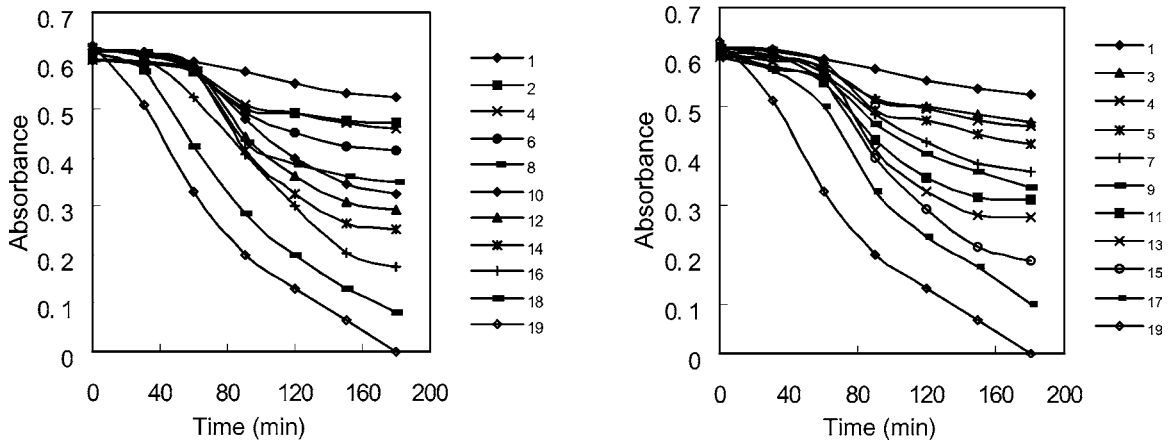

Figure 1. Antioxidant activities of lipophilic extracts from 16 species of seaweeds (at a concentration of 200 ppm). Numbers correspond to the samples in **Table 2**.

Table 2. Antioxidant Activities (AA) of Lipophilic Extracts of Various Seaweeds

	sample tested	AA[a] (%) 100 ppm	AA[a] (%) 200 ppm
1	BHT	72.8 ± 1.73	84.8 ± 0.24
2	*Rhodomela confervoides*	68.8 ± 0.97	79.2 ± 0.45
3	*Symphyocladia latiuscula*	64.9 ± 0.63	76.8 ± 1.33
4	PG	60.3 ± 2.12	74.3 ± 0.26
5	*Grateloupia filicina*	52.5 ± 0.97	68.6 ± 0.18
6	*Scytosiphon lomentarius*	53.2 ± 0.64	67.3 ± 0.11
7	*Chondrus ocellatus*	49.9 ± 2.10	60.3 ± 1.49
8	*Polysiphonia urceolata*	45.6 ± 0.61	58.4 ± 0.24
9	*Laminaria japonica*	42.5 ± 0.20	57.0 ± 0.32
10	*Porphyra yezoensis*	43.0 ± 2.07	52.9 ± 2.01
11	*Ceramium kondoi*	40.0 ± 2.07	51.3 ± 0.60
12	*Corallina officinalis*	40.6 ± 1.73	48.1 ± 0.24
13	*Chondria tenuissima*	41.5 ± 0.77	47.1 ± 1.88
14	*Porphyra haitanensis*	29.9 ± 0.78	40.5 ± 1.74
15	*Gloiopeltis furcata*	22.6 ± 0.71	33.0 ± 0.52
16	*Undaria pinnatifida*	11.3 ± 0.65	29.7 ± 1.74
17	*Chondria crassicaulis*	5.6 ± 0.50	17.6 ± 0.10
18	*Plocamium telfairiae*	2.9 ± 0.11	14.8 ± 0.29
19	control	0	0

[a] Values are mean ± standard deviation ($n = 3$).

shown in **Table 2**, the extracts of all tested seaweeds showed antioxidant activities. Of the 16 species of seaweeds, *R. confervoides* and *S. latiuscula* appeared to possess the highest antioxidant activity. At a concentration of 100 ppm, the lipophilic extracts of *R. confervoides* and *S. latiuscula* exhibited 68.8 and 64.9% antioxidant activity, respectively, which were comparable with that of the positive control antioxidant BHT (72.8%) and greater than that of PG (60.3%) in the same concentrations used in the assay. Similar results were also observed in the assay at a concentration of 200 ppm, at which the extracts of *R. confervoides* and *S. latiuscula* showed 79.2 and 76.8% antioxidant activity, respectively, whereas the positive control antioxidants, BHT and PG, revealed 84.8 and 74.3% antioxidant activity, respectively, at the same concentration. Both extracts of *R. confervoides* and *S. latiuscula* had higher antioxidant activities than BHT and PG at a concentration only one time higher (200 vs 100 ppm). The extracts of *Chondria crassicaulis* and *P. telfairiae* showed the weakest antioxidant activities, which were only 5.62 and 2.88%, respectively, at 100 ppm concentration, and 17.6 and 14.8%, respectively, at 200 ppm concentration. The antioxidant activities of the other samples were in the range of 29.8−68.6% at a concentration of 200 ppm. It deserves mention that the extracts

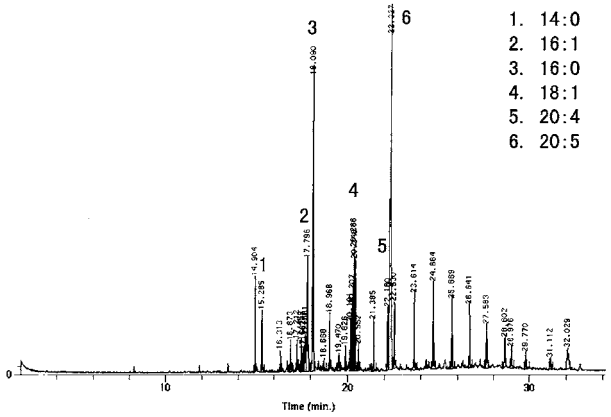

Figure 2. GC-MS total ion chromatogram of *R. confervoides*.

of three additional seaweeds, *Grateloupia filicina*, *Scytosiphon lomentarius*, and *Chondrus ocellatus*, also had antioxidant activities higher than or similar to that of PG at a concentration only one time higher (200 vs 100 ppm) (**Table 2**).

Identification of Lipophilic Chemical Composition. To study the relationship between the antioxidant activity and the content of antioxidant composition, four species of seaweeds, namely, *R. confervoides* and *S. latiuscula*, which exhibited higher antioxidant activities, and *L. japonica* and *P. telfairiae*, which showed moderate and lower antioxidant activities, respectively, were selected for further analysis of the chemical composition. The diethyl ether extracts from the above four species of seaweeds were methylated and analyzed with GC and GC-MS. The GC-MS total ion chromatograms are presented in **Figures 2**−**5**. The components identified and their relative amounts of individual composition, which are expressed as a percentage of peak area relative to total peak area, are summarized in **Table 3**. Totally, 25 compounds were identified from combined data, which consisted of 9 fatty acids, 14 alkanes, 1,1′-bicyclopentyl, and cholesta-3,5-diene. The latter two compounds were detected only in *R. confervoides*. The identified compounds constituented >90% of the total components. Fatty acids were the major components (>60%) in the lipophilic extracts of *R. confervoides* and *S. latiuscula*, whereas in the extracts of *L. japonica* and *P. telfairiae* the contents of fatty acids were only 48.293 and 26.052%, respectively. Two types of fatty acids, namely, saturated fatty acids (SFA) and unsaturated fatty acids (USFA), were classified in the four tested samples. Three SFAs, tetradecanoic acid, hexadecanoic acid, and octadecanoic acid, were identified in all four samples at

Table 3. Chemical Composition of Methylated Lipophilic Extracts from Four Selected Species of Seaweeds by GC-MS[a]

constituent	peak area (%)			
	R. confervoides	*S. latiuscula*	*L. japonica*	*P. telfairiae*
saturated fatty acid methyl esters				
tetradecanoic acid methyl ester	2.453	1.872	8.638	2.102
hexadecanoic acid methyl ester	16.376	22.555	10.871	16.358
octadecanoic acid methyl ester	0.426	1.229	0.632	0.400
unsaturated fatty acid methyl esters				
11-hexadecenoic acid methyl ester	5.459	6.509	2.124	6.371
9-octadecenoic acid (*Z*) methyl ester	13.401	10.918	12.536	1.221
10,13-octadecadienoic acid methyl ester		0.276	5.077	
9,12,15-octadecatrienoic acid methyl ester	1.836			
5,8,11,14-eicosatetraenoic acid methyl ester	2.186	1.924	3.542	
5,8,11,14,17-eicosapentaenoic acid methyl ester	23.795	15.007	4.872	
alkanes				
heptadecane	3.676	3.067		
octadecane	0.521	1.302		1.895
nonadecane	1.526	5.121	1.255	
eicosane	1.536	0.389	1.821	0.260
heneicosane	2.632	1.204	3.263	2.026
docosane	1.427	0.954	3.742	7.063
tricosane	2.730	2.404	4.926	10.122
tetracosane	2.529	4.301	6.682	13.060
pentacosane	1.874	3.994	6.461	14.192
hexacosane	1.536	1.637	5.133	7.302
heptacosane	2.071	3.300	4.727	4.228
octacosane	1.360	1.522	3.942	6.156
nonacosane	0.863	0.828	2.020	1.242
triacontane	0.302	0.501	0.728	0.633
others				
1,1′-bicyclopentyl	1.346			
cholesta-3,5-diene	0.594			
total identified amount	92.454	90.837	93.591	94.631
SFA	17.256	25.655	20.142	18.461
USFA	46.676	34.635	28.151	7.591
FA	63.930	60.291	48.293	26.052

[a] Compounds identified by standard compounds under the same chromatographic conditions and Christie's method (*21*), as well as by mass spectra.

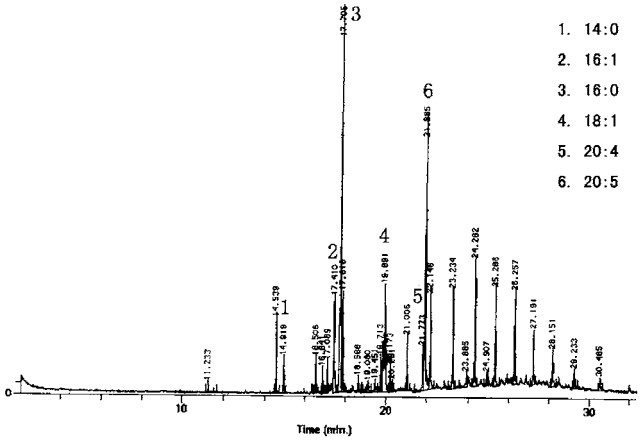

Figure 3. GC-MS total ion chromatogram of *S. latiuscula*.

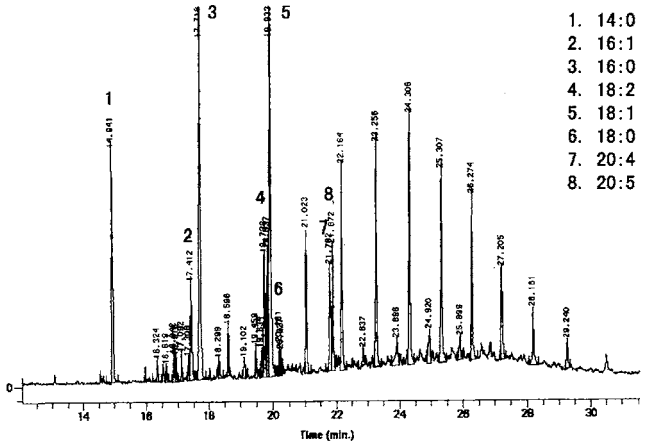

Figure 4. GC-MS total ion chromatogram of *L. japonica*.

various concentrations. The USFA identified included 11-hexadecenoic acid, 9-octadecenoic acid, 10,13-octadecadienoic acid, 9,12,15-octadecatrienoic acid, 5,8,11,14-eicosatetraenoic acid, and 5,8,11,14,17-eicosapentaenoic acid. The contents of the highly unsaturated fatty acid (5,8,11,14,17-eicosapentaenoic acid) were 23.795, 15.007, and 4.872% in the extracts of *R. confervoides*, *S. latiuscula*, and *L. japonica*, respectively, whereas in the extract of *P. telfairiae* this compound was not detected. According to the results presented in **Table 3**, it could be observed that the contents of SFA were in the order *S. latiuscula* (25.655%) > *L. japonica* (20.142%) > *P. telfairiae* (18.461%) > *R. confervoides* (17.256%). However, the contents of USFA were found to be in the order *R. confervoides* (46.676%) > *S. latiuscula* (34.635%) > *L. japonica* (28.151%) > *P. telfairiae* (7.591%). The latter was the same order as for their antioxidant activities. It obviously shows that an increase in the antioxidant activity related to an increase in the content of USFA. Therefore, USFA appears to be the main component that mainly contributed to the antioxidant activities of the lipophilic extracts from the seaweeds.

Antioxidants can be classified into two groups, namely, chain-breaking antioxidants and preventive antioxidants (*14*). It is well-known that USFAs with two or more double bonds are easily oxidized. Therefore, their activities are attributed to the electron-

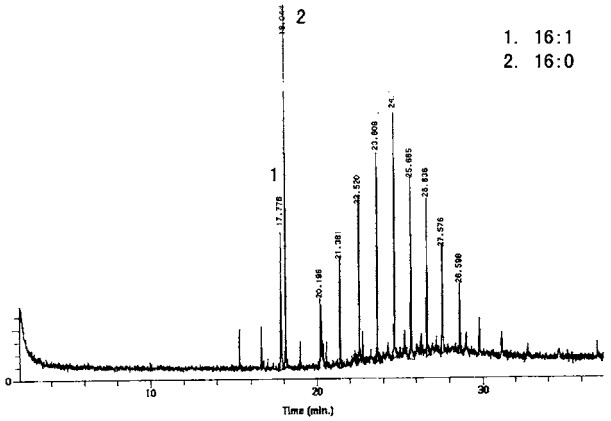

Figure 5. GC-MS total ion chromatogram of *P. telfairiae*.

donating ability, suggesting the role as a preventive antioxidant, that is, prooxidant.

In conclusion, the lipophilic extracts from 16 species of seaweeds studied for this paper exhibited potential antioxidant activities. The activities of *R. confervoides* and *S. latiuscula* were comparable with that of BHT and relatively higher than that of PG at the same concentration used in the β-carotene-linoleate assay system. The present findings appear to be useful in leading to the development of therapeutic products to protect against certain diseases. Our results also suggest that the consumption of these seaweeds as a dietary supplement or as a food ingredient has the potential to provide health benefits. In addition, seaweeds have a potential to be used as a natural antioxidants in the food industry to stabilize especially high-fat foodstuffs by retarding lipid oxidation to extend shelf life. To the best of our knowledge, this is the first report so far on the study of the content and antioxidant capacity of lipophilic extracts from seaweeds.

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