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Total Phenolic Contents and Biological Activities of Korean Seaweed Extracts

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Abstract Crude extracts of thirty seaweeds collected in Korea were obtained using 50% ethanol, and total phenolic contents and antioxidant activities were compared. Two brown algae, *Ecklonia cava* (*E. cava*) and *Sargassum siliquastrum* (*S. siliquastrum*), showing high antioxidant activity based on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity and superoxide anion radical scavenging activity were further investigated for their inhibitory effects on tyrosinase activity. The *E. cava* extract had the highest total phenolic content among the seaweeds extracts. Total phenolic contents were strongly correlated with antioxidant activity in the thirty seaweed extracts (R^2 = 0.9169). The *E. cava* and *S. siliquastrum* extracts exhibited higher inhibition to tyrosinase activity than butylated hydroxytoluene (BHT) and epigallocatechin gallate (EGCG).

Key words: total phenolic contents, antioxidant activity, physical activity, *Ecklonia cava*, *Sargassum siliquastrum*

Introduction

Seaweeds are a source of food in many maritime countries and have been applied to industrial products because they contain both essential minerals and various functional polysaccharides and proteins (1). Recently, seaweed resources have been used as the raw materials for food and cosmetics due to their antioxidant and antibacterial activities (2), and have been studied as new sources of a variety of compounds showing biological activities of potential medicinal value (3, 4). For instance, seaweeds such as fusiforme, gulfweed, sea-mustard, and sea-tangle were tested for antitumor activity in sarcoma implanted mice (5) and the potential antiangiogenic and antitumoral properties of a polysaccharide extracted from the brown marine alga *Sargassum stenophyllum* were studied in chick embryos and mice (6).

Much attention has been paid to their antioxidant activity because they are marine organisms and that are always exposed to a combination of light and high oxygen concentrations, which lead to the formation of free radicals and other strong oxidizing agents. Since their protective mechanisms and antioxidative compounds have been elucidated, seaweeds or their extracts have been studied as natural antioxidants (7). Recently, the active antioxidant compounds were identified as fucoxanthin in *Hijikia fusiformis* (8), phlorotannins in *Sargassum kjellmanianum* (9), and the crude extract of brown seaweed, *Sargassum siliquastrum*, collected in coastal waters was proved to have strong antioxidant activity (10).

In this study to screen the potential bioactivities of 50% ethanol extracts from seaweeds which were easily collected in coastal regions of Korea, we evaluated the total phenolic contents and antioxidant activities of seaweeds extracts and compared them with those of synthetic antioxidants such as butylated hydroxytoluene (BHT), ascorbic acid, α-tocopherol, (+)-catechin, and epigallocatechin gallate (EGCG). In addition, on the basis of the reports that other bioactivities such as antibacterial and anti-melanin forming activity were observed in various extracts showing antioxidant activity (11, 12), the antibacterial activity and effects on tyrosinase inhibition of seaweed extracts were investigated.

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Materials and Methods

Materials Thirty seaweeds were collected in the coastal regions of Geoje and Jeju islands, Korea, from March 2004 to August 2004 (Table 1). The samples were washed with running tap water, frozen, and lyophilized. Dry samples (0.5 g) were ground into powder and extracted with 50% ethanol (20 mL) in a shaking incubator (150 rpm) for 2 hr at room temperature. The extracts were evaporated in a dry oven at 45°C and stored at -20°C for further experiments. Gallic acid, tyrosinase, BHT, DPPH (1,1-diphenyl-2-picrylhydrazil), α-tocopherol, ascorbic acid, and (+)-catechin were purchased from Sigma Chemicals Co. (St. Louis, MO, USA). Other chemicals used were of 99% or greater purity.

Total phenolic contents Total phenolic contents of seaweed extracts were determined with Folin-Ciocalteu reagent according to the method of Capannesi and Palchetti (13). Briefly, extract (0.5 mL) was mixed with Folin-Ciocalteu reagent (0.5 mL) for 30 sec and then 7.5% Na_2CO_3 (1 mL) was added. The solution was mixed, brought to a volume of 10 mL with distilled water and incubated for 20 min at 65°C. The developed blue color

Table 1. List of 30 Korean seaweeds used for total phenolic contents and antioxidant activity

No.	Scientific name	Sampling	Type	No	Scientific name	Sampling	Type
1	*Chondrus occellatus*	Jeju	Red algae	16	*Sargassum hemiphyllum*	Geoje	Brown algae
2	*Caulerpa okamurae*	Jeju	Green algae	17	*Grateloupia elliptica*	Geoje	Brown algae
3	*Ishige okamurae*	Jeju	Brown algae	18	*Sargassum horneri*	Geoje	Brown algae
4	*Jania adhaerens*	Jeju	Red algae	19	*Colpomenia sinuosa*	Geoje	Green algae
5	*Codium fragile*	Geoje	Green algae	20	*Pterocladiella tenuis*	Jeju	Red algae
6	*Prionitis cornea*	Jeju	Red algae	21	*Enteromorpha intestinalis*	Geoje	Green algae
7	*Gelidium elegans*	Jeju	Red algae	22	*Sargassum thunbergii*	Geoje	Brown algae
8	*Cladophoropsis zollingeri*	Jeju	Green algae	23	*Sargassum ringgoldianum*	Jeju	Brown algae
9	*Cladophora opaca*	Jeju	Green algae	24	*Trematocarpus pygmaeus*	Jeju	Red algae
10	*Myelophycus simplex*	Jeju	Brown algae	25	*Ulva lactuca*	Geoje	Green algae
11	*Laurensia nipponica*	Geoje	Red algae	26	*Champia parvula*	Jeju	Red algae
12	*Lomentaria catenat*	Jeju	Red algae	27	*Sargassum yezoens*	Jeju	Brown algae
13	*Sargassum nigrifolium*	Geoje	Brown algae	28	*Prionitis divaricata*	Jeju	Red algae
14	*Sargassum siliquastrum*	Geoje	Brown algae	29	*Undaria pinnatifida*	Geoje	Brown algae
15	*Ecklonia cava*	Jeju	Brown algae	30	*Laminaria japonica*	Geoje	Brown algae

was measured using UV spectrophotometer at 765 nm with gallic acid as the standard phenolic compound. Analysis of seaweed extracts was performed in triplicate and total phenolic contents were expressed as GAE (gallic acid equivalent).

Free radical scavenging activity on DPPH Free radical scavenging activity was determined according to the method of Lu and Foo (14). Seaweed extract redissolved in 50% ethanol (0.2 mL) was added to 0.8 ml of DPPH solution and the mixture was shaken vigorously. After 10-min incubation at room temperature, the absorbance was measured at 517 nm and the free radical scavenging activity was calculated by the following equation:

Scavenging activity (%) = $[(A_0-A_1)/A_0] \times 100$

where, A_0 is the absorbance of control (mixture with 50% ethanol) at 517 nm, and A_1 is the absorbance of sample (mixture with seaweed extract) at the same wavelength.

Superoxide anion scavenging activity Superoxide anion scavenging activity of seaweed extracts was measured according to the method of Oktay *et al.* (15). One milliliter of nitroblue tetrazolium (NBT) solution (156 μM) and 1 mL of 468 μM NADPH solution were added to 0.1 mL of seaweed extract, and then mixed. The reaction was started by adding 100 μL of 60 μM phenazine methosulfate (PMS) solution to the previous mixture and the combined mixture was incubated at 25°C for 5 min. The absorbance was measured at 560 nm and the scavenging activity was calculated by the following equation:

Scavenging activity (%) = $[(A_0-A_1)/A_0] \times 100$

where, A_0 is the absorbance of control (mixture with 50% ethanol) at 560 nm, and A_1 is the absorbance of sample (mixture with seaweed extract) at the same wavelength.

Tyrosinase inhibition activity Tyrosinase inhibition activity was determined according to the method of Kim *et al.* (16). Tyrosinase (100 unit/mL), 60 mM potassium phosphate buffer (pH 6.8) and 0.4 mL of 10 mM DOPA (dihydroxyphenylalanine) were mixed. The mixture solution was added to 0.2 mL of seaweed extracts dissolved in distilled water and the absorbance was measured at 475 nm. Inhibition activity was calculated by the following equation:

Inhibition (%) = $[(A_0-A_1)/A_0] \times 100$

where, A_0 is the absorbance of control (mixture with distilled water) at 475 nm, and A_1 is the absorbance of sample (mixture with seaweed extract) at the same wavelength.

Antimicrobial activity The bacterial strains used to determine the antimicrobial activity of the seaweed extracts were *Escherichia coli*, *Bacillus subtilis*, and *Staphylococcus aureus*. The bacteria were cultured in LB media with 4% (v/v) inoculation for 10 hr at 37°C, and then 100 μL of bacterial culture solution was spread on agar plate. Filter paper (5 mm, diameter) was overlaid on the agar plate, followed by the injection of 20 μL of seaweed extract onto the filter paper. The plates were incubated at 37°C for 16 hr, and the diameter of clear zone around the filter paper was measured. As a control, 50% ethanol injected filter paper was also overlaid on the agar plate and the diameter of clear zone was compared.

Results and Discussion

Total phenolic contents Total phenolic contents were expressed as milligrams per gram of dry weight (dry seaweeds) based on a standard curve of gallic acid, which was expressed as milligrams per gram of GAE. As shown in Fig. 1, total phenolic content was the highest in extract of *E. cava* (2.27 mg GAE/g). The extracts of *Ishige okamurae* (0.63 mg GAE/g) and *Sargassum siliquastrum* (0.29 mg GAE/g) had relatively higher total phenolic contents than the remaining 27 seaweeds. All these three seaweeds showing relatively high total phenolic contents were brown algae, and especially *S. siliquastrum* showed the highest total phenolic contents among the seven

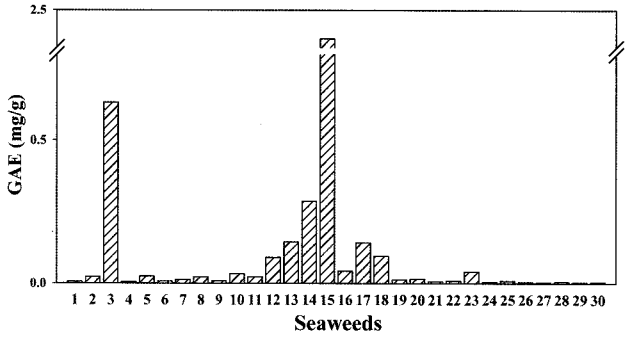

Fig. 1. Total phenolic contents of the 30 seaweed extracts. Total phenolic content is expressed as milligrams of phenolic contents/ gram of dry seaweed based on gallic acid as standard.

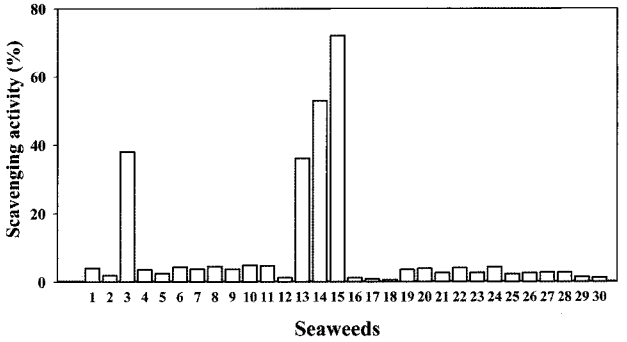

Fig. 3. Superoxide anion scavenging activities of the 30 seaweed extracts.

Sargassum spp. from the 30 seaweeds.

DPPH free radical and superoxide anion scavenging activities Free radical scavenging capacities of the seaweed extracts measured by DPPH assay are shown in Fig. 2. The *S. siliquastrum* (83.9%), *E. cava* (78.9%), and *I. okamurae* (42.3%) extracts showed strong DPPH scavenging activity among the 30 seaweeds. The other seaweed extracts exhibited less than 20% DPPH scavenging activity. The extracts of *S. siliquastrum* and *E. cava* possessed strong DPPH radical scavenging activity with a lower IC_{50} value (19.9 ± 1.5 and 18.1 ± 3.2 μg, respectively) than those of BHT (26.1 ± 8.5 μg), (+)-catechin (37.3 ± 9.7 μg), α-tocopherol (18.6 ± 2.8 μg), and EGCG (22.4 ± 3.8 μg) (data not shown).

The superoxide anion scavenging activity was determined by PMS/NADH-NBT system (Fig. 3). Superoxide anion scavenging activities of seaweed extracts were in the order of *E. cava* (72.0%) > *S. siliquastrum* (52.9%) > *I. okamurae* (38.0%) > *S. nigrifolium* (36.1%). According to the results, the extracts of *E. cava, S. siliquastrum, and I. okamurae* exhibited high values of total phenolic contents, DPPH radical scavenging activity, and superoxide anion scavenging activity. Total phenolic contents were in the order of *E. cava* > *I. okamurae* > *S. siliquastrum*, but the general antioxidant capacity [(DPPH scavenging activity + superoxide anion scavenging activity) / 2] of the three seaweeds extracts was in the order of *E. cava* (75.5%) > *S. siliquastrum* (68.4%) > *I. okamurae* (40.2%). Although the extract of *E. cava* had approximately 8-fold higher phenolic content than that of *S. siliquastrum*, they did not show a large difference in antioxidant activity. This result indicates that the antioxidant activity of *S. siliquastrum* extract might be induced by other antioxidative compounds such as fucoxanthin, as well as phenolic compounds (17).

Fig. 4 shows the correlation between total phenolic content and antioxidant activities in the 30 seaweed extracts. These results indicate the strong association between total phenolic content and antioxidant activities, suggesting that phenolic compounds play an important role in the antioxidant activities of those algae. A similar result was reported by Pyo *et al.* (18), who observed a positive correlation between radical scavenging activity and total phenolic content. In addition, phenolic compounds in fruit juice, some vegetables, and grains were suggested to be major contributors to the antioxidant activity (19, 20).

Antimicrobial activity To examine the antibacterial activity of seaweed extracts, two gram-positive (*B. subtilis, S. aureus*) and one gram-negative (*E. coli*) bacteria were tested (Table 2). Among the 30 seaweeds, the exhibited antibacterial activity against *E. coli* was the highest by the extract of *Jania adhaerens*, followed by *Caulerpa okamurae*, while *Codium fragil* and *Sargassum horneri*

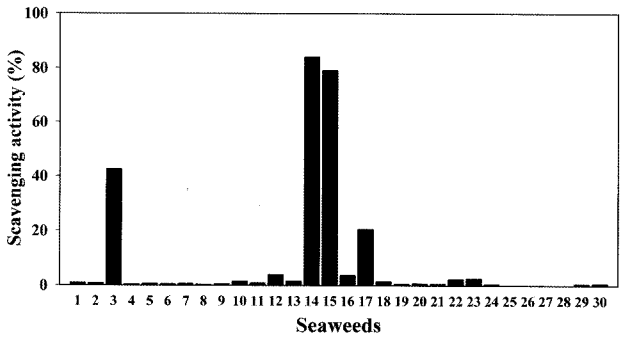

Fig. 2. DPPH radical scavenging activities of the 30 seaweed extracts.

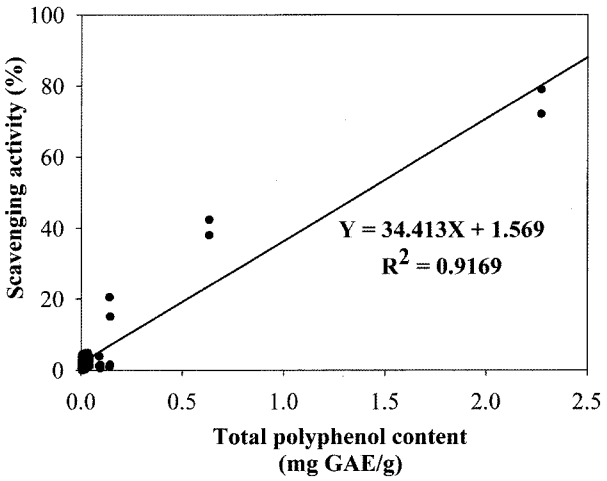

Fig. 4. Correlation between total phenolic content and scavenging activities of the 30 seaweed extracts.

Table 2. Antibacterial activities of the 30 seaweeds extracts

Algae	Diameter of clear zone (mm)*		
	E. coli	*B. subtilis*	*S. aureus*
Chondrus occellatus	0	3 ± 0.02	0
Caulerpa okamurae	7 ± 0.28	9 ± 0.16	2 ± 0.01
Ishige okamurae	3 ± 0.02	5 ± 0.21	6 ± 0.24
Jania adhaerens	7 ± 0.12	7 ± 0.11	0
Codium fragile	1 ± 0.04	10 ± 0.4	2 ± 0.04
Prionitis cornea	2 ± 0.02	6 ± 0.01	0
Gelidiun elegans	5 ± 0.34	6 ± 0.01	0
Cladophoropsis zollingeri	3 ± 0.32	9 ± 0.02	5 ± 0.24
Cladophora opaca	0	6 ± 0.21	3 ± 0.01
Myelophycus simplex	1 ± 0.01	7 ± 0.02	1 ± 0.03
Laurensia nipponica	0	8 ± 0.12	0
Lomentaria catenat	0	6 ± 0.04	3 ± 0.02
Sargassum nigrifolium	2 ± 0.24	6 ± 0.24	0
Sargassum siliquastrum	0	7 ± 0.01	0
Ecklonia cava	3 ± 0.03	7 ± 0.32	5 ± 0.04
Sargassum hemiphyllum	0	8 ± 0.02	0
Grateloupia elliptica	0	6 ± 0.32	0
Sargassum horneri	0	10 ± 0.28	2 ± 0.01
Colpomenia sinuosa	2 ± 0.03	7 ± 0.24	1 ± 0.01
Pterocladiella tenuis	4 ± 0.01	8 ± 0.02	0
Enteromorpha intestinalis	0	3 ± 0.01	3 ± 0.32
Sargassum thunbergii	0	0	0
Sargassum ringgoldianum	0	2 ± 0.24	0
Trematocarpus pygmaeus	0	0	0
Ulva lactuca	0	2 ± 0.16	0
Champia parvula	4 ± 0.02	0	0
Sargassum yezoens	0	0	0
Prionitis divaricata	0	0	0
Undaria pinnatifida	0	0	0
Laminaria japonica	0	3 ± 0.32	0

*The experiments were performed in triplicate and all values are expressed as mean ± S.D.

showed the highest antibacterial activities against *B. subtilis*. The antibacterial activity against *S. aureus* was in the order of *I. okamurae* > *Cladophoropsis zollingeri* = *E. cava* > *Cladophora opaca* = *Lomentaria catenat* ≥ *Enteromorpha intestinalis*. The extracts of *Sargassum thunbergii, Trematocarpus pygmaeu, Sargassum yezoens, Prionitis divaricata,* and *Undaria pinnatifida* had no antibacterial effects against any of the tested bacterial strains, and the extracts of *Chondrus occellatus, Laurensia nipponica, S. siliquastrum, Sargassum hemiphyllum, Grateloupia elliptica, Sargassum ringgoldianum, Ulva lactuca,* and *Laminaria japonica* showed antibacterial activities only against *B. subtilis*. The seaweed extracts exhibiting antibacterial effects on all three tested bacterial strains were those of *C. okamurae, I. okamurae, C. fragile, C. zollingeri, Myelophycus simplex, E. cava,* and *Colpomenia sinuosa*. Of the three algae showing high total phenolic content and antioxidant activities, the extracts of *E. cava* and *I. okamurae* had bacterial growth inhibitory effect on all tested strains but *S. siliquastrum* extract had an effect only on *B. subtilis*.

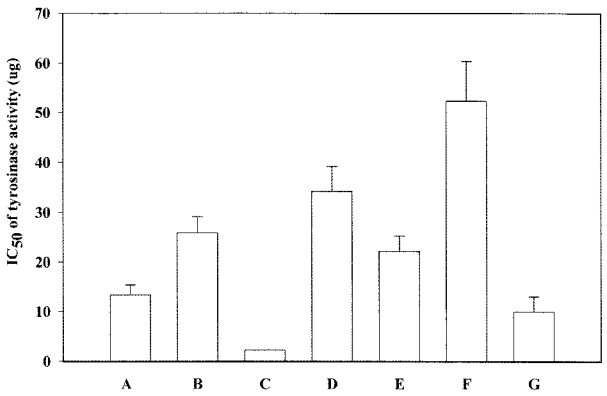

Fig. 5. Tyrosinase inhibitory activities of *E. cava* and *S. siliquastrum* extracts. The inhibitory activity is expressed as the concentration for 50% inhibition of tyrosinase activity. The values are expressed as mean ± S.D. (n=3). A, E. cava; B, S. siliquastrum; C, ascorbic acid; D, BHT; E, α-tocopherol; F, EGCG; G, (+)-catechin.

The inhibitory effects of phenolic compounds isolated from plants on growth and enzyme activity of bacteria and yeast have been reported (21, 22). Furthermore, extracts from several seaweeds were found to have antibacterial and antifungal compounds against gram-negative and gram-positive bacteria, mycobacteria, yeasts and fungi (23). In this study, except a few species, most of the seaweed extracts showed antibacterial effect with different activities against each strain, and specifically the seaweed extracts exhibiting high phenolic content and antioxidant activity had broad activity against the target micro-organisms.

Tyrosinase inhibition activity The tyrosinase inhibitory effects of *E. cava* and *S. siliquastrum* extracts which showed high antioxidant activities as well as high phenolic contents were investigated by recording the change in absorbance at 475 nm due to dopachrome formation from L-tyrosine or L-DOPA, and their IC_{50} values (μg) of tyrosinase activities were compared with those of synthetic antioxidants. As shown in Fig. 5, ascorbic acid (2.2 ± 0.1 μg) showed the highest inhibitory activity to tyrosinase. The *E. cava* (13.3 ± 2.0 μg) and *S. siliquastrum* (25.8 ± 3.2 μg) extracts had higher activities to tyrosinase inhibition than BHT (34.1 ± 5.0 μg) and EGCG (52.3 ± 8.0 μg). These results suggested that both seaweed extracts might include various antioxidative materials as well as phenolic compounds, which could have a higher tyrosinase inhibitory activity than well-known antioxidants.

In summary, to develop marine bioresources and functional marine materials, 50% ethanol extracts of thirty seaweeds that were collected in two coastal regions of Korea were screened for their total phenolic contents and antioxidant and antibacterial activities. The total phenolic contents of seaweed extracts varied in the range of 0.01-2.27 mg/g and the three algae which showed the highest DPPH radical and superoxide anion scavenging activities had the highest phenolic contents, suggesting that total phenolic content was correlated with antioxidant activity in the three seaweeds. *E. cava* and *S. siliquastrum* extracts had higher DPPH radical scavenging activity than those of

(+)-catechin, BHT, and EGCG and showed stronger tyrosinase inhibitory activity than those of BHT and EGCG.

The present data indicated that antioxidative property increased with increasing phenolic compound content, confirming that seaweeds can be considered as a potential source for the extraction of phenolic compounds for use as natural antioxidants, pharmaceutical agents, dietary supplements or products in the food industry. Considering the acute need for efficient dietary antioxidants to moderate against the exposure to excessive stresses in modern life, the development of natural antioxidant resources able to effectively block or delay the oxidation pathway, unlike the synthetic antioxidant compounds such as BHT or BHA which have found limited use due to toxicity, is important (24). Consequently, of the seaweeds used for this study, *S. siliquastrum* and *E. cava* have shown potential as marine natural antioxidant resources and raw materials for food supplements. Further studies are needed to isolate and characterize individual compounds of the extracts and to elucidate possible mechanisms for the results observed in this study.

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