Algae
Volume 21(1): 149-156, 2006

Antioxidant Activities of Red Algae from Jeju Island

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The aim of the present study was to evaluate the antioxidant activity of red algae in Jeju Island. The algal extracts were obtained with MeOH and fresh water at 20 and 70°C, and screened for antioxidant activities using hydroxyl radical ($HO^{\cdot}$), superoxide anion (O_{2}^{-}), hydrogen peroxide (H_2O_2) and DPPH free radical scavenging assays. Among them, *Gracilaria verrucosa* methanolic extract at 20°C (20ME, 96.85%), *G. textorii* aqueous extract at 20°C (20AE, 88.01%), *Grateloupia filicina* 20AE (85.35%), and *Polysiphonia japonica* 20ME (94.92%) exhibited the highest scavenging activities against $HO^{\cdot}$, O_{2}^{-}, H_2O_2, and DPPH free radicals, respectively. Moreover, *P. japonica* (20ME and 70ME) is correlated between DPPH free radical scavenging activity and polyphenolic contents. These results indicate that some red algae in Jeju Island could be potential candidates for development of antioxidants.

Key Words: antioxidant, free radical, Jeju Island, reactive oxygen, red algae

INTRODUCTION

Free radicals can be generated in biological systems in the form of reactive oxygen species (ROS), such as superoxide anion radicals (O_{2}^{-}), hydroxyl radicals ($HO^{\cdot}$), hydrogen peroxide (H_2O_2), and singlet oxygen ($^1O_2$). These ROS are an entire class of highly reactive molecules derived from the normal metabolism of oxygen or from exogenous factors and agent (Chung *et al.* 2006). Oxidative damage of DNA, protein, lipid, and other molecules caused by ROS is associated with a number of pathological processes, including atherosclerosis, arthritis, diabetes, cataractogenesis, muscular dystrophy, pulmonary dysfunction, ischemia-reperfusion tissue damage, and neurological disorders such as Alzheimer's disease (Frlich and Riederer 1995).

Antioxidants are used to preserve food quality mainly by prevention of oxidative deterioration of constituent of lipids. There is an increasing interest in natural antioxidants because of the safety and toxicity problems of synthetic antioxidants, such as butylated hydroxylanisol (BHA) and butylated hydroxytoluene (BHT), which are commonly used in lipid-containing food (Amarowicz *et al.* 2000). Natural antioxidants can protect the human body from reactive oxygen species and free radicals, and retard the progress of many chronic diseases as well as lipid oxidative rancidity in food (Kinsella *et al.* 1993). Therefore, the studies on natural antioxidant are attracted by investigators and consumers for use in foods or medicinal materials to replace synthetic antioxidant.

Marine algae have been consumed in Asia since ancient times, which are rich in vitamins, minerals, dietary fiber, protein, and various functional polysaccharides (Kuda *et al.* 2005). Moreover, seaweeds are considered to be a rich source of antioxidant. Hence, many types of seaweeds have been examined to identify new and effective antioxidant compounds, as well as to elucidate the mechanisms of cell proliferation and apoptosis (Siriwardhana *et al.* 2004; Athukorala *et al.* 2005; Heo *et al.* 2005a, b, c; Park *et al.* 2005). Recently, the active antioxidant compounds were identified as fucoxanthin, phlorotannins, and other polyphenolic compounds (Yan *et al.* 1996; Yan *et al.* 1999; Yoshie *et al.* 2002).

The aim of present study is to investigate the *in vitro* antioxidant activities of different extracts from red algae present in Jeju Island in order to evaluate their potential as functional food materials and natural antioxidative resources for food and medicinal industry. Also we want to find out an effective methodology for extraction of different antioxidative compounds by MeOH and H_2O in low and high temperatures (i.e. 20 and 70°C, respectively). This study is a part for construction of a seaweed extract bank in Jeju Island for various biological activities, with

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the aim of identifying new seaweed species and novel molecules with potentially useful therapeutic activities. Total antioxidant potential has been examined using hydroxyl radical, superoxide anion, hydrogen peroxide and DPPH free radical scavenging assays.

MATERIALS AND METHODS

Red algal materials and extraction

Red algae were collected along Jeju Island coast of Korea from February 2004 to March 2005. From the 26 red algal species, salt, epiphytes and sand were removed using tap water. Finally the algae were carefully rinsed 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 with 80% MeOH for 24 h under continuous shake at 20°C and 70°C, and then with freshwater at the same condition. Four different extracts named 20ME (methanolic extract at 20°C), 70ME (methanolic extract at 70°C), 20AE (aqueous extract at 20°C) and 70AE (aqueous extract at 70°C), respectively. 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 $mg \cdot ml^{-1}$.

Hydroxyl radical scavenging activity

Hydroxyl radical scavenging activity was determined by a slightly modified method of the 2-deoxyribose oxidation method (Chung *et al.* 1997). Hydroxyl radical was generated from H_2O_2 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.

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 completely 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 $[(A_{blank\,1} - A_{blank\,2})-(A_{sample} - A_{blank\,2})] / (A_{blank\,1} - A_{blank\,3}) \times 100$.

Hydrogen peroxide scavenging activity

Hydrogen peroxide scavenging activity was determined by the method of Muller (1985). A hundred micro liter of 0.1 M phosphate buffer (pH 5.0) and the sample solution were mixed in a 96 micro well 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 algal 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 algal extract and 100 μl of freshly prepared DPPH solution were completely mixed. The reaction mixture was incubated in the room temperature for 1 h. After one hour, 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 the algal extract, 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

Table 1. Hydroxyl radical scavenging activity of methanolic and aqueous extracts from red algae in Jeju Island

Scientific name	Hydroxyl radical scavenging activity (%)			
	20ME[1)]	70ME[2)]	20AE[3)]	70AE[4)]
Porphyra tenera	45.03 ± 0.01[5)]	34.29 ± 0.90	51.31 ± 0.02	52.88 ± 0.20
Scinaia okamurae	27.23 ± 0.60	26.70 ± 0.01	27.49 ± 0.06	38.48 ± 0.01
Bonnemaisonia hamifera	21.62 ± 0.65	31.58 ± 0.55	32.63 ± 0.01	49.46 ± 0.05
Gelidium amansii	55.41 ± 0.02	57.57 ± 0.03	25.14 ± 0.00	30.27 ± 0.09
Pterocladiella capillacea	23.56 ± 0.06	20.16 ± 0.07	29.06 ± 0.40	36.91 ± 4.10
Lithophyllum okamurae	23.82 ± 0.02	16.23 ± 0.00	47.12 ± 0.02	38.22 ± 0.01
Carpopeltis affinis	67.13 ± 0.33	58.74 ± 6.66	71.33 ± 4.15	75.52 ± 0.55
Prionitis cornea	39.73 ± 1.50	48.65 ± 3.21	53.24 ± 1.00	51.08 ± 1.00
Grateloupia filicina	6.99 ± 0.50	21.68 ± 3.00	75.87 ± 0.10	86.71 ± 2.00
Sinkoraena lancifolia	8.74 ± 0.01	48.60 ± 2.00	87.76 ± 2.11	83.22 ± 0.55
Halymenia dilatata	13.35 ± 0.11	3.93 ± 0.05	34.03 ± 1.00	38.48 ± 0.06
Grateloupia elliptica	54.20 ± 1.10	49.30 ± 1.00	87.76 ± 0.05	87.06 ± 0.20
Grateloupia lanceolate	74.13 ± 0.01	60.14 ± 3.00	76.22 ± 2.00	3.15 ± 0.01
Gloiopeltis furcata	12.83 ± 0.01	15.45 ± 0.05	38.74 ± 0.10	46.86 ± 1.00
Schizymenia dubyi	20.94 ± 1.00	15.97 ± 1.50	42.15 ± 0.01	45.29 ± 0.02
Phacelocarpus sp.	42.97 ± 0.50	48.42 ± 1.00	19.46 ± 0.05	45.68 ± 0.55
Gracilaria textorii	96.15 ± 0.30	76.22 ± 5.33	88.46 ± 2.00	74.83 ± 1.00
Gracilaria verrucosa	76.57 ± 0.05	78.32 ± 3.22	94.06 ± 5.00	96.85 ± 5.33
Ahnfeltiopsis flabelliformis	63.64 ± 5.12	76.22 ± 0.02	87.76 ± 2.56	95.10 ± 1.12
Chondrus crispus	27.23 ± 0.01	23.82 ± 0.55	39.27 ± 0.60	59.16 ± 0.01
Lomentaria catenata	19.11 ± 0.01	20.94 ± 0.01	39.79 ± 0.06	21.47 ± 0.02
Martensia denticulata	8.12 ± 0.02	6.02 ± 0.00	48.42 ± 1.00	35.08 ± 0.01
Chondria crassicaulis	67.48 ± 0.76	68.88 ± 2.00	88.46 ± 2.32	86.36 ± 2.22
Laurencia okamurae	61.19 ± 0.05	56.99 ± 1.00	79.02 ± 1.00	85.31 ± 2.00
Chondrophycus undulatus	53.24 ± 0.30	41.35 ± 0.33	54.05 ± 0.14	55.14 ± 0.02
Polysiphonia japonica	12.97 ± 0.10	30.00 ± 0.02	52.63 ± 3.00	62.97 ± 3.00

[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.

calibration of phenolic content.

RESULTS AND DISCUSSION

Hydroxyl radical scavenging activity

Hydroxyl radical is a highly reactive species attacking almost all biological molecules. It is formed from hydrogen peroxide in a reaction catalyzed by metal ions (Cu^{2+} or Fe^{2+}) known as the Fenton reaction (Nordberg and Arner 2001). Hydroxyl radical scavenging activity of red algal extracts was measured as the percentage of inhibition of hydroxyl radicals generated in the Fenton reaction mixture and the results was shown in Table 1. *Gracilaria verrucosa* 70AE indicated the highest scavenging activity (96.85%) on hydroxyl radical, and *G. textorii* 20ME, *G. verrucosa* 20AE, *Ahnfeltiopsis flabelliformis* 70AE also showed similar scavenging effects (around 95%) to *G. verrucosa* 70AE. In addition, *Sinkoraena lancifolia* (20AE and 70AE), *Grateloupia elliptica* (20AE and 70AE), *G. textorii* 20AE, *A. flabelliformis* 20AE, *Chondria crassicaulis* (20AE and 70AE), *G. filicina* 70AE, and *Laurencia okamurae* 70AE also exhibited higher scavenging activities above 83%. Moreover, respective extracts (methanolic and aqueous extracts) on different conditions (20 and 70°C) of *G. textorii*, *G. verrucosa*, *A. flabelliformis*, and *C. crassicaulis* recorded more than 60% scavenging activities. Hydroxyl radicals are known to be capable of abstracting hydrogen atoms from membranes and they bring about peroxidic reactions of lipids (Kitada *et al.* 1979). Therefore, the strong hydroxyl radical scavenging activity in aqueous extract of red algae indicates that some aqueous extract could be used as an application of antioxidant source.

Superoxide anion scavenging activity

Superoxide and hydroxyl radical are the two most effective representative free radicals. In cellular oxidation reactions, superoxide radical is normally formed first

Table 2. Superoxide anion scavenging activity of methanolic and aqueous extracts from red algae in Jeju Island

Scientific name	Superoxide anion scavenging activity (%)			
	20ME[1)]	70ME[2)]	20AE[3)]	70AE[4)]
Porphyra tenera	62.50 ± 0.01[5)]	35.89 ± 6.12	12.50 ± 2.00	25.69 ± 2.30
Scinaia okamurae	25.22 ± 3.22	37.50 ± 1.22	20.00 ± 5.00	12.50 ± 6.33
Bonnemaisonia hamifera	75.13 ± 9.22	87.48 ± 6.33	45.00 ± 5.12	25.33 ± 7.25
Gelidium amansii	42.50 ± 3.20	62.58 ± 3.77	50.33 ± 4.55	55.77 ± 0.99
Pterocladiella capillacea	12.50 ± 3.33	25.83 ± 1.11	25.06 ± 3.33	44.12 ± 1.66
Lithophyllum okamurae	75.00 ± 4.12	20.33 ± 0.59	37.50 ± 5.78	25.00 ± 0.99
Carpopeltis affinis	25.00 ± 0.10	84.56 ± 1.05	50.80 ± 0.03	10.50 ± 0.40
Prionitis cornea	25.69 ± 3.00	29.66 ± 0.32	66.67 ± 6.05	83.33 ± 5.55
Grateloupia filicina	5.00 ± 3.00	37.50 ± 5.10	51.04 ± 5.00	25.04 ± 3.22
Sinkoraena lancifolia	12.50 ± 0.50	47.50 ± 1.20	37.50 ± 3.12	24.08 ± 0.55
Halymenia dilatata	75.39 ± 3.66	50.75 ± 6.62	35.00 ± 3.22	25.39 ± 1.11
Grateloupia elliptica	25.00 ± 0.05	12.50 ± 0.01	25.25 ± 0.20	12.50 ± 0.75
Grateloupia lanceolate	87.30 ± 3.33	61.48 ± 6.12	19.55 ± 6.77	75.00 ± 6.12
Gloiopeltis furcata	15.00 ± 3.33	35.67 ± 8.77	12.50 ± 3.33	38.99 ± 3.99
Schizymenia dubyi	15.00 ± 1.22	75.09 ± 2.25	12.50 ± 2.22	25.40 ± 3.33
Phacelocarpus sp.	25.35 ± 5.45	37.50 ± 0.22	26.67 ± 0.06	50.10 ± 3.33
Gracilaria textorii	87.50 ± 6.61	25.00 ± 1.11	88.01 ± 6.33	37.50 ± 0.55
Gracilaria verrucosa	62.50 ± 0.55	62.50 ± 6.01	75.88 ± 5.12	25.00 ± 3.10
Ahnfeltiopsis flabelliformis	85.70 ± 6.11	75.70 ± 1.12	5.00 ± 0.55	25.00 ± 1.12
Chondrus crispus	37.50 ± 6.35	50.00 ± 3.66	25.33 ± 4.21	40.22 ± 1.11
Lomentaria catenata	51.06 ± 6.99	25.48 ± 5.44	75.00 ± 9.11	51.09 ± 5.98
Martensia denticulata	86.58 ± 3.33	22.50 ± 1.11	62.34 ± 9.12	25.07 ± 5.55
Chondria crassicaulis	10.00 ± 0.55	63.57 ± 3.12	25.00 ± 0.21	50.00 ± 3.22
Laurencia okamurae	50.06 ± 0.55	62.50 ± 9.11	42.50 ± 1.12	37.50 ± 1.12
Chondrophycus undulatus	50.22 ± 5.00	41.00 ± 1.00	66.67 ± 3.22	16.67 ± 0.01
Polysiphonia japonica	13.00 ± 3.00	15.00 ± 2.00	18.02 ± 5.33	23.99 ± 4.11

[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.

and its effects can be magnified because it produces other kinds of cell damaging free radicals and oxidizing agents (Lui and Ng 1999). Table 2 exhibited the scavenging effect of superoxide anion on various algal extracts. *Gracilaria textorii* 20AE showed the highest scavenging activity (88.01%) while *G. textorii* 20ME showed the second highest activity (87.50%) on superoxide anion. Of the *G. textorii*, the low temperature (20°C) extracts recorded higher scavenging activities compared to the high temperature (70°C). Moreover, *Grateloupia lanceolate* 20ME, *Ahnfeltiopsis flabelliformis* 20ME, *Martensia denticulate* 20ME, *Bonnemaisonia hamifera* 70ME, *Carpopeltis affinis* 70ME, and *Prionitis cornea* 70AE also showed relatively higher superoxide anion scavenging activities (over 83%). Studies concerning superoxide anion scavenging activity of algae have been increasing due to the harmful reactivity of superoxide anion. Qi *et al.* (2005) recorded a good superoxide anion scavenging activity in a polysaccharide extracted from *Ulva pertusa*, and Kuda *et al.* (2005) found significant superoxide anion scavenging activities in four algal water extracts. Superoxide radical is not only formed in the body but also in the early products of protein glycation, such as the Schiff base and Amadori compound which may be the key structural components involved in the generation of superoxide radical (Ukeda *et al.* 2002). Therefore this good superoxide anion scavenging activity of some red algal extracts may be important both in medicinal and functional food fields.

Hydrogen peroxide scavenging activity

Hydrogen peroxide can be formed in vivo by an antioxidant enzyme such as superoxide dismutase. It can cross membranes and may slowly oxidize a number of compounds. The ability of red algae to scavenge hydrogen peroxide is shown in Table 3. *Grateloupia filicina* 20AE showed the highest scavenging on hydrogen peroxide (85.35%) while the other aqueous

Table 3. Hydrogen peroxide scavenging activity of methanolic and aqueous extracts from red algae in Jeju Island

Scientific name	Hydrogen peroxide scavenging activity (%)			
	20ME[1)]	70ME[2)]	20AE[3)]	70AE[4)]
Porphyra tenera	7.40 ± 0.03[5)]	12.29 ± 0.04	2.31 ± 0.07	3.72 ± 0.08
Scinaia okamurae	1.72 ± 0.21	9.48 ± 0.00	2.03 ± 0.09	5.10 ± 0.06
Bonnemaisonia hamifera	7.75 ± 0.16	4.50 ± 0.03	5.40 ± 0.11	10.14 ± 0.02
Gelidium amansii	1.55 ± 0.10	3.97 ± 0.12	4.00 ± 0.16	6.66 ± 0.01
Pterocladiella capillacea	5.64 ± 0.03	15.23 ± 0.02	12.91 ± 0.29	3.88 ± 0.01
Lithophyllum okamurae	5.19 ± 0.04	14.40 ± 0.03	15.05 ± 0.17	0.15 ± 0.04
Carpopeltis affinis	26.29 ± 0.05	68.81 ± 3.11	18.09 ± 0.02	0.17 ± 0.01
Prionitis cornea	32.29 ± 0.05	18.82 ± 0.49	6.60 ± 0.18	7.97 ± 0.09
Grateloupia filicina	12.39 ± 0.02	16.54 ± 0.02	85.35 ± 0.33	0.80 ± 0.00
Sinkoraena lancifolia	18.78 ± 3.33	21.38 ± 2.00	6.20 ± 0.05	1.65 ± 0.01
Halymenia dilatata	10.92 ± 0.00	7.48 ± 0.01	1.28 ± 0.04	7.96 ± 0.01
Grateloupia elliptica	19.77 ± 0.05	15.80 ± 0.01	5.01 ± 0.20	1.42 ± 0.75
Grateloupia lanceolate	15.91 ± 0.02	15.08 ± 0.33	48.35 ± 1.66	2.13 ± 3.33
Gloiopeltis furcata	9.64 ± 0.04	8.06 ± 0.01	7.36 ± 0.31	1.95 ± 0.02
Schizymenia dubyi	18.69 ± 0.07	10.48 ± 0.01	6.76 ± 0.20	9.35 ± 0.21
Phacelocarpus sp.	0.69 ± 0.04	25.21 ± 0.01	0.94 ± 0.00	24.09 ± 0.19
Gracilaria textorii	5.03 ± 0.03	11.14 ± 0.02	8.21 ± 0.03	1.37 ± 0.03
Gracilaria verrucosa	12.39 ± 0.05	14.04 ± 0.11	2.40 ± 0.01	3.02 ± 0.01
Ahnfeltiopsis flabelliformis	55.44 ± 1.11	14.04 ± 0.02	2.40 ± 0.00	3.02 ± 0.04
Chondrus crispus	3.80 ± 0.04	6.97 ± 0.06	5.72 ± 0.18	0.28 ± 0.01
Lomentaria catenata	6.71 ± 0.05	17.95 ± 0.01	43.71 ± 0.05	34.72 ± 0.03
Martensia denticulata	59.54 ± 0.01	73.80 ± 0.02	43.71 ± 0.66	34.72 ± 1.12
Chondria crassicaulis	17.96 ± 3.00	3.87 ± 0.11	0.41 ± 0.00	1.35 ± 0.03
Laurencia okamurae	2.68 ± 1.22	6.73 ± 2.11	11.36 ± 0.41	0.59 ± 0.00
Chondrophycus undulatus	6.53 ± 0.05	16.55 ± 0.10	9.92 ± 0.35	1.62 ± 0.00
Polysiphonia japonica	13.72 ± 0.17	2.98 ± 0.09	7.46 ± 0.02	0.17 ± 0.02

[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.

extracts exhibited relatively lower activities. Of the methanolic extracts, *Martensia denticulate* 70ME and *Carpopeltis affinis* 70ME showed relatively higher scavenging activity (73.80 and 68.81%, respectively). Hydrogen peroxide itself is not very reactive; however it can sometimes be toxic to cell because it may give rise to hydroxyl radical in the cells. Addition of hydrogen peroxide into living cells can lead to metal ion-dependent hydroxyl radicals mediated oxidative DNA damage. In our previous study (Heo *et al.* 2005a, b, c), water-soluble enzymatic extracts from *Sargassum horneri* have high scavenging activity on hydrogen peroxide and inhibitory effect on DNA damage. Siriwardhana *et al.* (2003) and Karawita *et al.* (2005) also recorded aqueous and organic extracts of *Hizikia fusiformis* exhibited higher hydrogen peroxide scavenging effects.

DPPH free radical scavenging activity

DPPH is a free radical generating compound and has been widely used to evaluate the free radical scavenging ability of various antioxidative compounds (Hatano *et al.* 1989). The effect of antioxidant on DPPH radical scavenging was thought to be due to their hydrogen donating ability (Ilhami *et al.* 2004). The reduction capability of DPPH radical was determined by the decrease induced by antioxidative compounds and those results are shown in Table 4. Among them, *Polysiphonia japonica* 20ME showed the highest scavenging activity (94.92%) and *P. japonica* 70ME also exhibited similar scavenging activity (93.86%) on DPPH free radical. Moreover, *Ahnfeltiopsis flabelliformis* 20ME and *Lithophyllum okamurae* 70ME also showed higher DPPH free radical scavenging activities (88.07 and 75.75%, respectively). Of the tested samples, methanolic extracts recorded a little higher than that of aqueous extracts (less than 50%). Many reports have recently described the ability to scavenge DPPH free radical on seaweeds. Athukorala *et al.* (2003) and Heo *et al.* (2005a, b, c) also

Table 4. DPPH free radical scavenging activity of methanolic and aqueous extracts from red algae in Jeju Island

Scientific name	DPPH free radical scavenging activity (%)			
	20ME[1)]	70ME[2)]	20AE[3)]	70AE[4)]
Porphyra tenera	40.58 ± 0.73[5)]	40.93 ± 0.73	18.85 ± 0.98	27.02 ± 0.90
Scinaia okamurae	17.75 ± 1.01	22.03 ± 0.96	33.64 ± 0.80	26.14 ± 0.89
Bonnemaisonia hamifera	26.00 ± 0.90	57.98 ± 0.31	31.18 ± 0.84	14.29 ± 1.05
Gelidium amansii	33.63 ± 0.81	49.98 ± 0.65	25.56 ± 0.91	22.58 ± 0.95
Pterocladiella capillacea	24.14 ± 0.93	44.67 ± 0.68	20.44 ± 0.97	18.30 ± 0.99
Lithophyllum okamurae	24.12 ± 0.93	75.75 ± 0.31	32.51 ± 0.81	23.07 ± 0.93
Carpopeltis affinis	15.28 ± 1.17	22.47 ± 1.07	28.62 ± 1.04	5.53 ± 1.33
Prionitis cornea	46.39 ± 0.69	13.06 ± 1.06	39.35 ± 0.74	20.90 ± 0.97
Grateloupia filicina	20.60 ± 1.09	31.24 ± 0.95	13.95 ± 1.22	12.31 ± 1.24
Sinkoraena lancifolia	29.52 ± 0.97	38.71 ± 0.84	12.82 ± 1.24	10.34 ± 1.26
Halymenia dilatata	29.00 ± 0.87	57.73 ± 0.52	21.42 ± 0.95	24.79 ± 0.91
Grateloupia elliptica	24.56 ± 1.04	20.29 ± 1.10	9.61 ± 1.21	3.71 ± 1.36
Grateloupia lanceolate	37.94 ± 0.86	45.96 ± 0.75	15.40 ± 1.19	29.48 ± 1.04
Gloiopeltis furcata	29.85 ± 0.86	57.16 ± 0.53	20.77 ± 0.96	33.59 ± 0.80
Schizymenia dubyi	45.61 ± 0.67	31.45 ± 0.84	29.92 ± 0.86	29.54 ± 0.85
Phacelocarpus sp.	51.54 ± 0.59	42.15 ± 0.42	14.93 ± 1.05	27.92 ± 0.88
Gracilaria textorii	18.01 ± 1.13	22.51 ± 1.08	10.55 ± 1.26	13.29 ± 1.22
Gracilaria verrucosa	29.65 ± 0.97	36.75 ± 0.84	6.98 ± 1.31	11.18 ± 1.26
Ahnfeltiopsis flabelliformis	88.07 ± 0.17	19.97 ± 1.10	5.53 ± 1.33	9.01 ± 1.28
Chondrus crispus	16.35 ± 1.03	30.25 ± 0.86	19.12 ± 0.97	17.54 ± 0.99
Lomentaria catenata	22.17 ± 0.96	49.15 ± 0.65	47.99 ± 0.63	16.24 ± 1.01
Martensia denticulata	18.11 ± 1.01	50.42 ± 0.64	44.51 ± 0.67	15.67 ± 1.02
Chondria crassicaulis	44.97 ± 0.76	48.85 ± 0.71	18.80 ± 1.15	22.39 ± 1.10
Laurencia okamurae	12.67 ± 1.20	12.33 ± 1.22	11.22 ± 1.25	26.61 ± 1.07
Chondrophycus undulatus	31.48 ± 0.84	48.80 ± 0.63	24.99 ± 0.92	21.40 ± 0.96
Polysiphonia japonica	94.92 ± 0.06	93.86 ± 0.08	30.37 ± 0.85	37.41 ± 0.77

[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.

reported that each seaweed organic and aqueous extract have positive effect in DPPH free radical scavenging.

Determination of total phenolic compounds

Phenolic compounds are very important constituents because of their scavenging ability due to their hydroxyl groups. Many researchers reported that phenolic compounds were associated with DPPH fee radical scavenging activity. This study also reported the total phenolic contents for comparing with DPPH free radical scavenging activity. As shown in Table 5, only *Polysiphonia japonica* (20ME and 70ME) showed a positive correlation between DPPH free radical scavenging activity and polyphenolic contents, which values are 176.90 and 133.60 mg/g, respectively. A number of researches have point out that seaweed polyphenols are associated with antioxidant activity and play an important role in stabilizing lipid peroxidation (Yen *et al.* 1993). Phenols are particularly effective antioxidants for polyunsaturated fatty acid; in fact they easily transfer a hydrogen atom to lipid peroxyl radicals and form the aryloxyl, which being incapable of acting as a chain carrier, couples with another radical thus quenching the radical process (Ruberto *et al.* 2001).

In conclusion, the methanolic and aqueous extracts of the examined red algae contain antioxidative compounds that can strongly scavenge ROS such as hydroxyl radical, superoxide anion, hydrogen peroxide, and DPPH free radical. These results indicate that the metanolic and aqueous extracts can be used as easily accessible source of natural antioxidants and as a possible food supplement or in pharmaceutical industry. However, the responsible compounds related to the antioxidant activity of the algal extracts are not yet cleared. Therefore, it is suggested that further works should be performed on the isolation and identification of the antioxidant component in seaweeds.

Table 5. Total phenolic contents of methanolic and aqueous extracts from red algae in Jeju Island

Scientific name	Total phenolic contents (mg/g)			
	20ME[1)]	70ME[2)]	20AE[3)]	70AE[4)]
Porphyra tenera	26.78 ± 3.21[5)]	34.61 ± 1.31	10.14 ± 0.33	9.21 ± 0.12
Scinaia okamurae	10.47 ± 1.55	10.28 ± 1.01	8.41 ± 1.11	7.19 ± 0.66
Bonnemaisonia hamifera	8.27 ± 1.00	13.75 ± 1.11	12.06 ± 1.02	11.88 ± 0.01
Gelidium amansii	24.06 ± 0.09	24.67 ± 1.00	7.28 ± 1.02	7.00 ± 1.00
Pterocladiella capillacea	23.97 ± 1.97	37.89 ± 1.06	13.80 ± 1.11	8.36 ± 0.12
Lithophyllum okamurae	21.72 ± 1.97	34.42 ± 2.00	33.20 ± 1.01	10.14 ± 0.50
Carpopeltis affinis	31.80 ± 0.01	38.08 ± 0.01	18.39 ± 0.03	14.36 ± 0.05
Prionitis cornea	10.42 ± 0.05	4.75 ± 0.03	8.08 ± 0.06	8.50 ± 1.02
Grateloupia filicina	30.48 ± 0.06	31.23 ± 1.11	10.52 ± 2.22	6.96 ± 1.00
Sinkoraena lancifolia	37.19 ± 0.02	36.81 ± 0.96	12.02 ± 1.00	9.67 ± 0.56
Halymenia dilatata	30.34 ± 1.17	31.19 ± 1.01	10.66 ± 1.00	10.05 ± 0.67
Grateloupia elliptica	36.48 ± 1.00	28.75 ± 1.02	12.81 ± 0.02	10.80 ± 0.02
Grateloupia lanceolate	33.67 ± 1.12	36.67 ± 2.22	9.91 ± 1.17	15.02 ± 2.33
Gloiopeltis furcata	61.18 ± 1.02	51.11 ± 1.06	13.14 ± 0.66	8.92 ± 0.11
Schizymenia dubyi	13.10 ± 0.03	29.92 ± 1.11	8.83 ± 0.05	7.28 ± 0.05
Phacelocarpus sp.	17.74 ± 0.45	11.88 ± 0.06	4.85 ± 0.05	19.28 ± 1.00
Gracilaria textorii	17.03 ± 0.01	71.63 ± 0.90	13.00 ± 0.05	7.05 ± 0.01
Gracilaria verrucosa	21.63 ± 0.12	23.31 ± 1.21	8.46 ± 1.01	10.24 ± 1.11
Ahnfeltiopsis flabelliformis	17.17 ± 1.11	13.10 ± 2.00	8.69 ± 0.08	10.24 ± 1.00
Chondrus crispus	26.17 ± 3.02	27.81 ± 1.00	7.00 ± 1.22	10.24 ± 1.17
Lomentaria catenata	18.11 ± 1.01	24.48 ± 1.21	9.02 ± 1.01	8.36 ± 0.06
Martensia denticulata	49.93 ± 1.89	56.92 ± 1.11	23.97 ± 1.09	22.47 ± 1.17
Chondria crassicaulis	17.64 ± 1.22	19.05 ± 0.03	8.50 ± 1.12	6.77 ± 0.60
Laurencia okamurae	19.66 ± 1.21	26.31 ± 1.66	10.05 ± 1.00	7.89 ± 0.09
Chondrophycus undulatus	7.14 ± 0.05	26.27 ± 1.11	11.60 ± 0.32	7.61 ± 0.88
Polysiphonia japonica	176.90 ± 1.11	133.60 ± 1.11	10.85 ± 0.98	8.27 ± 0.66

[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.

ACKNOWLEDGEMENTS

This work was supported by the Korea Research Foundation Grant funded by the Korean Government (MOEHRD) (2004-F00038).

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Received 4 January 2006
Accepted 23 February 2006