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Antioxidant Activities of Chlorophyta and Phaeophyta from Jeju Island

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Antioxidative activities of Chlorophyta and Phaeophyta in Jeju Island were measured by superoxide anion ($O_2^{\cdot-}$), hydroxyl radical ($HO^{\cdot}$), hydrogen peroxide (H_2O_2) and DPPH free radical scavenging assays. Methanolic and aqueous extracts of the seaweeds were prepared at both temperatures, higher (70°C) and room temperature (20°C), and screened for the construction of an extract bank from seaweeds in Jeju Island. A variety of extracts showed positive effect against reactive oxygen species (ROS). Especially, *Sargassum thunbergii* methanolic extract at 70°C (70ME, 97.41%), *S. fulvellum* methanolic extract at 20°C (20ME, 84.66%), *Codium fragile* aqueous extract at 70°C (70AE, 96.61%) and *S. thunbergii* 20ME (97.44%) exhibited the highest scavenging activities against $O_2^{\cdot-}$, $HO^{\cdot}$, H_2O_2 and DPPH free radicals, respectively. Total phenolic contents also examined but did not show a positive correlation with ROS scavenging abilities (except for a few extracts). These results indicate that further investigation is needed to identify and purify the responsible antioxidative components.

Key Words: antioxidant, aqueous extract, methanolic extract, ROS, seaweeds

INTRODUCTION

Lipid peroxidation is mostly an undesirable deteriorative reaction in food materials, which can produce rancid odors and flavors during storage and processing. Moreover the nutritional quality and safety of foods also can easily change due to their toxic compound formation (Yagi 1987; Zainol *et al.* 2003). These lipid peroxidation by-products, called free radicals, give damage to healthy cells, and thereby create harmful molecules. Free radicals and other reactive oxygen species (ROS) are generated continuously not only via normal physiological processes but also by external stimulations. Normal physiological processes need oxygen in order to carry out their operations as a resultant by-product like ROS are produced within the human body. If these harmful factors accumulate in cell, tissue and other vital organs of the body, then our bodies exposed to dangerous circumstances. Of the external stimulations, ROS can be induced by tobacco smoke, ionizing radiation, certain pollutants, organic solvents, and pesticides (Robinson *et al.* 1997; Karawita *et al.* 2005). ROS including superoxide ($O_2^{\cdot-}$), hydroxyl radical ($HO^{\cdot}$), and hydrogen peroxide (H_2O_2), have the ability to react with a large variety of easily oxidizable cellular components, such as proteins, lipids, nucleic acids, and carbohydrates. Their oxidative damages cause aging and many other diseases, including arthritis, strokes, heart diseases, atherosclerosis, diabetes, cancers and neurodegenerative disorders (Alho and Leinonen 1999; Lemberkovices *et al.* 2002).

Antioxidants are important inhibitors against lipid peroxidation, therefore these compounds are utilized to delay or prevent lipid peroxidation in foods and the oxidation of cellular substrates. They exert their effects by scavenging ROS, activating a battery of detoxifying proteins, or by preventing the generation of ROS (Jun *et al.* 2001). The most commonly used antioxidants are butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), *tert*-butylhydroquinone (TBHQ), and propyl gallate (PG). However, there has been growing concern over their safety and toxicity. Moreover, some studies (Lu and Foo 2000) have been reported that there is an inverse relationship between dietary intake of antioxidant-rich foods and the incidence of human diseases. Therefore, development and utilization of more effective antioxidant from natural resources are desired for use in foods or medicinal materials to replace the synthetic antioxidants.

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Many marine bio-resources have been attracted attention in the search for natural bioactive compounds to develop new drugs and health foods. Seaweeds have many phytochemicals with various bioactivities including antioxidant, anti-inflammatory and anticancer. Among them, antioxidant activity is intensively focused due to the current growing demand from the pharmaceutical industry where they are interested in antiaging and anticarcinogenic compounds, which possess health benefits. Therefore, many seaweeds have been examined to identify new and effective antioxidant compounds, as well as to elucidate the mechanisms of cell proliferation and apoptosis (Pietta *et al*. 1998; Athukorala *et al.* 2003; Heo *et al.* 2003; Lee *et al.* 2004). Recently, the active antioxidant compounds were identified as fucoxanthin and phlorotannins in *Hizikia fusiformis* and *Sargassum kjellamanianum*, respectively (Yan *et al.* 1996; Yan *et al.* 1999).

The aim of present study was to investigate the *in vitro* antioxidant activities of different extracts of seaweed in order to evaluate their potential as functional food materials and natural antioxidative sources for food and medicinal industry. And also, we wanted to find out an effective methodology for extraction of different antioxidative compounds by readily available solvents (i.e. MeOH and H_2O) in low and high temperatures (i.e. 20 and 70°C, respectively). This study is a part of construction of an extract bank from seaweeds in Jeju Island for a variety of biological activities, with the aim of identifying new seaweed species and novel molecules with potentially useful therapeutic activities. Total antioxidant potential has been examined using superoxide anion, hydroxyl radical, hydrogen peroxide and DPPH free radical scavenging assays.

MATERIALS AND METHODS

Seaweed materials and extraction

Seaweeds were collected along Jeju Island coast of Korea from February 2004 to March 2005. Of the Jeju coastal seaweeds, 10 species of Chlorophyta and 25 species of Phaeophyta samples were collected, and then salt, epiphytes and sand were removed using tap water. Finally the seaweeds were rinsed carefully with freshwater and stored in a medical refrigerator at -20°C. The frozen samples were lyophilized and homogenized with a grinder before extraction. The powdered samples were then extracted for 24 h first with 80% MeOH under continuous shaking at 70°C and 20°C, and then aqueous extracts were prepared with the residue after MeOH extraction of the seaweeds. Resultant four different extracts namely 70ME (methanolic extract at 70°C), 20ME (methanolic extract at 20°C), 70AE (aqueous extract at 70°C) and 20AE (aqueous extract at 20°C), respectively were obtained. The extracts were then concentrated under a vacuum in a rotate-vapor at 40°C. The solid mass obtained was then dissolved in water and the concentration of all the extracts was adjusted to 2 mg mL^{-1}.

Superoxide anion scavenging activity

Superoxide anion scavenging activity was determined using SOD Assay Kit-WST (Dojindo, Japan). Briefly, a 20 μL of sample solution was added into each well and blank 2, and 20 μL of ddH_2O was added into blank 1 and blank 3 wells. Water-soluble tetrazolium salts (WST) working solution was thoroughly mixed with reaction mixture. Dilution buffer was added to each sample and blank 1. The reaction mixture was incubated at 37°C for 20 min and absorbance was measured at 450 nm by ELISA reader. The inhibition rate was calculated as $[(A_{blank\,1} - A_{blank\,2})-(A_{sample} - A_{blank\,2})] / (A_{blank\,1} - A_{blank\,3}) \times 100$.

Hydroxyl radical scavenging activity

Hydroxyl radical scavenging activity was determined according to a slightly modified method of the 2-deoxyribose oxidation method (Chung *et al*. 1997). Hydroxyl radical was generated by Fenton reaction in the presence of $FeSO_4 \cdot 7H_2O$. A reaction mixture containing each 0.2 mL of 10 mM $FeSO_4 \cdot 7H_2O$, 10 mM EDTA and 10 mM 2-deoxyribose was mixed with 0.2 mL of the extract solution and 0.1 M phosphate buffer (pH 7.4) was added into the reaction mixture until the total volume reached to 1.8 mL. Then 0.2 mL of 10 mM H_2O_2 was finally added to the reaction mixture and incubated at 37°C for 4 h. After incubation, each 1 mL of 2.8% TCA (trichloroacetic acid) and 1.0 % TBA (thiobarbituric acid) were added. Then, the mixture was placed in a boiling water bath for 10 min. The absorbance was measured at 532 nm.

Hydrogen peroxide scavenging activity

Hydrogen peroxide scavenging activity was determined according to the method of Muller (1985). A hundred microliter of 0.1 M phosphate buffer (pH 5.0) and the sample solution were mixed in a 96 microwell plate. A 20 μL of hydrogen peroxide was added to the

mixture, and then incubated at 37°C for 5 min. After the incubation, 30 μL of 1.25 mM ABTS and 30 μL of peroxidase (1 unit $\cdot$ mL^{-1}) were added to the mixture, and then incubated at 37°C for 10 min. The absorbance was read with an ELISA reader at 405 nm.

DPPH free radical scavenging activity

Free radical scavenging activity of the seaweeds extracts was determined by using a stable free radical, DPPH, according to a slightly modified method of Blois (1958). DPPH solution was prepared at the concentration of 4×10^{-4} M in dimethyl sulfoxide (DMSO). During the assay, a 100 μL seaweed extract and 100 μL of freshly prepared DPPH solution were thoroughly mixed. The reaction mixture was incubated in the room temperature for 1 h. After standing for 1 h, the absorbance was recorded at 517 nm by ELISA reader (ELX tek Instrument Inc). The percentage inhibition was calculated as $[1-(A_i-A_j)/A_c] \times 100$; A_i is the absorbance of extract mixed with DPPH solution, A_j is the absorbance of same extract mixed with 100 μL DMSO, A_c is the absorbance of control with particular solvent (without seaweed extract).

Determination of total phenolic compound

Phenolic contents were determined using a protocol similar to Chandler and Dodds (1983) described by Shetty *et al.* (1995). Each 1 mL of seaweeds extracts, 1 mL of 95% EtOH, 5 mL of distilled water, and 0.5 mL of 50% Folin-Ciocalteu reagent (Sigma Chemical, St. Louis, MO) were mixed. The mixtures were allowed to react for 5 min, and then 1 mL of 5% Na_2CO_3 was added, and placed in the dark for 1 h. Absorbance was measured at 725 nm and gallic acid standard curve was obtained for the calibration of phenolic content.

RESULTS AND DISCUSSION

Superoxide anion scavenging activity

Superoxide anion ($O_2^{\cdot -}$) is formed in living cells during several biochemical reaction and its negative effect can be magnified because it produce other kinds of free radicals and oxidizing agents inducing cell damage (Lui and Ng 1999). The inhibition rate of superoxide anion by seaweed extracts was evaluated in Table 1. The highest superoxide anion scavenging effect was recorded in *S. thunbergii* 70ME (97.41%) in this study. Especially, all extracts of *S. thunbergii* on different extraction conditions showed more than 87% and *Ecklonia cava*, *Dictyota dichotoma*, *Pachydictyon* sp., *H. fusiformis*, *S. coreanum*, *S. fulvellum*, *S. piluliferum*, and *S. siliquastrum* also exhibited more than 50% scavenging activity under all the extraction conditions. Among the Chlorophyta, *Enteromorpha intestinalis* 20ME showed the highest effect on superoxide anion scavenging (70.01%) while the other extracts exhibited relatively lower activities. Studies about seaweeds concerning superoxide anion scavenging activity have been increasing due to the harmful reactivity of superoxide anion. Nagai and Yukimoto (2003) recorded a significant superoxide anion scavenging activity for a beverage made from *H. fusiformis* and Kuda *et al.* (2005) reported a good superoxide anion scavenging activity in edible brown seaweed, *Nemacystus decipiens*. In agreement, our study also exhibited strong superoxide anion inhibitory effect, specially in *Sargassum* spp.. This result indicates that *Sargassum* spp. can be easily used as an application of antioxidant source.

Hydroxyl radical scavenging activity

Hydroxyl radical scavenging activity was measured as the percentage inhibition of hydroxyl radicals generated in the Fenton reaction. In the model system, a mixture of $FeSO_4$, EDTA and H_2O_2 is incubated with deoxyribose, the hydroxyl radical generated attacks the deoxyribose and results in a series of reaction forming TBARS (Halliwell *et al.* 1987). Therefore, the ability to diminish the color formation is a measurement of hydroxyl radical scavenging. Table 2 exhibited the scavenging effect of hydroxyl radical on various seaweed extracts. *S. fulvellum* 20ME showed the highest scavenging activity on hydroxyl radical and *Petrospongium rugosum* 20ME also showed similar effect to *S. fulvellum* 20ME (around 84%). *Leathesia difformis* 20ME, *Endarachne binghamiae* 70ME, *E. cava* 70ME and, *Colpomenia sinuosa* 20ME of Phaeophyta had 83.30, 81.64, 81.62 and 80.09% hydroxyl radical scavenging activity, respectively. *Chaetomorpha linum* 20ME showed the highest scavenging activity among the Chlorophyta tested (81.36%). Moreover, all the 20ME of Chlorophyta exhibited more than 50% scavenging activities. Hydroxyl radical is a major reactive oxygen species causing lipid peroxidation as it can abstract a hydrogen atom from phospholipids membranes (Namiki 1990). In this study, each methanolic extract showed similar effect compared to the effect exhibited by phenolic compounds in the studies carried out by Shon *et al.* (2003) suggesting that they can be used to minimize the adverse effects from the hydroxyl radical.

Table 1. Superoxide anion scavenging activity of methanolic and aqueous extracts from seaweeds

Scientific name	Superoxide anion scavenging activity (%)			
	20ME[1)]	70ME[2)]	20AE[3)]	70AE[4)]
Chlorophyta				
Monostroma nitidum	6.82±1.32[5)]	56.82±3.24	6.67±1.49	7.69±0.53
Enteromorpha compressa	21.03±0.32	32.07±1.19	44.48±2.21	45.69±3.38
Enteromorpha intestinalis	70.01±3.73	7.14±2.11	28.85±0.72	9.62±1.72
Enteromorpha linza	22.86±3.27	12.86±1.94	20.00±1.47	12.22±0.38
Enteromorpha sp.	28.18±2.31	44.55±2.14	64.10±1.13	44.87±2.18
Ulva conglobata	6.74±0.46	34.09±1.46	28.85±2.81	30.77±1.04
Ulva pertusa	-	-	42.31±1.29	19.23±1.34
Chaetomorpha linum	13.33±0.21	36.36±2.87	56.41±2.19	42.95±2.12
Codium contractum	8.18±1.73	10.91±0.63	44.23±3.12	43.59±1.32
Codium fragile	54.35±1.87	32.61±2.86	14.17±1.43	25.01±2.73
Phaeophyta				
Papenfussiella kuromo	32.69±2.46	70.45±2.16	54.55±1.74	47.37±3.41
Ishige okamurai	39.47±1.63	57.89±2.28	43.10±1.84	27.59±1.15
Ishige sinicola	65.79±0.84	84.21±3.29	27.59±1.73	68.97±2.21
Leathesia difformis	41.67±1.49	61.67±1.87	26.79±1.73	12.50±0.62
Petrospongium rugosum	48.33±2.29	76.67±2.49	-	87.50±3.21
Colpomenia sinuosa	65.38±2.18	88.46±3.17	-	42.19±1.71
Endarachne binghamiae	17.39±0.32	26.09±1.75	80.36±1.84	17.65±1.43
Hydroclathrus clathratus	72.73±2.73	90.01±3.54	6.82±0.97	47.37±2.42
Scytosiphon lomentaria	89.47±3.28	86.84±2.85	27.59±1.65	41.38±2.39
Myelophycus simplex	21.03±1.75	32.93±2.32	77.41±2.94	52.76±1.74
Undaria pinnatifida	-	4.35±1.42	13.24±1.89	14.71±2.84
Ecklonia cava	26.09±1.93	52.75±2.81	67.65±1.64	38.24±1.03
Laminaria ochotensis	32.61±2.31	19.57±2.64	-	47.06±1.18
Dictyopteris prolifera	-	31.82±1.94	-	78.95±2.43
Dictyota dichotoma	94.23±3.27	50.00±2.34	95.31±4.83	75.04±1.97
Pachydictyon sp.	92.31±4.38	94.23±2.29	70.31±2.14	62.50±1.78
Padina arborescens	22.76±1.87	24.31±1.98	67.41±2.75	63.28±2.54
Myagropsis myagroides	9.09±1.62	29.55±2.74	51.92±3.36	76.92±2.83
Hizikia fusiformis	62.73±3.42	81.82±1.45	82.69±2.41	87.18±3.76
Sargassum coreanum	90.91±4.38	79.55±2.43	80.46±2.57	69.23±1.85
Sargassum fulvellum	92.86±3.74	85.71±2.86	80.03±1.96	74.44±3.54
Sargassum horneri	41.30±2.14	34.78±1.63	51.47±3.47	57.35±2.95
Sargassum piluliferum	65.04±3.29	90.23±3.48	69.64±2.14	80.36±3.59
Sargassum siliquastrum	72.73±3.29	88.64±2.62	90.18±3.75	94.23±3.42
Sargassum thunbergii	94.14±4.37	97.41±2.49	93.10±3.75	87.93±3.59

[1)]20ME: methanolic extract at 20°C, [2)]70ME: methanolic extract at 70°C, [3)]20AE: aqueous extract at 20°C, [4)]70AE: aqueous extract at 70°C, [5)]Mean ± SE of determinations was made in triplicate experiments.

Hydrogen peroxide scavenging activity

Hydrogen peroxide is a product derived from normal metabolism, but is not an inherently reactive non-radical compound. However, hydrogen peroxide can be converted into highly reactive and deleterious products such as singlet oxygen ($^1O_2$) and hydroxyl radical. Therefore, the effort on inhibition of hydrogen peroxide is very important. The hydrogen peroxide scavenging ability is shown in Table 3. *Codium fragile* 70AE showed the highest scavenging on hydrogen peroxide (96.61%) and *Ishige okamurae* 20AE, *Scytosiphon lomentaria* 20AE, *Myelophycus simplex* 20AE, and *Myagropsis myagroides* 20AE also showed higher scavenging activity (over 90%). Of the methanolic extracts, *C. linum* 20EM showed the highest scavenging activity (88.01%) and each *E. cava* extract (20ME and 70ME) showed relatively higher scavenging activity (86.19 and 84.76%, respectively). Moreover, each extract on different conditions of *I. okamurae*, *E. cava*, *M. myagroides*, and *S. siliquastrum*

Table 2. Hydroxyl radical scavenging activity of methanolic and aqueous extracts from seaweeds

Scientific name	Hydroxyl radical scavenging activity (%)			
	20ME[1)]	70ME[2)]	20AE[3)]	70AE[4)]
Chlorophyta				
Monostroma nitidum	74.64±1.68[5)]	34.37±2.92	53.59±0.96	-
Enteromorpha compressa	65.63±1.14	-	66.38±0.19	-
Enteromorpha intestinalis	56.92±0.41	62.36±4.00	58.23±3.46	-
Enteromorpha linza	73.36±1.41	62.79±0.91	52.09±4.37	20.86±2.68
Enteromorpha sp.	79.16±2.09	73.59±1.14	62.60±1.18	35.07±2.18
Ulva conglobata	66.84±3.73	71.71±3.96	21.87±1.14	58.96±2.68
Ulva pertusa	71.07±0.67	76.73±5.18	55.21±5.91	24.32±1.79
Chaetomorpha linum	81.36±1.50	79.81±0.32	47.65±0.18	58.97±0.77
Codium contractum	79.15±2.09	73.58±1.14	62.59±1.18	35.06±2.18
Codium fragile	77.93±0.36	56.72±3.69	71.24±0.05	41.63±1.94
Phaeophyta				
Papenfussiella kuromo	71.28±0.02	79.65±0.98	19.17±0.23	0.23±0.01
Ishige okamurai	41.95±0.82	20.69±2.30	22.29±4.32	29.36±0.23
Ishige sinicola	17.83±3.20	25.77±0.23	65.73±6.39	24.37±3.12
Leathesia difformis	83.30±0.27	78.95±4.41	8.63±1.12	53.92±3.05
Petrospongium rugosum	84.05±0.23	76.15±5.83	37.95±1.50	48.85±5.73
Colpomenia sinuosa	80.09±0.02	71.83±0.08	16.76±2.11	0.25±0.08
Endarachne binghamiae	74.74±1.33	81.64±0.41	18.22±2.82	37.65±4.52
Hydroclathrus clathratus	72.13±0.06	70.35±0.98	14.17±1.21	0.23±0.02
Scytosiphon lomentaria	22.23±2.19	68.96±1.56	1.32±0.28	41.45±5.02
Myelophycus simplex	21.80±2.57	43.75±0.66	51.12±1.56	20.19±3.70
Undaria pinnatifida	65.07±3.54	72.64±2.87	8.67±2.79	6.67±1.19
Ecklonia cava	75.02±1.18	81.62±2.10	34.40±0.96	26.95±0.51
Laminaria ochotensis	66.40±3.85	33.82±1.39	20.25±0.23	15.98±1.98
Dictyopteris prolifera	72.81±0.03	68.27±1.28	26.67±1.12	0.18±0.02
Dictyota dichotoma	77.59±0.08	61.19±1.83	21.67±2.19	0.36±0.04
Pachydictyon sp.	75.20±0.04	69.78±1.19	9.17±1.12	0.27±0.03
Padina arborescens	51.24±3.28	37.26±1.56	33.13±3.97	31.21±2.30
Myagropsis myagroides	75.36±6.97	-	-	-
Hizikia fusiformis	79.79±0.18	76.95±5.32	23.55±3.01	34.68±1.41
Sargassum coreanum	39.76±3.91	-	21.73±2.41	-
Sargassum fulvellum	84.66±1.10	34.83±2.21	36.35±4.63	-
Sargassum horneri	74.18±1.13	69.30±0.46	5.91±0.56	1.31±0.34
Sargassum piluliferum	75.58±1.05	72.28±1.09	7.08±1.73	25.55±4.82
Sargassum siliquastrum	54.92±5.15	-	45.56±2.79	-
Sargassum thunbergii	61.02±2.73	38.49±2.84	20.96±3.98	11.37±2.83

[1)]20ME: methanolic extract at 20°C, [2)]70ME: methanolic extract at 70°C, [3)]20AE: aqueous extract at 20°C, [4)]70AE: aqueous extract at 70°C, [5)]Mean ± SE of determinations was made in triplicate experiments.

exhibited more than 50% scavenging activities. The measurement of H_2O_2 scavenging activity is one of the useful methods determining the ability of antioxidants to decrease the level of pro-oxidants such as H_2O_2 (Czochra and Widensk 2002). Many species of seaweeds possess the ability to scavenge hydrogen peroxide. Heo *et al.* (2005) reported that water-soluble enzymatic extracts from brown seaweeds have high scavenging activities on hydrogen peroxide and inhibitory effect on DNA damage. Karawita *et al.* (2005) also recorded each aqueous and organic fraction of *H. fusiformis* exhibited higher H_2O_2 scavenging effects. The activities of some aqueous and methanolic extracts observed in the present study imply that high polar compounds of seaweeds have potential antioxidative sources.

DPPH free radical scavenging activity

Free radicals are continuously produced in cells either as by-products of metabolisms or as derivatives in phagocytosis (Cheesman and Slater 1993). DPPH is a

Table 3. Hydrogen peroxide scavenging activity of methanolic and aqueous extracts from seaweeds

Scientific name	Hydrogen peroxide scavenging activity (%)			
	20ME[1)]	70ME[2)]	20AE[3)]	70AE[4)]
Chlorophyta				
Monostroma nitidum	62.36±2.215)	21.58±2.97	45.78±1.10	30.29±0.42
Enteromorpha compressa	44.01±3.27	71.24±0.04	56.72±3.15	41.63±1.64
Enteromorpha intestinalis	65.63±2.12	-	53.70±4.08	5.81±1.48
Enteromorpha linza	74.64±3.14	34.37±1.02	42.07±5.05	-
Enteromorpha sp.	77.93±0.36	75.41±4.60	4.74±1.07	54.32±2.57
Ulva conglobata	73.36±2.63	62.79±1.71	47.99±4.47	20.86±5.01
Ulva pertusa	60.08±0.67	56.73±3.58	25.31±2.34	32.46±1.79
Chaetomorpha linum	88.01±1.53	69.28±0.32	55.63±0.18	48.15±1.95
Codium contractum	49.16±1.45	47.59±1.14	53.05±0.96	65.33±0.37
Codium fragile	66.84±4.21	74.57±1.25	11.86±0.12	96.61±3.38
Phaeophyta				
Papenfussiella kuromo	30.36±0.03	38.56±0.02	17.76±0.08	2.67±0.05
Ishige okamurai	82.14±2.18	73.63±0.13	92.64±0.16	55.25±1.55
Ishige sinicola	75.88±3.92	74.38±0.36	41.55±3.08	37.56±0.27
Leathesia difformis	12.40±0.78	11.95±2.17	27.01±0.67	19.47±4.51
Petrospongium rugosum	25.88±1.36	39.53±1.38	57.32±1.61	31.89±1.11
Colpomenia sinuosa	66.09±0.02	58.02±0.02	45.44±0.01	44.20±0.04
Endarachne binghamiae	21.49±1.20	24.61±0.49	38.89±2.61	6.31±1.78
Hydroclathrus clathratus	26.75±0.09	17.04±0.01	2.10±0.02	22.33±0.07
Scytosiphon lomentaria	6.08±0.56	8.81±0.36	93.50±0.04	84.46±1.58
Myelophycus simplex	58.09±6.29	55.44±0.31	91.36±0.38	64.71±4.93
Undaria pinnatifida	0.41±0.27	-	1.64±0.39	-
Ecklonia cava	86.19±0.38	84.76±0.08	75.82±0.92	53.28±1.13
Laminaria ochotensis	8.08±0.41	15.30±2.35	47.81±1.89	22.38±5.24
Dictyopteris prolifera	76.75±0.09	50.55±0.03	19.41±0.04	36.76±0.03
Dictyota dichotoma	38.96±0.03	17.04±0.05	61.55±0.43	39.06±1.21
Pachydictyon sp.	34.78±0.03	58.05±0.01	16.04±0.04	24.79±0.02
Padina arborescens	28.67±0.31	46.97±1.18	77.38±0.16	74.47±1.75
Myagropsis myagroides	52.50±9.42	59.05±0.26	90.06±0.08	52.92±4.69
Hizikia fusiformis	9.44±0.91	21.57±0.83	21.57±0.83	15.18±1.53
Sargassum coreanum	80.01±0.45	72.64±0.39	59.01±0.34	36.67±5.31
Sargassum fulvellum	43.48±1.79	54.04±0.71	61.52±1.42	45.54±6.36
Sargassum horneri	49.12±5.07	62.76±0.41	86.26±0.23	37.10±4.25
Sargassum piluliferum	53.62±0.56	55.82±0.22	59.14±0.81	42.77±0.93
Sargassum siliquastrum	76.96±1.06	75.51±0.41	60.14±0.53	54.35±4.05
Sargassum thunbergii	52.67±0.66	43.43±0.58	84.66±0.45	77.34±0.19

[1)]20ME: methanolic extract at 20°C, [2)]70ME: methanolic extract at 70°C, [3)]20AE: aqueous extract at 20°C, [4)]70AE: aqueous extract at 70°C, [5)]Mean ± SE of determinations was made in triplicate experiments.

stable free radical which is widely used as a substrate to evaluate antioxidative activity of natural compounds. The reduction capability of DPPH free radicals was determined by the decrease induced by antioxidative compounds and those results are shown in Table 4. Many kinds of methanolic extracts showed relatively higher scavenging activities on DPPH free radicals. Especially, *S. thunbergii* 20ME showed the highest scavenging activity (97.44%) and *I. okamurae* (20ME and 70ME), *I. sinicola* 20ME, *P. rugosum* 70ME, *D. dichotoma* (20ME and 70ME), *Pachydictyon* sp. 20ME, *S. coreanum* 70ME, *S. piluliferum* (20ME and 70ME), and *S. siliquastrum* 70ME also exhibited higher scavenging activities above 90%. Moreover, almost all *Sargassum* spp. (such as *S. coreanum*, *S. fulvellum*, *S. piluliferum*, *S. siliquastrum*, and *S. thunbergii*) showed higher scavenging activity (over 67%, except *S. horneri* 20ME) and each extract under different extraction conditions of *S. siliquastrum* exhibited over 75%. *E. compressa* 20AE showed the highest scavenging activity (85.20%) in

Table 4. DPPH free radical scavenging activity of methanolic and aqueous extracts from seaweeds

Scientific name	DPPH free radical scavenging activity (%)			
	20ME[1)]	70ME[2)]	20AE[3)]	70AE[4)]
Chlorophyta				
Monostroma nitidum	27.90±0.34[5)]	20.86±0.89	66.28±1.06	30.67±3.63
Enteromorpha compressa	39.28±2.35	43.06±0.11	85.20±1.13	19.15±1.07
Enteromorpha intestinalis	65.12±0.03	37.42±1.37	61.63±0.02	28.22±0.92
Enteromorpha linza	69.76±3.21	25.77±2.01	44.19±0.03	29.44±1.21
Enteromorpha sp.	58.04±1.98	8.47±1.08	75.37±0.91	18.07±2.19
Ulva conglobata	53.48±2.84	31.90±0.94	59.30±1.54	36.80±4.20
Ulva pertusa	63.04±4.23	28.04±0.22	56.89±0.13	26.90±2.19
Chaetomorpha linum	71.83±0.71	15.25±0.03	57.46±0.02	18.07±1.18
Codium contractum	45.97±0.64	27.12±0.02	48.51±0.03	26.55±2.11
Codium fragile	43.37±0.37	46.89±1.12	79.59±1.02	35.41±0.05
Phaeophyta				
Papenfussiella kuromo	86.11±1.11	76.85±4.21	39.35±2.11	44.88±2.13
Ishige okamurai	95.40±1.81	91.83±2.67	43.06±1.92	62.23±2.58
Ishige sinicola	94.89±1.74	83.67±3.34	49.28±2.10	21.05±3.26
Leathesia difformis	48.27±2.11	51.49±1.25	7.90±2.96	10.73±1.44
Petrospongium rugosum	82.18±1.39	94.02±2.98	24.85±2.87	21.46±1.89
Colpomenia sinuosa	69.91±0.97	71.76±2.19	38.71±3.21	49.10±2.22
Endarachne binghamiae	67.93±0.29	62.87±3.42	10.58±0.20	36.04±2.06
Hydroclathrus clathratus	63.89±0.03	69.91±3.29	40.97±1.29	57.83±1.23
Scytosiphon lomentaria	86.73±4.27	89.28±2.57	38.27±1.21	45.93±1.30
Myelophycus simplex	83.67±2.34	79.59±1.18	35.40±1.66	16.74±2.64
Undaria pinnatifida	69.02±1.15	74.25±1.14	16.40±2.75	57.86±2.80
Ecklonia cava	89.67±3.20	86.22±1.97	29.62±4.96	50.25±2.38
Laminaria ochotensis	76.63±4.73	62.87±2.42	33.33±1.19	36.54±1.82
Dictyopteris prolifera	31.48±1.22	45.37±0.82	37.42±2.19	45.18±2.21
Dictyota dichotoma	90.28±1.11	93.06±3.29	41.61±2.12	56.02±3.12
Pachydictyon sp.	96.30±0.81	83.80±2.39	41.61±3.11	53.31±1.19
Padina arborescens	85.20±1.41	82.65±1.30	42.17±2.19	29.66±3.50
Myagropsis myagroides	65.11±1.71	74.41±1.86	26.99±3.38	36.80±1.98
Hizikia fusiformis	83.33±2.33	81.34±1.32	64.40±0.67	44.63±1.27
Sargassum coreanum	86.04±3.21	90.69±2.76	87.11±1.65	52.14±0.72
Sargassum fulvellum	67.44±2.19	72.09±2.30	19.01±2.84	36.80±2.98
Sargassum horneri	23.91±1.92	80.23±3.95	19.57±1.61	27.41±3.11
Sargassum piluliferum	93.67±4.25	95.52±1.23	1.69±0.49	11.29±1.94
Sargassum siliquastrum	75.58±0.32	94.18±1.60	85.27±2.92	88.95±1.70
Sargassum thunbergii	97.44±1.89	88.77±0.55	9.56±1.93	22.00±2.95

[1)]20ME: methanolic extract at 20°C, [2)]70ME: methanolic extract at 70°C, [3)]20AE: aqueous extract at 20°C, [4)]70AE: aqueous extract at 70°C, [5)]Mean ± SE of determinations was made in triplicate experiments.

Chlorophyta, and *C. fragile* 20AE, *Enteromorpha* sp. 20AE, *C. lunum* 20ME exhibited 79.59, 75.37 and 71.83% scavenging activities, respectively. Many reports have recently described antioxidant activity and the responsible compounds present in fruits, plants, algae and marine organisms (Dykens *et al.* 1992; Pietta *et al.* 1998; Linda *et al.* 2004; Kuda *et al.* 2005). These natural products have shown higher scavenging ability indicating that they are potential free-radical inhibitors. Similarly, our results also exhibited higher scavenging activities against DPPH free radicals, implying that seaweeds have vast potential as natural antioxidative resources.

Determination of total phenolic compound

Recently, many studies have shown that phenolic compounds, such as flavonoid, isoflavone, antocyanin, and lignin are known to be very important inhibitors of oxidative molecules. These phenolic compounds have many hydroxyl groups in their structure, thus the

Table 5. Total phenolic contents of methanolic and aqueous extracts from seaweeds

Scientific name	Total phenolic contents (mg/g)			
	20ME[1)]	70ME[2)]	20AE[3)]	70AE[4)]
Chlorophyta				
Monostroma nitidum	5.49±1.075)	15.62±1.04	1.70±0.64	1.18±0.60
Enteromorpha compressa	16.03±0.64	8.54±0.67	11.53±0.80	3.99±0.64
Enteromorpha intestinalis	5.59±1.53	4.51±0.77	2.96±0.60	0.62±0.20
Enteromorpha linza	5.02±1.33	4.23±0.67	2.68±0.60	0.67±0.18
Enteromorpha sp.	6.06±0.90	1.42±0.64	3.15±0.87	2.59±0.97
Ulva conglobata	3.38±0.67	3.81±0.77	1.60±0.60	0.67±0.12
Ulva pertusa	2.59±0.67	3.10±1.20	1.70±0.70	1.98±0.67
Chaetomorpha linum	2.87±0.70	1.65±0.60	6.76±1.04	5.59±0.94
Codium contractum	3.20±0.70	1.04±0.74	3.15±0.70	3.24±0.97
Codium fragile	5.73±0.97	10.79±1.30	7.46±0.67	2.63±1.10
Phaeophyta				
Papenfussiella kuromo	4.93±1.14	6.38±0.70	6.38±0.90	42.99±0.90
Ishige okamurai	53.52±0.97	44.58±0.80	8.49±0.90	5.07±0.60
Ishige sinicola	43.68±0.70	43.74±1.76	49.31±0.70	3.15±0.64
Leathesia difformis	4.70±0.80	1.51±1.00	6.76±0.77	10.87±0.60
Petrospongium rugosum	4.93±0.70	1.84±0.77	21.57±0.70	22.59±0.67
Colpomenia sinuosa	21.19±1.18	29.77±1.07	21.99±1.27	16.18±0.60
Endarachne binghamiae	9.71±0.97	9.57±0.97	5.35±0.74	3.95±0.60
Hydroclathrus clathratus	15.24±0.80	8.63±0.90	11.16±0.94	11.26±1.10
Scytosiphon lomentaria	12.75±0.60	5.49±0.64	59.15±0.60	5.12±0.64
Myelophycus simplex	24.00±1.07	18.52±1.37	20.73±0.67	6.62±0.60
Undaria pinnatifida	1.56±0.84	1.65±0.64	3.81±0.84	2.40±0.64
Ecklonia cava	109.50±0.97	109.36±1.60	34.51±0.90	14.96±0.60
Laminaria ochotensis	1.09±0.70	1.46±0.80	4.09±1.07	3.99±0.64
Dictyopteris prolifera	43.13±1.07	41.77±1.10	5.87±2.26	5.31±0.67
Dictyota dichotoma	7.51±0.74	2.35±0.60	19.13±1.27	4.74±0.60
Pachydictyon sp.	5.31±1.07	8.63±1.10	14.45±1.00	5.59±0.87
Padina arborescens	13.69±0.80	8.77±0.77	10.79±0.97	7.56±0.80
Myagropsis myagroides	7.37±1.00	13.93±0.94	12.38±0.70	1.74±0.67
Hizikia fusiformis	4.32±0.84	1.65±0.64	5.12±0.87	4.13±0.60
Sargassum coreanum	38.11±0.84	88.36±6.04	93.89±0.77	2.35±0.70
Sargassum fulvellum	7.56±0.77	10.70±0.60	22.51±0.94	1.32±0.70
Sargassum horneri	6.81±0.77	9.01±1.43	27.71±1.04	1.65±0.77
Sargassum piluliferum	14.91±0.94	2.31±0.80	4.09±0.94	18.15±0.67
Sargassum siliquastrum	75.00±4.55	50.30±4.62	102.93±0.74	1.70±0.84
Sargassum thunbergii	23.06±0.60	16.98±1.37	21.71±0.80	15.52±0.67

[1)]20ME: methanolic extract at 20°C, [2)]70ME: methanolic extract at 70°C, [3)]20AE: aqueous extract at 20°C, [4)]70AE: aqueous extract at 70°C, [5)]Mean ± SE of determinations was made in triplicate experiments.

hydroxyl groups have ability to catch free radicals (Hatano *et al.* 1989). Total polyphenol contents are shown in Table 5. The highest content was observed in *E. cava* 20ME and 70ME (about 109 mg/g). These values were well correlated with hydroxyl radical, hydrogen peroxide and DPPH free radical scavenging activities. *S. siliquastrum* 20AE also showed higher polyphenolic contents (102.93 mg/g), which was correlated with superoxide anion and DPPH free radical scavenging abilities. Polyphenols in Phaeophyta are called as phlorotannins, which reported to have potential bioactivities specially as antioxidant, anticancer, antibacterial, antiplasmin compounds (Nakamura *et al.* 1996; Nagayama *et al.* 2002; Shibata *et al.* 2002). Of the results, some Phaeophyta showed higher phenolic contents which results indicate that Phaeophyta extracts have high contents of phlorotannins. In case of Chlorophyta, phenolic contents were very less than Phaeophyta. Nevertheless, some extracts of Chlorophyta showed high ROS scavenging effect. This result indicates

that other factors like chlorophyll may affect to antioxidant activities of marine algae. Many researchers have been reported a positive correlation between total phenolic compound and antioxidant activity. Pellati *et al.* (2004) observed that the radical scavenging activity of *Echinacea* root extract reflected its phenolic composition. Yen *et al.* (1993) also reported that phenolic compounds were associated with antioxidant activity and play an important role in stabilizing lipid peroxidation. However, according to this study, variation of reactive oxygen species scavenging ability recorded for different extracts and variation of phenolic content among different extraction suggested that no relationship between total phenols and total antioxidant activity (except in a few algae). Similar observation was revealed by Velioglu *et al.* (1998). They examined 28 plant products, and recorded high antioxidant activity but no correlation was observed between the phenolic content and antioxidant activity. It is thought that other compounds in natural products, such as small molecular weight polysaccharide, protein, pigments or peptides, probably influence the antioxidant activities.

In conclusion, the methanolic and aqueous extracts of the examined seaweeds contain antioxidative compounds that can strongly scavenge ROS such as superoxide anion, hydroxyl radical, hydrogen peroxide and DPPH free radical. These results indicate that the methanolic and aqueous extracts of seaweeds can be potential natural antioxidants in foods and pharmaceutical industries. However, the responsible compounds related to the antioxidant activity of seaweed extracts are not yet cleared. Therefore, further studies are required in order to identify the antioxidant compounds.

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