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Evaluation of Antioxidative Activity of Extracts from a Brown Seaweed, *Sargassum siliquastrum*

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Antioxidative activities of the extracts from *Sargassum siliquastrum* were determined using the inhibition of red blood cell (RBC) hemolysis induced by 2,2′-azobis(2-amidinopropane) dihydrochloride (AAPH) radicals, suppression of lipid peroxidation using rat brain homogenate, and scavenging activity of superoxide radicals. The dichloromethane fraction isolated from the methanol crude extract by differential solvent extractions exhibited the strongest antioxidant activity in both RBC hemolysis and lipid peroxidation assays. This fraction was further fractionated into four subfractions F1−F4 by silica gel column chromatography. F1 was found to be most effective in protecting RBC against AAPH radicals and in inhibiting lipid peroxidation. On the basis of thin-layer chromatography and UV and IR spectra analyses, all subfractions contained phenolic compounds. However, there was no correlation between the above antioxidant potency and total phenolic compounds estimated by using the Folin−Ciocalteau method.

KEYWORDS: Antioxidant; brown seaweed; phenolic compounds; *Sargassum siliquastrum*

INTRODUCTION

Lipid peroxidation is a major cause of many pathological effects such as cardiovascular disease, cancer, and brain dysfunction as well as aging processes. It also leads to the development of food rancidity and off-flavors. Reactive oxygen species such as superoxide anions, hydroxyl radicals, and hydrogen peroxide are important mediators for the initiation of lipid peroxidation, which occurs mainly in biomembranes where the content of unsaturated fatty acids is relatively high, leading to destruction of the cellular membrane, and is related to the development of many chronic diseases (*1*). Epidemiological studies have found that intake of antioxidants such as vitamin E and vitamin C reduced the risk of coronary heart disease, stroke, and cancer (*2*, *3*). Antioxidant is an inhibitor of lipid peroxidation. Therefore, consumption of antioxidants plays an important role in protecting against these events. Several synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and butylated hydroxyquinone (TBHQ) are commercially available and currently used; however, their safety and toxicity are some problems of concern (*4*, *5*). Hence, the development of alternative antioxidants from natural origins has drawn more and more attention. Many investigators have found different types of antioxidants in various kinds of land plants (*6*). Tocopherol, vitamin C, carotenoid, and flavonoid are good sources of antioxidants found in fresh fruits and vegetables. Plant phenolic compounds are known to possess antioxidant activity and have the ability to scavenge reactive oxygen species and free radicals. The phenolic compounds from berries were demonstrated to exhibit antioxidant activity on human low-density lipoprotein (LDL) and liposome oxidation (*7*). The phenolic antioxidants from the spice *Aframomum danielli* were shown to be more effective than BHT and α-tocopherol in stabilizing refined peanut oil (*8*).

Seaweeds are considered to be a rich source of antioxidants (*9*, *10*). Recently, the active antioxidant compounds were identified as fucoxanthin in *Hijikia fusiformis* (*11*) and phlorotannins in *Sargassum kjellamanianum* (*12*). We have screened 13 species of seaweed commonly found in the coastal waters of the South China Sea for their antioxidant activity (*13*). Among them, the crude extracts of the brown seaweed, *Sargassum siliquastrum*, had the strongest antioxidant activity. Therefore, in this study, further work on the isolation and identification of the potent antioxidative compounds in *S. siliquastrum* is reported. Antioxidant activities of the isolated fractions from *S. siliquastrum* were evaluated, and attempts were made to correlate the phenolic content with the antioxidant activities of the fractions.

MATERIALS AND METHODS

Extraction and Isolation of Antioxidant Components from *S. siliquastrum*. *S. siliquastrum* collected from Tung Ping Chau Island, Hong Kong, was rinsed with fresh water, frozen, and lyophilized. Dry seaweed sample (10 g) was ground into small pieces by a Waring blender and extracted with methanol (150 mL) in a Soxhlet extractor for 6 h. The extraction was repeated twice. The combined methanolic extracts were evaporated under reduced pressure to a dark green semisolid and dissolved in distilled water. The aqueous suspension was partitioned sequentially in three different solvents, dichloromethane (DCM), ethyl acetate, and *n*-butanol, to fractionate the polar and

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nonpolar compounds in the methanol crude extract. The resulting four extracts were evaporated to dryness in a rotary evaporator to give the dichloromethane [6.42% dry weight (DW) of seaweed], ethyl acetate (0.35% DW of seaweed), butanol (0.87% DW of seaweed), and water (2.41% DW of seaweed) fractions. They were kept in the dark and stored at 4 °C under nitrogen.

The dichloromethane extract was further purified because it showed the highest activity in subsequent antioxidant assays. The dichloromethane fraction (100 mg/mL) was applied to a column (1.7 × 20 cm) packed with silica gel 60 (70−230 mesh, Merck). Elution was performed with chloroform/methanol in the following ratios: 99:1, 95:5, 90:10, 80:20, and 50:50 successively. The eluates were combined according to thin-layer chromatography (TLC) analytical results to produce four subfractions, F1, F2, F3, and F4. Solvent in each subfraction was removed under a stream of nitrogen gas, and the antioxidant activity of the residue in each subfraction was determined.

Hemolysis Assay. Male Sprague−Dawley rats (200 g) were used in this experiment. Blood obtained from the posterior vena cava of the animals was collected into a heparinized tube. The red blood cells (RBC) were centrifuged and washed three times with phosphate-buffered saline (PBS; 125 mM NaCl and 10 mM sodium phosphate buffer, pH 7.4). The plasma and buffy coat were carefully removed by aspiration after each wash. During the last washing, RBC were centrifuged at 1000*g* for 10 min to obtain an evenly packed cell preparation. Washed RBC were finally resuspended in PBS to obtain a 20% RBC suspension.

Hemolysis of RBC mediated by 2,2′-azobis(2-amidinopropane) dihydrochloride (AAPH; a peroxyl radical initiator) was measured according to the method of Miki et al. (*14*). A portion of RBC suspension (0.1 mL) was mixed with 0.1 mL of PBS solution containing different concentrations of seaweed solvent fractions and subfractions (F1−F4). An aliquot of 200 mM AAPH (0.2 mL, in PBS) was then added to the mixture. The reaction mixture was shaken and incubated in a water bath at 37 °C for 3 h. After incubation, one of the reaction mixtures was diluted with 8 mL of PBS (A), and the other was diluted with 8 mL of distilled water (B) to induce hemolysis. Both reactions were centrifuged at 1000*g* for 10 min. The absorbance of the supernatants from A and B (A_{abs} and B_{abs}, respectively) at 540 nm was recorded by a spectrophotometer (Milton Roy Spectronic 3000). Vitamin C was used as a control in this experiment. The percent inhibition was calculated by using the following equation:

$$\% \text{ inhibition} = (1 - A_{abs}/B_{abs}) \times 100$$

Lipid Peroxidation Assay. The brain was removed quickly from male Sprague−Dawley rats weighing 150 g and washed with ice-cold 20 mM Tris-HCl buffer (pH 7.4). The brain was homogenized in 2 volumes of ice-cold Tris-HCl buffer using a Polytron PT 3000 homogenizer, followed by centrifugation at 3000*g* for 10 min. The supernatant was used for the study of lipid peroxidation.

Lipid peroxidation was assayed using the formation of malondialdehyde (MDA) as an indicator. The effect of different seaweed fractions and subfractions on rat brain homogenate induced by Fe^{2+}/ascorbate-stimulated lipid peroxidation was determined according to the method of Liu et al. (*15*). The reaction mixture was composed of 0.1 mL of brain homogenate, 0.1 mL of 10 μM $FeSO_4$, 0.1 mL of 0.1 mM ascorbic acid, and 0.2 mL of seaweed fractions with different concentrations. The mixture was incubated at 37 °C for 1 h. The reaction was stopped by adding trichloroacetic acid (TCA) and thiobarbituric acid (TBA). The mixture was then heated at 80 °C for 20 min. After cooling, centrifugation was carried out for 10 min. The absorbance of the MDA−TBA complex in the supernatant was determined at 532 nm by a spectrophotometer. Butylated hydroxyanisole (BHA) was used as the control in this experiment.

Superoxide Anions Scavenging Activity. Superoxide anions were generated in a nonenzymatic system (*15*). The influence of extracts on the generation of superoxide was evaluated by spectrophotometric measurement of the product on reduction of nitroblue tetrazolium (NBT). The reaction medium contained 16 mM Tris-HCl buffer (pH 8.0), 78 μM β-nicotinamide adenine dinucleotide (reduced form, NADH), 50 μM NBT, 10 μM phenazine methosulfate (PMS), and seaweed extracts with different concentrations. The blank sample contained no NADH. The reaction mixture was measured at the absorbance at 560 nm by spectrophotometer, against the blank sample. Vitamin C and glucose were used as positive and negative controls, respectively, in this experiment.

Thin-Layer Chromatography. TLC was performed on a silica gel plate (5 × 20 cm, Kieselgel 60F, 0.25 mm, Merck). An aliquot of each fraction was spotted on the silica gel plate with a solvent system of chloroform/ethanol/acetic acid/water (98:10:2:2 v/v). The spots were visualized by spraying the plates with two spraying solutions (*8*).

Spray 1 was a 1% solution of potassium ferricyanide in water and a 1% solution of ferric chloride in water. Blue color spots produced indicate that the fractions may be phenolic compounds.

Spray 2 was an ammoniacal silver nitrate solution. Thirty milliliters of ammonium hydroxide was mixed with 70 mL of water. Silver nitrate solution (3.4 g/100 mL of water) was then added to the solution. The sprayed plate was heated for 10 min at 105 °C. Brown, black, and gray spots produced indicate that they are reducing compounds.

Infrared Spectra (IR). The infrared spectra (500−4000 cm^{-1}) of all the subfractions (F1−F4) were recorded in potassium bromide (KBr) disks with a Fourier transform IR spectrophotometer (Nicolet Magna 750). One milligram of dry sample was mixed with 100 mg of dry KBr, and the mixture was pressed into a disk.

Ultraviolet and Visible (UV−Vis) Spectrophotometry. UV−vis absorption of all the subfractions (F1−F4) dissolved in ethanol was recorded in a spectrophotometer. The bathochromic shift of the absorption maxima was determined by adding 2 drops of a 2 M solution of sodium hydroxide to the sample solutions in a cuvette (*16*).

Determination of Total Phenolics. The concentration of total phenolic compounds in the fractions was determined spectrophotometrically using Folin−Ciocalteu reagent (*17*). The seaweed fraction (0.1 mL) was diluted with deionized water (7.9 mL). Folin−Ciocalteu phenol reagent (0.5 mL) was added, and the contents were mixed thoroughly. After 1 min, 1.5 mL of 20% sodium carbonate solution was added, and the mixture was mixed thoroughly. The mixture was allowed to stand for 1 h. The absorbance of the blue color produced was measured with a spectrophotometer at 750 nm. Phenolic content was expressed in milligrams per gram of dry weight (seaweed fraction) based on a standard curve of gallic acid (GA), which was expressed as milligrams per gram of gallic acid equivalent (GAE).

Statistics. All results are expressed as mean ± SEM of three different trials and analyzed with SigmaStat software. Analysis of variance (ANOVA) and the Student−Newman−Keuls test were used to assess significant differences ($p < 0.05$) between fractions.

RESULTS AND DISCUSSION

Antioxidant Activity. Three assays, namely, inhibition of RBC hemolysis, suppression of lipid peroxidation using rat brain homogenate, and scavenging of superoxide radicals, were used to measure the antioxidant activity of four fractions isolated by differential solvent extraction from methanol crude extract and the subfractions (F1−F4) obtained by column chromatography from the dichloromethane fraction of *S. siliquastrum*.

Among the four fractions isolated by differential solvent extraction, the dichloromethane fraction appeared to possess the highest antioxidant activity ($p < 0.05$) in hemolysis assay initiated by AAPH to protect RBC (**Table 1**) at a concentration of 10 μg/mL. AAPH is a peroxyl radical initiator that generates free radicals by its thermal decomposition and will attack the RBC to induce the chain oxidation of lipid and protein, disturbing the membrane organization and eventually leading to hemolysis (*14*). The dichloromethane fraction was also found to be the most effective in inhibiting Fe^{2+}/ascorbate-induced lipid peroxidation in rat brain homogenate (**Table 2**). The activity of the dichloromethane fraction was observed to be significantly higher ($p < 0.05$) than that of the crude methanol extract and the control, BHA, in the above two assays. The water fraction did not show any protective effect on RBC hemolysis

Table 1. Inhibitory Effects of Different Fractions from the Methanol Crude Extract of *S. siliquastrum* Separated by Different Solvents on Hemolysis of Rat RBC Initiated by AAPH

	hemolysis inhibition[a] (%)			
fraction	0.4 μg/mL	2 μg/mL	10 μg/mL	50 μg/mL
methanol	8.80 ± 0.97a	12.9 ± 1.45a	54.2 ± 2.63f	42.9 ± 1.53d
DCM	5.03 ± 2.12a	11.8 ± 1.26ab	78.6 ± 0.11a	9.85 ± 3.64a
ethyl acetate	8.80 ± 0.97a	8.86 ± 1.18ab	45.7 ± 2.10e	46.1 ± 1.49d
butanol	1.65 ± 0.61a	2.88 ± 0.24b	7.33 ± 2.07b	28.5 ± 2.01c
water	2.62 ± 0.20a	4.34 ± 0.32ab	11.1 ± 0.77bc	13.5 ± 1.88a
BHA	3.61 ± 1.73a	6.72 ± 0.24ab	16.3 ± 2.07c	51.5 ± 0.60b
vitamin C	4.12 ± 0.78a	6.50 ± 1.74ab	22.2 ± 3.71d	44.3 ± 3.52d

[a] Each value is the mean ± SEM of three replicates. Means within each column with different letters (a–f) differ significantly ($p < 0.05$).

Table 2. Inhibitory Effects of Different Fractions from the Methanol Crude Extract of *S. siliquastrum* Separated by Different Solvents on Fe^{2+}/Ascorbate-Induced Lipid Peroxidation in Rat Brain Homogenate

	lipid peroxidation inhibition[a] (%)			
fraction	0.016 μg/mL	0.08 μg/mL	0.4 μg/mL	2 μg/mL
methanol	3.59 ± 0.65b	7.98 ± 1.79b	9.06 ± 2.35b	96.1 ± 0.10a
DCM	23.9 ± 3.90ac	86.1 ± 3.24a	97.9 ± 0.25a	98.3 ± 0.19a
ethyl acetate	24.5 ± 1.18a	28.1 ± 3.49c	33.4 ± 1.54c	97.6 ± 0.55a
butanol	2.05 ± 0.50b	2.83 ± 0.29b	7.97 ± 1.59b	24.8 ± 2.73c
water	1.34 ± 0.45b	1.63 ± 0.55b	4.51 ± 1.47b	5.22 ± 1.41b
BHA	20.3 ± 0.97c	30.1 ± 3.59c	32.0 ± 3.95c	96.2 ± 0.44a

[a] Each value is the mean ± SEM of three replicates. Means within each column with different letters (a–f) differ significantly ($p < 0.05$).

Table 3. Inhibitory Effects of Different Fractions of Dichloromethane Fraction from *S. siliquastrum* Separated by Silica Gel Column Chromatography on Hemolysis of Rat RBC Initiated by AAPH

	hemolysis inhibition[a] (%)			
fraction	0.4 μg/mL	2 μg/mL	10 μg/mL	50 μg/mL
DCM	5.03 ± 2.12a	11.8 ± 1.26a	78.6 ± 0.11a	9.85 ± 3.64b
F1	6.89 ± 0.73a	11.4 ± 3.89a	57.0 ± 2.63d	81.8 ± 3.39a
F2	5.13 ± 1.00	9.67 ± 1.51a	39.7 ± 2.82c	69.0 ± 4.57e
F3	4.46 ± 1.25a	14.1 ± 1.82a	75.6 ± 4.83a	2.58 ± 0.52b
F4	10.5 ± 1.26a	11.4 ± 0.79a	59.3 ± 1.30d	7.11 ± 0.46b
BHA	3.61 ± 1.73a	6.72 ± 0.24a	16.3 ± 2.07b	51.5 ± 0.60d
vitamin C	4.12 ± 0.78a	6.50 ± 1.74a	22.2 ± 3.71b	44.3 ± 3.52c

[a] Each value is the mean ± SEM of three replicates. Means within each column with different letters (a–e) differ significantly ($p < 0.05$).

and was the weakest one in preventing lipid peroxidation (**Tables 1** and **2**). This suggested that the potent antioxidant components seemed to be more soluble in a nonpolar solvent, such as dichloromethane.

Because the dichloromethane fraction had the highest antioxidant activity from the above two assays, it was further purified using liquid column chromatography. **Tables 3** and **4** show the antioxidative effects of four subfractions (F1−F4) on RBC hemolysis and lipid peroxidation assays, respectively. It was found that F1 and F2 were more effective as antioxidants in the protection of RBC hemolysis than the dichloromethane fraction itself at a concentration of 50 μg/mL, but the effect was more pronounced in F1. F1 and F2 showed a concentration-dependent manner in the hemolysis assay (**Table 3**). Although F3 showed the highest activity at a concentration of 10 μg/mL, which was similar to the dichloromethane fraction, F1 had the most significant contribution to protect RBC against AAPH radical, as F3 would be toxic to RBC at a higher concentration (**Table 3**).

Table 4. Inhibitory Effects of Different Fractions of Dichloromethane Fraction from *S. siliquastrum* Separated by Silica Gel Column Chromatography on Fe^{2+}/Ascorbate-Induced Lipid Peroxidation in Rat Brain Homogenate

	lipid peroxidation inhibition[a] (%)			
fraction	0.016 μg/mL	0.08 μg/mL	0.4 μg/mL	2 μg/mL
DCM	23.9 ± 3.90c	86.1 ± 3.24a	97.9 ± 0.25a	98.3 ± 0.19a
F1	63.0 ± 7.22a	94.1 ± 0.70a	95.7 ± 0.04a	97.6 ± 0.12a
F2	7.40 ± 2.03b	22.9 ± 3.68bc	40.3 ± 5.21b	84.1 ± 5.89a
F3	11.6 ± 1.74bc	17.3 ± 4.20b	96.0 ± 0.58a	98.2 ± 0.10a
F4	13.1 ± 1.38bc	23.1 ± 1.41bc	95.0 ± 0.32a	97.8 ± 0.32a
BHA	20.3 ± 0.97c	30.1 ± 3.59c	32.0 ± 3.95b	96.2 ± 0.44a

[a] Each value is the mean ± SEM of three replicates. Means within each column with different letters (a–c) differ significantly ($p < 0.05$).

Concerning lipid peroxidation assay, F1 exhibited >60% inhibition of lipid peroxidation at a concentration of 0.016 μg/mL. This was significantly ($p < 0.05$) higher than that of the dichloromethane fraction. F3 and F4 had only moderate inhibitory activity on lipid peroxidation (**Table 4**). This indicated that F1 isolated from *S. siliquastrum* was the most effective subfraction in inhibiting lipid peroxidation.

All of the fractions isolated from solvent extraction and column chromatography had little effect on superoxide radical scavenging activities (data not shown).

Antioxidants can be classified into two groups, namely, primary or chain-breaking antioxidants and secondary or preventive antioxidants. F1 isolated from *S. siliquastrum* exerted strong protection of RBC against hemolysis by peroxyl radical and had potent inhibition of rat brain lipid peroxidation induced by Fe^{2+}/ascorbate. However, it had very little effect on superoxide radical scavenging activity, suggesting its role as a chain-breaking antioxidant. It might break the chain reaction of lipid peroxidation by rapid donation of electrons to lipid peroxyl radicals, terminating the chain reaction by forming a corresponding radical that was less reactive than the peroxyl radical and thus slowing the process of peroxidation. Tocopherol is an example of a chain-breaking antioxidant, as evidenced by the regeneration of tocopherol from its radical by ascorbic acid in vitro (*1*). Hence, F1 might be useful in the food industry to protect against lipid peroxidation in foods because the low polarity of the chemical components in this subfraction could be readily dissolved in the lipid fraction of the food.

Identification of Phenolic Compounds. Tentative identification of antioxidative compounds from *S. siliquastrum* was based on TLC, IR spectra, and UV−vis spectra.

The four fractions isolated from the methanol crude extract by solvent extraction were separated by using TLC. Two blue and two gray spots were observed in the methanol, dichloromethane, and ethyl acetate fractions after spraying with spray solutions 1 and 2, respectively (data not shown). This indicated the presence of phenolic compounds with reducing properties in these three fractions.

Table 5 shows the R_f values and colors of spots from the TLC chromatograms of the four subfractions after spraying with spray solution 1. F1 and F3 each contained one phenolic compound with R_f values of 0.88 and 0.78, respectively, whereas F4 appeared to contain two phenolic compounds with R_f values of 0.82 and 0.76 (**Table 5**). These spots also produced gray color after spraying with spray solution 2. Therefore, on the basis of TLC analysis, at least four types of phenolic compounds with reducing properties were identified from F1, F3, and F4 of *S. siliquastrum* having higher antioxidant activity than that of BHA.

Table 5. R_f Value and Color of Spots Separated on TLC Plate from the Different Subfractions of the Dichloromethane Extract of *S. siliquastrum*

	spray 1		spray 2	
fraction	color	R_f	color	R_f
DCM	blue	0.90, 0.79	gray	0.88, 0.78
	yellow	0.87	yellowish green	0.85
F1	blue	0.88	gray	0.86
F2				
F3	blue	0.78	gray	0.76
	yellow	0.84	yellowish green	0.83
F4	blue	0.82, 0.76	gray	0.80, 0.77
	orange	0.87	orange	0.86

Table 6. UV Spectral Data of Different Subfractions Isolated from the Dichloromethane Fraction of *S. siliquastrum*

fraction	λ_{max} (nm)	bathochromic shift[a] λ_{max} (nm)
DCM	298*, 410, 667	314*, 401, 665
F1	298*, 404	314*, 401
F2	296*	307*
F3	298*	314*
F4	298*, 450	315*, 426, 450

[a] When bathochromic shift reagent (sodium hydroxide) was added, the wavelength of the absorption maxima of a peak corresponding to a phenolic compound (*) would shift to a longer λ.

According to the UV−vis spectral data (**Table 6**), the four subfractions isolated from the dichloromethane extract contained different components despite some fractions containing similar absorption maxima. Modifying reagents such as sodium hydroxide (NaOH) confirmed that the peak corresponded to phenolic compound as shifting of the wavelengths of absorption maxima to a longer ones was observed. This effect is known as a bathochromatic shift caused by the ionization of the phenol with the production of the corresponding phenolate ion (*16*). The present study suggested that the absorption maxima of F1, F3, and F4 had a bathochromatic shift (16 nm) with NaOH, confirming the presence of phenolic compound(s).

The presence of a hydroxyl group (3415 cm^{-1}) and an aromatic ring (1260, 1620, 2970, and 2850−2970 cm^{-1}) in the IR spectrum (figure not shown) of the four subfractions also suggested the presence of phenolic compounds. However, it was difficult to use the spectra to interpret the structure of the compounds in the fraction unless they were compared with an authentic standard.

Table 7 shows the total phenolic content (determined by using the Folin−Ciocalteu method) of the various fractions isolated from *S. siliquastrum* with F3 having the highest amount of phenolic compounds. However, we found that there was no positive correlation between the total phenolic content and the antioxidant activities of the various subfractions obtained from the dichloromethane extract at concentrations of 0.08 and 50 μg/mL in both the lipid peroxidation assay ($r = -0.1579$) and hemolysis assay ($r = -0.8351$), respectively.

A number of studies have shown that marine seaweed extracts and their polyphenols have antioxidant activity. The phenolic compounds in brown algae are derived from phloroglucinol and its derivatives (*18*). Yan et al. (*11*) reported that the major active compound from a brown seaweed, *Hijikia fusiforms*, was identified as fucoxanthin. Cahyana et al. (*10*) showed that pyropheophytin *a*, which was one of the chlorophyll *a*-related compounds in *Eisenia biscyclis*, had an antioxidative activity higher than that of α-tocopherol using the TBA method.

Table 7. Polyphenol Content of Different Solvent Fractions from the Methanol Crude Extract and Subfractions of the Dichloromethane Fraction from *S. siliquastrum*[a]

fraction	GAE
methanol crude extract	51.0 ± 2.15a
DCM	62.2 ± 0.86b
ethyl acetate	64.1 ± 0.33b
butanol	33.0 ± 1.64c
water	28.9 ± 3.71c
F1	50.7 ± 1.41a
F2	29.8 ± 1.07c
F3	80.0 ± 0.95d
F4	71.2 ± 3.75e

[a] Total polyphenol is expressed as gallic acid equivalents (GAE; mg/g of GAE). Each value is the mean ± SEM of three replicates. Means within the column with different letters (a–e) differ significantly ($p < 0.05$).

Recently, phlorotannin was isolated as an active antioxidant component from *Sargassum kjellmanianum* and *Ecklonia stolonifera* (*12*, *19*). We also found that the chemical components in the subfractions of *S. siliquastrum* contained an aromatic ring, hydroxyl groups, and an aromatic ether linkage from their IR spectra (data not shown), which could be identified as phenolic compounds. However, these subfractions displayed different degrees of antioxidative activities in various assays, suggesting that several chemical components might be involved in the antioxidative activities of the *S. siliquastrum* extract.

Conclusion. Four subfractions were isolated from the dichloromethane fraction by column chromatography. F1, F3, and F4 were positively identified to have phenolic compounds on the basis of TLC analysis, bathochromatic shift in UV spectra, and the presence of a hydroxyl group and an aromatic ring in their IR spectra. However, the total phenolic content did not show a positive correlation with their antioxidant activity, which might be due to the interference of some chemical species present in the fractions that affect the specificity of the Folin−Ciocalteau method.

The four isolated subfractions exhibited potent antioxidative activities. They all showed excellent activity in protecting RBC against hemolysis and inhibiting lipid peroxidation, with F1 being the most effective. They had relatively higher antioxidant activity than BHA and vitamin C in the hemolysis assay, but F3 and F4 showed some toxic effect to RBC at high concentration. They also had exceptionally higher antioxidant activity than BHA in the lipid peroxidation assay with the exception of F2, which had an activity comparable to that of BHA.

The present study demonstrated that F1 from *S. siliquastrum* contained the most potent antioxidant compound, which is probably a nonpolar chain-breaking antioxidant. There is no report so far on the study of the antioxidant activity of the methanol extract of *S. siliquastrum* and its active components. The present findings would be useful in leading to further work on the identification of the active components and the development of therapeutic products to protect against certain diseases, subject to toxicity evaluation. In addition, F1 has a potential to be used as a natural antioxidant in the food industry to stabilize especially high-fat foodstuffs by retarding lipid oxidation to extend shelf life.

ABBREVIATIONS USED

AAPH, 2,2′-azobis(2-amidinopropane) dihydrochloride; BHA, butylated hydroxyanisole; BHT, butylated hydroxytoluene; DCM, dichloromethane; MDA, malondialdehyde; PBS, phos-

phate-buffered saline; RBC, red blood cell; TBA, thiobarbituric acid; TBHA, butylated hydroxyquinone.

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