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Identification of Sulfoglycolipid Bioactivities and Characteristic Fatty Acids of Marine Macroalgae

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ABSTRACT: The fatty acid compositions of 21 species of marine macroalgae, including 5 species of Chlorophyta (green algae), 13 of Rhodophyta (red algae), and 3 of Heterokontophyta (brown algae), were collected from northeastern Taiwan to survey their functional lipids. The lipid contents of green algae ranged from 15.36 to 20.15 mg/g, dry basis (db), and were characterized by a high content of C18:2 and C18:3, red algae (18.57−28.34 mg/g db) were high in C20:4 and C20:5, and brown algae (13.11−19.56 mg/g db) were high in C18:4, C20:4, and C:20:5. All algal lipids contained fatty acids of odd-number carbons, C17:0, and C17:1. Red algae had relatively higher levels of polyunsaturated fatty acids (PUFAs) and were richer in eicosapentaenoic acid (EPA) than green and brown algae. A red alga, *Porphyra crispata*, was extracted with ethanol and separated on a hydrophobic column (Diaion HP-20 column) to obtain sulfoglycolipids (sulfoquinovosyldiacylglycerols, SQDGs). The main fatty acids in SQDGs were palmitic acid (C16:0), 33.3%; EPA (C20:5), 30.0%; arachidonic acid (C20:4), 12.7%; oleic acid (C18:1), 7.52%; and stearic acid (C18:0), 6.83%. The n-3/n-6 ratio was 1.9, whereas the authentic standard, spinach SQDG, did not contain n-3 fatty acids. Sulfoglycolipids inhibited the growth of human hepatocellular carcinoma cell line (HepG2). The IC_{50} was 126 μg/mL, which is lower than that of the spinach SQDG (255 μg/mL).

KEYWORDS: *macroalgae, Porphyra crispata, odd-carbon-number fatty acid, polyunsaturated fatty acids, PUFAs, eicosapentaenoic acid, EPA, n-3/n-6 fatty acid ratio, sulfolipids, sulfoquinovosyldiacylglycerols, SQDG, human hepatocellular carcinoma, HepG2*

INTRODUCTION

Algae are abundant in coastal waters. They have long been used in Asian cuisines and traditional remedies. Marine maroalgae are rich in nutrients and have been used in food, medicine, body-care products, aquaculture feeds, biofuel products, and agricultural fertilizer.[1] In recent years, marine macroalgae are considered to be promising resources of bioactive compounds with pharmaceutical and biomedical potentials being applied as anticoagulant, antioxidant, antiviral, antiallergic, anticancer, anti-inflammatory, and antiobesity agents.[2] Some bioactive materials from marine maroalgae have been studied, including polysaccharides, functional lipids, enzymes and bioactive peptides, natural pigments, essential minerals, and vitamins.[3]The insoluble dietary fiber separated from red macroalgae *Gracilaria* was able to enhance the excretion of cholesterol and bile acids into feces.[4] Therefore, it has the potential to prevent hypercholesterolemia.

The lipid contents in marine algae are unique in their sulfoglycolipids and polyunsaturated fatty acids (PUFAs) of high n-3/n-6 ratio. The sulfoglycolipids are anionic glycolipids present in the photosynthetic membranes of various marine algae.[5] They have specific biological inhibitory activities against DNA polymerase,[6] certain types of virus,[7] telomerase,[8] angiogenesis,[9] and inflammation/proliferation.[10] The spinach sulfoglycolipids were found to inhibit mammalian cancer cell growth and mouse sarcoma and colon tumor proliferation influenced by the antiangiogenesis and antiproliferation of tumor cells.[9,11] Therefore, sulfoglycolipids were suggested as preventive to human cancer diseases and have the potential to become functional foodstuffs with cytotoxic effects. Macroalgae are also a good source of PUFAs,[12] which can effectively reduce the risk of cardiovascular diseases, cancer, and diabetes. PUFAs are precursors of a variety of eicosanoids in animals and the plant oxylipin family.[13] Oxylipins in algae protect the algae against infections and pathologies.[14,15]

In addition, we found that in marine algae PUFAs catalyzed by the endogenous high lipoxygenase (LOX) activity were responsible for the fresh seaweed or fresh oyster aroma.[16] Treatment of edible oil with immobilized algal LOX was able to improve the aroma of fish oil and chicken oil.[17−19]

The objective of this study was to investigate the lipid content of the marine algal species to identify the species of high n-3 PUFAs and high sulfoglycolipid contents. Their unique fatty acids may be used for algal taxonomy. The macroalgae rich in n-3 PUFA may have the potential to substitute for fish oil in vegan diets and aquaculture feeds, whereas the sulfoglycolipids may be used as functional lipids with cytotoxic efficacy at 20 mg/day by oral administration without side effect.[9]

MATERIALS AND METHODS

Lipid Extraction. The 21 species of algae were collected from the northeastern coast of Taiwan (E 121° 65′−122° 15′, N 24° 50′−25° 25′) during January to April. The species studied and the taxonomy are shown in Table 1. The samples collected were washed with fresh

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Table 1. Taxonomy of the 21 Marine Macroalgae Species Studied

DIVISION CLASS Order Family *Genus species*	sample code
CHLOROPHYTA (green algae)	
CHLOROPHYCEAE	
Ulvales	
Ulvaceae	
Ulva fasciata Delile, 1813:155	G1
Enteromorpha prolifera (Muller) J. Agardh ,1883:129	G2
Cladophorales	
Cladophoraceae	
Chaetomorpha antennina (Bory) Kutzing ,1847:166	G3
Siphonocladales	
Siphonocladaceae	
Cladophora vagabunda (Linnaeus) Hoek, 1963:144-162	G4
BRYOPSIDOPHYCEAE	
Bryopsidales	
Codiaceae	
Codium mamillosum Harvey, 1855:565	G5
RHODOPHYTA (red algae)	
BANGIOPHYCEAE	
Bangiales	
Bangiaceae	
Porphyra crispata Kjellman, 1897:15	R6
FLORIDEOPHYCEAE	
Gigartinales	
Gigartinaceae	
Chondrus verrucosa Mikami, 1965:248	R7
Chondracanthus intermedius (Suringar) Hommersand, 1993:115	R8
Halymeniales	
Halymeniaceae	R9
Grateloupia filicina (Lamouroux) C. Agardh, 1822:223	
Carpopeltis maillardii (Montagne et Millardet) Chiang, 1970:68	R10
Plocamiales	
Plocamiaceae	
Plocamium telfairiae (Hooker et Harvey) Harvey ex Kutzing, 1849:885	R11
FLORIDEOPHYCEAE	
Corallinales	
Corallinaceae	
Cheilosporum acutilobum (Decaisne) Piccone, 1886:66	R12
Corallina pilulifera Postels et Ruprecht, 1840:20	R13
Gracilariales	
Gracilariaceae	
Gracilaria coronopifolia J. Agardh, 1852:592	R14
Gracilaria chorda Holmes, 1896:253	R15
Ceramiales	
Rhodomelaceae	
Chondrophycus intermedius (Yamada) Garbary & Harper, 1994: 195	R16
Laurencia brongniartii J. Agardh, 1841:20	R17
Ceramiaceae	
Centroceras clavulatum (C. Agardh) Montagne, 1846:140	R18
HETEROKONTOPHYTA (brown algae)	
PHAEOPHYCEAE	
Fucales	
Sargassaceae	B19
Sargassum crassifolium J. Agardh, 1848	B20
Sargassum cristaefolium C. Agardh, 1820:13	
Ectocarpales	
Scytosiphonaceae	
Petalonia binghamiae J. Agardh, 1896: 27	B21

water followed by distilled water and cleaned of epiphytes. The algae were freeze-dried (FreeZone 4.5 model 77500, Labconco Co., Kansas City, MO, USA) and ground (D3 V-10, Yu Chi Machinery Inc., Chang Hua, Taiwan) to 200 mesh fine powder, which was kept at −40 °C until use. The powder was extracted at room temperature with 5 volumes of chloroform/methanol (2:1, v/v) for 1 h with shaking and then filtered. The extract was evaporated to dryness, redissolved with solvent to a known volume, and assayed for fatty acids.

Fatty Acid Esterification. The method of the AOAC[20] was used. About 25 mg of algal lipid extract or SQDG was mixed with 0.5 mL of an internal standard, methyl tricosanoate (methyl-C23:0, 40 mg/mL in diethyl ether) in 1.5 mL of 0.5 N methanolic NaOH, blanketed with a N_2 cap, mixed, and heated at 100 °C for 5 min. Subsequently, 2.0 mL of 14% BF_3 (Sigma-Aldrich Co., St. Louis, MO, USA) in CH_3OH was added, and the mixture was boiled for 30 min and then cooled to 30−40 °C; 1 mL of isooctane was added, blanketed with a N_2 cap, and the mixture was shaken vigorously for 30 s while still warm. The reaction was terminated with the addition of 5.0 mL of saturated NaCl solution and cooled to room temperature. The isooctane layer was collected and dried. The methyl ester was then dissolved in isooctane.

Fatty Acid Analysis. The methyl ester was analyzed with a gas chromatograph (GC-14A, Shimadzu, Kyoto, Japan) equipped with an Rtx-2330 fused-silica capillary column of 30 m × 0.25 mm (10% cyanopropylphenyl, 90% biscyanopropyl polysiloxane, Restek, Bellefonte, PA, USA) and flame ionization detector at 250 °C and injector at 300 °C. The oven temperature was programmed from 130 to 230 at 4 °C/min and held for 10 min at 230 °C. High-purity nitrogen was used as carrier gas. The inlet pressure was 1.0 kg/cm^2, with a flow rate of 3.0 mL/min. The sample split was 50:1 (v/v). Peaks were identified by comparison with fatty acid standards (FAMEs, GLC-461, Nu-Chek Prep, Elysian, MN, USA) and quantified in reference to the peak area of the incorporated internal standard (methyl tricosanoate, C23:0).[20]

Purification Methods of the Sulfoglycolipids from Algae. Fifty grams of algal powder was extracted with 2 L of absolute ethanol and sonicated (Branson 8510, Danbury, CT, USA) for 60 min at room temperature under nitrogen. After centrifugation for 20 min at 9000*g* at 4 °C, the clear supernatant was removed, and residue was re-extracted twice, each time with 2 L of absolute ethanol. The supernatants were combined and concentrated to dryness under vacuum. The dried ethanol extract was dissolved in 80% ethanol and subjected to adsorption chromatography on a Dianion HP-20 column (Mitsubishi Chemical, Tokyo, Japan), followed by stepwise elution with 1000 mL of 80% ethanol and then 95% ethanol. The eluent was collected and lyophilized as the glycolipid fraction.[21] Then the glycolipid fraction was separated by DEAE-cellulose acetate (Whatman D52 Microgranular, Reeve Angel, Clifton, NJ, USA) column (2.5 × 30 cm),

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which was prewashed with chloroform/methanol (3:2, v/v). After successive elution of monogalactosyldiacylglycerol (MGDG) and digalactosyldiacylglycerol (DGDG) with chloroform/methanol (98:2 and 90:10, v/v), sulfoquinovosylglycolipids (SQDG) were collected with chloroform/methanol (4:1, v/v) containing 2% NaOH and 50 mM CH_3COONH_4. The salt was removed by washing with chloroform/methanol/water (1:1:1, v/v/v). The crude fraction was dried under nitrogen. The residue was solubilized in cold acetone, evaporated under nitrogen, and stored at −35 °C[10] until further use.

Thin Layer Chromatography (TLC) of SQDG. The glycolipid fractions were identified by TLC. The TLC plates (20 × 20 cm; 0.25 mm layer thickness) (Merck, Darmstadt, Germany) were activated by baking at 120 °C for 2.5 h. The samples were loaded as spots smaller than 0.5 cm in diameter and developed in a solvent system of dichloromethane/acetone/methanol/acetic acid/water (50:10:15:5, by volume) in a glass sealed TLC chamber at room temperature for about 90 min. The method was modified after Ramani et al.,[22] who used a solvent system of chloroform/methanol/0.02% $CaCl_2$ (60:40:9, by volume), but the TLC spots exhibited a tailing effect (Figure 1). The TLC-separated SQDG was visualized by using anthrone reagent (0.2% in concentrated H_2SO_4).

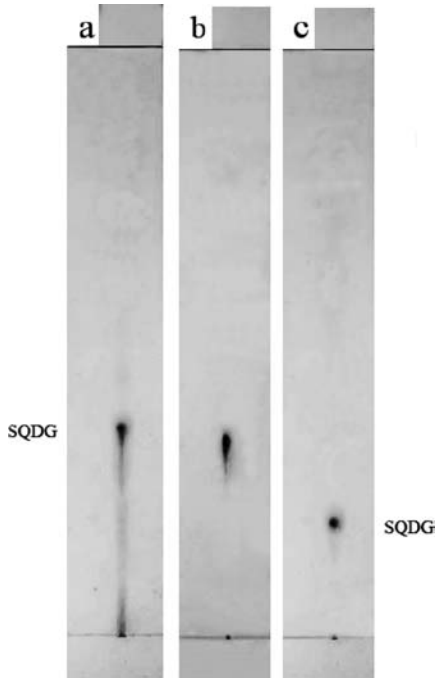

Figure 1. Thin-layer chromatogram of the SQDG isolated from *Porphyra crispata*: developing solvent (a) chloroform/methanol/0.02% $CaCl_2$ (60:40:9, by volume); (b) acetone/toluene/water (91:30:7, by volume); (c) dichloromethane/acetone/methanol/acetic acid/water (50:20:10:15:5 by volume), respectively. TLC was visualized with anthrone reagent (0.2% in concentrated H_2SO_4).

Quantitative Determination of SQDG. Algal SQDG was analyzed by a normal-phase HPLC using Shimadzu LC-10AD (Kyoto, Japan) equipped with an evaporative light scattering detector (ELSD 70 °C, 350 kPa, Sedex 75, Sedere, France).[23] A binary gradient system (Table 2) was used as mobile phase at a flow rate of 0.9 mL/min. The injection volume was 10−20 μL and the column temperature, 35 °C. The column was a LiChrospher 100 Diol (250 × 4 mm i.d.; particle size 5 μm, Merck, Darmstadt, Germany) with a LiChroCART 4-4 guard column. Peak identification and quantification of SQDG were calculated usingthe calibration curve of the authentic SQDG from spinach (Larodan Fine Chemicals AB, Malmö, Sweden).

MTT Assay. Human HepG2 cells (liver carcinoma cell line, BCRC 60025, Bioresources Collection and Research Center of the Food Industry Research and Development Institute, Hsin Chu, Taiwan) were cultured and maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% (v/v) fetal bovine serum (FBS), 1.5 μg/mL sodium bicarbonate, 1.0 mM sodium pyruvate, 100 IU/mL penicillin, and 100 μg/mL streptomycin and incubated with 5% CO_2 at 37 °C. The HepG2 cells (1 × 10^4) were seeded onto a 96-well plate

Table 2. HPLC Elution Program of the Binary Gradient Solvent System for SQDG Quantification[a]

time (min)	A (%)	B (%)
1	100	0
2	70	30
6	70	30
8	50	50
15	50	50
17	0	100
19	0	100
20	100	0

[a]A, dichloromethane; B, methanol/acetone/water/acetic acid (30:60:9:1 by volume).

Table 3. Lipid Content and Ratio of n-3/n-6 PUFA of Marine Macroalgae

species	code	lipid content (mg/g dry basis, mean ± SD)	n-3/n-6
Chlorophyta: green algae			
Ulva fasciata	G1	16.54 ± 1.38	1.05
Enteromorpha prolifera	G2	20.15 ± 3.71	2.10
Chaetomorpha antennina	G3	15.36 ± 2.44	0.64
Cladophora vagabunda	G4	18.66 ± 1.32	1.11
Codium mamillosum	G5	19.67 ± 1.95	1.10
Rhodophyta: red algae			
Porphyra crispata	R6	28.34 ± 2.98	4.11
Chondrus verrucosa	R7	24.86 ± 2.13	2.75
Chondracanthus intermedius	R8	19.42 ± 1.94	2.06
Grateloupia filicina	R9	21.97 ± 2.43	2.80
Carpopeltis maillardii	R10	20.40 ± 3.38	3.36
Plocamium telfairiae	R11	19.96 ± 2.11	1.99
Cheilosporum acutilobum	R12	22.42 ± 1.31	1.75
Corallina pilulifera	R13	21.04 ± 2.03	5.40
Gracilaria coronopifolia	R14	19.31 ± 1.55	0.08
Gracilaria chorda	R15	18.57 ± 1.48	0.04
Chondrophycus intermedius	R16	21.15 ± 1.95	2.36
Laurencia brongniartii	R17	21.87 ± 2.11	3.04
Centroceras clavulatum	R18	23.17 ± 2.31	1.83
Phaeophyceae: brown algae			
Sargassum crassifolium	B19	13.11 ± 2.31	0.81
Sargassum cristaefolium	B20	13.89 ± 2.68	0.66
Petalonia binghamiae	B21	19.56 ± 1.98	2.18

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Table 4. Major Fatty Acids (Milligrams per Gram Dry Basis) Characteristic of the 21 Marine Macroalgal Species Studied

fatty acid	green	red	brown
odd-numbered	**C17:0**[a]	**C17:0**	**C17:0**
	C17:1[b]	**C17:1**	**C17:1**
		C21:0[c]	
major FA (% total FA)			
saturated	**C16:0** (13.0−38.7%)	**C16:0** (18.4−36.5%)	**C16:0** (26.9−30.1%)
monoenoic	**C16:1n7** (1.7−8.8%)		
	C18:1n9 (16.1−24.0%)	**C18:1n9** (2.6−18.4%)	**C18:1n9** (5.7−13.3%)
polyenoic	**C18:2n6** (3.5−22.7%)	**C20:4n6** (35.1−45.7%)	**C18:4n3** (1.7 −16.4%)
	C18:3n3 (8.2 −18.4%)	**C20:5n3** (23.5−47.3%)	**C20:4n6** (9.5−11.3%)
			C20:5n3 (3.4 −12.3%)
total polyenoic (%)	21.8−46.6	30.4−58.4	25.9−72.5
n-3/n-6 ratio	0.64−2.10	1.75−4.11[d]	0.66−2.18
total (mg/g db)	13.82−18.34	16.50−26.55	11.88−21.09

[a]C17:0 was found in green algae (G1, G2, and G5), red algae (R6, R11, R12, R16, and R17), and brown algae (B19 and B20). [b]C17:1 was found in green algae (G1, G2, G4, and G5), red algae (R12 and R17), and brown algae (B21). [c]Found only in *Chondrus verrucosa* (R7) and *Chondracanthus intermedius* (R8). [d]All red algae except *G. coronopifolia* (R14) and *G. chorda* (R15).

and allowed to grow for 1 day before treatment with different concentrations (0−400 μg/mL) of the SQDG. After 48 h, the cell viability was measured on the basis of the reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) (MTT, Sigma, St. Louis, MO, USA) in mitochondria. The absorbance at 570 nm was measured[24] with a microplate reader (Tecan Sunrise Absorbance Reader, Software Magellan V.4, Crailsheim, Germany).

RESULTS AND DISCUSSION

Macroalgae Lipids. The 21 algae species from the three Divisions of Chlorophyta (green algae), Rhodophyta (red algae), and Phaeophyta (brown algae) are shown in Table 3. The algal lipid contents on dry basis (db) ranging from 13.11 to 28.34 mg/g db are similar to those of the 34 edible seaweed species previously analyzed, being about 20 mg/g db.[25] The red alga *Porphyra crispata* (R6) had the highest lipid content (28.34 mg/g db) followed by *Centroceras clavulatum* (R18, 23.17 mg/g db). No significant differences in lipid contents were found among the red alga classes. Among all of the algae studied, the lowest lipid level was found in brown algae *Sargassum crassifolium* (B19) and *Sargassum cristaefolium* (B20). Brown algae *Hizikia* sp. and Arame were also low in lipid content (7.0−9.0 mg/g db).[26] The 12 species of marine algae from the southeastern coast of India showed total lipid content ranged from 13.3 to 46.0 mg/g db, which varied with species, genetic origin, season, and biogeography.[27]

Odd-Carbon-Number Fatty Acids. Odd-numbered fatty acids C17:0 and C17:1 were present in all three divisions of algal species studied (Table 4), whereas C21:0 was found only in *Chondrus verrucosa* (R7) and *Chondracanthus intermedius* (R8) in minor quantities, being 1.3 and 0.2%, respectively. The odd-numbered fatty acids were most frequently found in microbes and lower plants.[28] They are formed through elongation of propionate or valerate and synthesized through incorporation of propionyl-CoA instead of acetyl-CoA.[29] Land plants normally contain fatty acids of only even-numbered carbon chains.[28]

Total PUFAs. The total PUFAs in the green, red, and brown algae ranged from 21.8 to 46.6%, from 30.4 to 58.4%, and from 25.9 to 72.5%, respectively. Green algae are characterized by the high contents of C18:2 and C18:3, similar to land plants; red algae consisted of C20:4 and C20:5 and brown algae, C18:4, C20:4, and C20:5 (Table 4).

PUFAs of the n-3 series were abundant in most of the red algae. The highest content was found in red alga *Corallina pilulifera* (R13), being 10.9 mg/g db, whereas *G. coronopifolia* (R14) and *G. chorda* (R15) were rich in n-6 PUFAs. The green alga G2 and brown alga B21 had higher levels of n-3 PUFAs and n-3/n-6 ratio >2 in their respective divisions.

The n-3/n-6 ratio was ≥1 in 16 species of marine algae (Table 3). The highest ratio was found in *P. crispata* (R6), of which the n-3/n-6 ratio was 4.11, whereas its lipid content was also the highest (28.34 ± 2.98 mg/g db). Anthropological and epidemiological studies and studies at the molecular level suggest that human beings evolved on a diet with a ratio of n-3 to n-6 essential fatty acids (EFA) of approximately 1. At present, the Western diet is relatively deficient in n- 3 fatty acids and high in n- 6 fatty acids, resulting in n-3/n-6 ratios in the range of 1:10−30.[30] Increases in the ratio of n-3/n-6 PUFA and availability of eicosapentaenoic acid (EPA) are beneficial to human health. Even in aquatic animals, such as freshwater clams, filter-fed microalgae containing high EPA showed higher growth rate than those fed microalgae of low EPA content.[31] EPA or n-3 PUFA has been generally supplied by fish oil in human diets or animal feeds. The red algae (R6) having high EPA content may be used to substitute for fish oil.

Glycolipids Content of Algae. *P. crispata* was extracted for total lipids using absolute ethanol and fractionated to obtain glycolipids by hydrophobic column chromatography then to isolate sulfoglycolipids by ion exchange chromatography followed by purification of SQDG with acetone precipitation. Yields at each step are summarized in Table 5. The purity of

Table 5. Yield of SQDGs Isolated and Purified from *Porphyra crispata* (R6)

purification step	amount (g)	yield % dry basis	yield % total lipid
biomass (dry)	50.000	100.000	
ethanol extract	1.450	2.900	100.00
adsorption chromatography (HP-20), glycolipid fraction	0.287	0.574	19.79
ion-exchange chromatography (DEAE)	0.012	0.024	0.83
acetone precipitation, SQDG	0.010	0.020	0.69

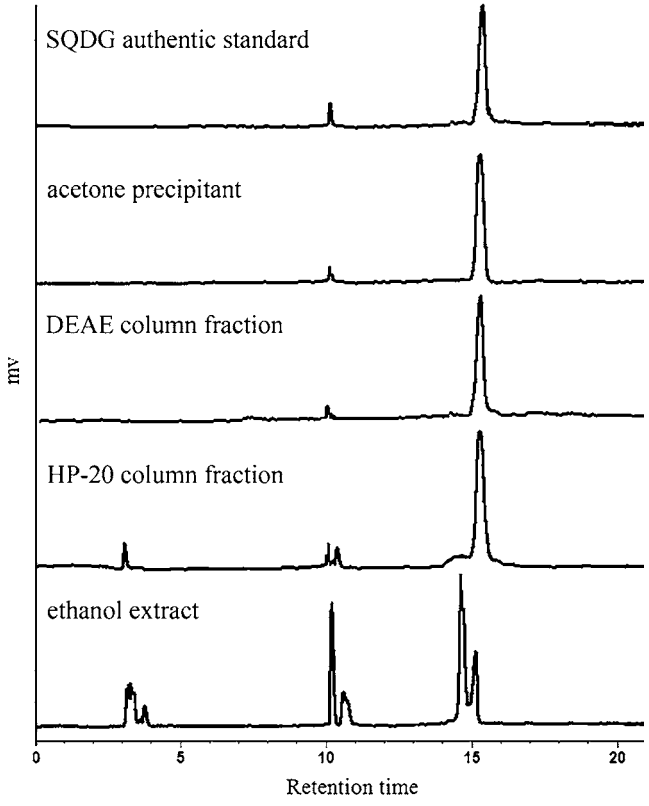

Figure 2. HPLC chromatogram of the SQDG purified from *Porphyra crispata* by ethanol extraction, HP-20, DEAE-cellulose acetate column chromatography, and acetone precipitation in comparison to the spinach SQDG authentic standard.

SQDG after each purification step was examined by TLC (Figure 1) and HPLC-ELSD (Figure 2). The pure SQDG showed a single spot with R_f value of 0.23 and a single peak at the retention time of 15 min. The SQDG authentic standard was also detected by TLC and HPLC, respectively. The sample and the standard showed the same R_f by TLC and the same retention time by HPLC-ELSD.

The glycolipid fraction contributed 19.79% of the total lipids of *P. crispata* (Table 5), whereas the two species of red algae reported by El-Baroty et al.[32] contained glycolipids ranging from 11.1 to 22.5% of total lipids. In the 14 species of red algae sampled from the Black Sea, the glycolipids content ranged from 1.64 to 3.18% of total lipids.[33]

The amount of SQDG content in the glycolipids fraction was 5.74 mg/g of dried biomass of the algae (Table 5). In the eight species of vegetables reported by Kuriyama et al.[21] SQDG content ranged from 0.046 to 0.824 mg/g db, whereas the red alga *Porphyridium cruentum* contained about 1.5 mg/g db.[10] Therefore, red algae are richer in SQDG than vegetables.

Table 6. Fatty Acid Profile of Algal SQDG and Authentic Standard of Spinach SQDG and the IC_{50} against HepG2

fatty acid	alga[a] mg/g SQDG	alga[a] % total FA	spinach[b] % total FA
saturated	250.2	40.2	28
C16:0	207.6	33.3	28
C18:0	42.6	6.8	
monoenoic	46.8	7.5	5
C18:1 (cis-9)	46.8	7.5	3
polyenoic	325.9	52.4	67
n-3	213.4	34.3	
C20:3 (cis-11,14,17)	8.1	1.3	
C20:5 (cis-5,8,11,14,17)	205.3	33.0	
n-6	112.5	18.1	67
C18:2 (cis-9,12)	17.4	2.8	6
C18:3 (cis-6,9,12)			61
C20:3 (cis-8,11,14)	16.2	2.6	
C20:4 (cis-5,8,11,14)	78.9	12.7	
n-3/n-6	1.9	1.9	0
total	622.9	100	100
IC_{50} (μg/mL)	126		255

[a] *Porphyra crispata*. [b] Cited from Larodan Fine Chemicals (Malmoe, Sweden) and Maeda et al.[9]

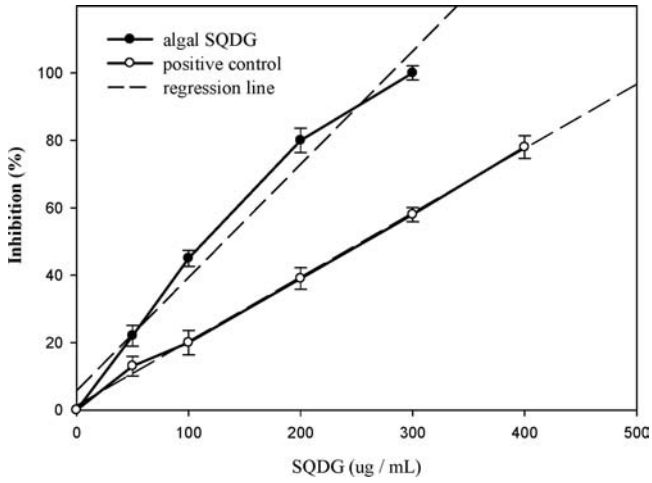

Figure 3. Inhibitory effect of SQDGs from *Porphyra crispata* and the spinach SQDG authentic standard as positive control against HepG2 cell activity. Results are expressed as the mean ± standard deviation (n = 3).

Fatty Acid Composition of SQDG. The glycolipid fraction of *P. crispata* was separated into three major glycolipids: monogalactosyldiacylglycerol (MGDG), digalactosyldiacylglycerol (DGDG), and sulfoquinovosyldiacylglycerol (SQDG). The fatty acid compositions of SQDG are shown in Table 6. Eight fatty acids were identified in algal SQDG, which was found to be rich in palmitic acid (C16:0) 33.3% and EPA 33.0% of total fatty acids similar to those reported on red algae.[33−35] Arachidonic acid (AA, C20:4) contributed 12.7% of total fatty acids. The SQDG authentic standard isolated from spinach (Larodan Fine Chemicals AB) consisted of two major fatty acids, γ-linolenic acid (C18:3, 61%) and C16:0 (28%).

Cytotoxic Activity of Algal SQDG. Algal SQDG was assessed in the range of 0−400 μg/mL on its inhibition against

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human hepatic carcinoma HepG2 cells in comparison to the spinach SQDG authentic standard (STD) as positive control. The HepG2 viability after 48 h of incubation is shown in Figure 3. The inhibitory effects on HepG2 cells by SQDG and STD were both dose-dependent, but algal SQDG was more potent against proliferation of HepG2 cells, with an IC_{50} of 126 μg/mL, than that of the spinach STD, being 255 μg/mL. The results on HepG2 are in agreement with that of the SQDG of *P. cruentum*, which was inhibitory to human colon adenocarcinoma DLD-1, breast adenocarcinoma MCF-7, prostatic adenocarcinoma PC-3 cells, and malignant melanoma cells M4-Beu with IC_{50} values in the range of 20−46 μg/mL, whereas the IC_{50} of synthetic SQDGs against proliferation of colon carcinoma DLD-1 cells was 94−250 μg/mL.[10]

The algal SQDG and the authentic STD differed mainly in their unsaturated fatty acids composition. The algal SQDG had EPA and AA, whereas the spinach SQDG did not have the highly unsaturated fatty acids. EPA was considered to be very important to the cytotoxic activity. Similar results were found by Eitsuka et al.,[36] who found that SQDG from *Porphyra yezoensis* inhibited the activity of human telomerase. The SQDG consisted of EPA-inhibited telomerase activity much more effectively then the SQDG containing linolenic acid, linoleic acid, and oleic acid. The potency of SQDGs on tumor inhibition might depend on their esterified FA. The cancer chemopreventive activity of SQDGs was attributed to the inhibition of DNA polymerases and telomerase, leading to apoptosis in tumor cells.[11,36−38] However, how the SQDGs are metabolized and absorbed in vivo to exert the inhibitory effect on hepatocarcinoma cell still needs further investigation.

Conclusion. *P. crispata* (R6) appears to have the potential to be a natural source of bioactive lipids due to its high proportion of PUFAs and EPA and its n-3/n-6 ratio in addition to its sulfolipid structure and content. Aside from the essential sulfate moiety of SQDG, the EPA and AA moieties seemed to be more critical than the C18:3 on its potency of carcinoma inhibition.

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Notes

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