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Antioxidant Activity of Sulfated Polysaccharides Isolated from *Sargassum fulvellum*

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Abstract

***Sargassum fulvellum*, a marine brown alga, is a popular low priced edible plant in Korean markets. The polysaccharide fraction of the alga was separated and investigated for its radical scavenging activities and the results compared with those of commercial fucoidans (*Fucus vesiculosus* and *Undaria pinnatifida*), BHA and α-tocopherol. The polysaccharide fraction of *S. fulvellum* showed a promising DPPH radical scavenging activity than did other fucoidans. Moreover, the sample exhibited a dose-dependent activity on hydrogen peroxide scavenging activity in the V79-4 cell line. Interestingly, all the tested polysaccharide counterparts were more potent NO· scavengers than were the commercial antioxidants, BHA and α-tocopherol. The sulfated polysaccharide of *S. fulvellum* had an approximate molecular weight of 529 kDa and mainly consisted of fucose and galactose, and minor amounts of mannose, rhamnose and xylose.**

Key words: *Sargassum fulvellum*, sulfated polysaccharide, antioxidant activity, hydrogen peroxide scavenging

INTRODUCTION

Edible seaweeds have good nutritional value as a source of minerals, vitamins, and non-caloric dietary fiber. The dietary fibers are considered to be important treatments for constipation, colon cancer, cardiovascular diseases and obesity. Seaweeds contain higher amounts of soluble and non-soluble fiber (polysaccharides) than fruits and vegetables. Moreover, sulfated polysaccharides of brown algae exert diverse biological activities, including anticoagulant, antihyperlipidaemic, antiviral and antitumour activities. In recent years, algal polysaccharides have been demonstrated to play an important role as free radical scavengers and antioxidants for the prevention of oxidative damage in living organisms. Recently, because of demand as a health food, the average seaweed production in Korea has increased dramatically. Seaweeds have been used traditionally as food in Korea and Japan for long time, however it has also been reported that seaweeds contain a spectrum of untapped biologically active substances (1,2).

It is of interest that inhabitants of many of the longevous regions in the world have a long history of seaweed consumption. Jeju island in Korea has been known as one of the most longevous provinces of Korea since the 1980s (3). Okinawa, where seaweed consumption is very high, has been known as the most longevous region in the world. It is interesting to note that the dietary pattern and lifestyle of these two regions have been observed to be similar, where the main food dishes are comprised of boiled pork, fishes and brown seaweeds. Edible brown algae, like *Cladosiphon okamuranus Tokida* (*C. o. Tokida*) and *Sargassum fulvellum* are considered a delicacy in both Okinawa and Jeju Island, respectively, hence people of those areas have incorporated edible algal species into their diets for many years. The bioactivities of *C. o. Tokida* have been the subject of considerable research revealing that its polysaccharides have multiple health effects (4-8); however, studies on the bioactivities of *S. fulvellum* are quite limited.

Therefore, the biological activities of *S. fulvellum* polysaccharides are still unknown. In this paper, we studied the antioxidative activity of a polysaccharide fraction from *S. fulvellum* on DPPH, superoxide anion, hydrogen peroxide, hydroxyl radical and nitric oxide radical scavenging; and compared the results with those of commercially available polysaccharides.

MATERIALS AND METHODS

Materials

Commercial fucoidan (from *Fucus vesiculosus*), butylated hydroxytoluene (BHT), α-tocopherol, dimethyl sulfoxide (DMSO), 1,1-diphenyl-2-picrylhydrazyl (DPPH),

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nitro blue tetrazolium salt (NBT), xanthine, xanthine oxidase (XOD), folin-ciocalteu reagent, sodium nitroprusside, sulfanilic acid xanthine, ethylenediaminetetra acetic acid (EDTA) and 2',7'-dichlorodihydrofluorescein diacetate (DCFH-DA), were purchased from Sigma Co. (St. Louis, USA). N-(1-Naphthyl) ethylenediamine dihydrochloride was purchased from Hayashi Pure Chemical Industries Ltd. (Osaka, Japan). Peroxidase, 2,2-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) (ABTS), $FeSO_4 \cdot 7H_2O$ and deoxyribose were purchased from Fluka Co. (Buchs, Switzerland).

Preparation of algal polysaccharide samples

S. fulvellum was obtained from Dongmun market in Jeju City of S. Korea in November 2006. Epiphytes, salt and sand were removed using tap water and the samples were rinsed with deionized water before freeze-drying. Then, freeze dried *S. fulvellum* was pulverized into a fine powder. The extraction and partial purification of *S. fulvellum* was followed by a slightly modification of the methods of Koo et al. (9) and Duarte et al. (10). A twenty-gram of the dried *S. fulvellum* powder was refluxed in 85% methanol (1,000 mL) 2 times for 2 hr and vacuum filtered using Whatman No.1 filter paper. Residue was extracted with diluted-HCl (pH 2, 100°C) for 1 hr. After neutralizing, the filtrate was precipitated with $CaCl_2$ and supernatant was dialyzed (MWCO 10～12 kDa) against water, and precipitated again with ethanol. The precipitate was lyophilized and designated as crude polysaccharide sample. The crude polysaccharide sample was dissolved in water (11%) and was precipitated with cetylpyridiniumchloride. The precipitate was dissolved in 3 M $CaCl_2$ and precipitated again with ethanol and then centrifuged at 8,000 rpm for 20 min. The supernatant was dialyzed against water and lyophilized. This was used as a partially purified sample for bioactivity test. The yield of the separated polysaccharide was 2.5% from raw material on a dry weight basis. For its comparison, a galacto-fucoidan purified from *U. pinnatifida* was purchased from Heawon Biotech Inc. (Seoul, Korea) as commercial counterpart. However, the sample was not readily dissolved in water, so the sample was treated as mentioned above and the resultant fraction was investigated for its antioxidant activity. An 85% purified fucoidan isolated from *Fucus vesiculosis* was purchased from Sigma and used for comparison. All the purified materials from *S. fulvellum* and *U. pinnatifida* were dissolved with PBS (Sigma) and sterilized by a 0.45 μm syringe filter (Millipore, Carrigtwohill, Ireland).

DPPH radical scavenging assay

DPPH radical scavenging activities of *S. fulvellum* and *U. pinnatifida* polysaccharides were measured according to a modified method of Brand-Williams (11). Freshly prepared 2 mL DPPH (3×10^{-5} M in DMSO) solution was thoroughly mixed with 2 mL of polysaccharide samples. The reaction mixture was incubated for 1 hr at room temperature. An absorbance of the resultant mixture was recorded at 517 nm using a UV-VIS spectrophotometer (Opron 3000 Hanson Tech. Co. Ltd., Seoul, Korea). Scavenging activity was calculated by the following formula:

$$\text{Scavenging activity} = [1-(Ai-Aj)/Ac]\times100$$

where Ai is the absorbance of organic solvent extract mixed with DPPH solution; Aj is the absorbance of same organic extract mixed with 2 mL DMSO; Ac is the absorbance of DPPH solution adding 2 mL DMSO.

Superoxide anion ($O_2^{\cdot-}$) scavenging assay

The superoxide scavenging abilities of the polysaccharide samples were assessed by the method of Nagai (12). The reaction mixture contained 0.48 mL of 0.05 M sodium carbonate buffer (pH 10.5), 0.02 mL of 3 mM xanthine, 0.02 mL of 3 mM EDTA, and 0.02 mL of 0.15% bovine serum albumin, 0.02 mL of 0.75 mM NBT and 0.02 mL of the polysaccharide samples. After incubation at 25°C for 20 min, 6 mU XOD was added to the mixture to initiate the reaction, which was carried out at 25°C for 20 min. The reaction was terminated by adding 0.02 mL of 6 mM $CuCl_2$. An absorbance of the mixture was recorded at 560 nm.

Hydrogen peroxide (H_2O_2) scavenging assay

The hydrogen peroxide scavenging abilities of the polysaccharide samples were investigated based on the scavenging of the hydrogen peroxide in ABTS-peroxidase system described by Muller (13). Eighty microliter of each polysaccharide sample and 20 μL of 10 mM hydrogen peroxide were mixed with 100 μL of phosphate buffer (pH 5.0, 0.1 M) in a 96-microwell plate and the samples were incubated at 37°C for 5 min. Subsequently, 30 μL of freshly prepared ABTS (1.25 mM) and 30 μL of peroxidase were added and incubated at 37°C for another 10 min. An absorbance of the resulting mixture was recorded using an ELISA reader (Sunrise; Tecan Co. Ltd., Salzburg, Austria) at 405 nm.

Hydroxyl radical (HO·) scavenging assay

Ability of the polysaccharide samples to scavenge HO· generated by Fenton reaction was measured according to the following method (14). The Fenton reaction mixture containing 200 μL of 10 mM $FeSO_4 \cdot 7H_2O$, 200 μL of 10 mM EDTA and 200 μL of 10 mM 2-deoxyribose was mixed with 1.2 mL of 0.1 M phosphate buffer (pH 7.4) containing 200 μL of polysaccharide samples.

Thereafter, 200 μL of 10 mM H_2O_2 was added to the mixture before incubation at 37°C for 4 hr. After incubation, 1 mL of 2.8% TCA and 1 mL of 1% TBA were added and placed in the boiling water bath for 10 min. Then, the resultant mixture was allowed to cool to room temperature and centrifuged at 395×*g* for 5 min. The absorbance was recorded at 532 nm.

Nitric oxide radical (NO·) scavenging assay

Aqueous solutions of sodium nitroprusside at physiological pH (7.4) spontaneously produce nitric oxide, which reacts with oxygen to produce nitrite ions, which can then be determined by the use of Griess Illosvoy reaction (15). Griess Illosvoy reagent was slightly modified using naphthylethylenediamine dihydrochloride (0.1% w/v) instead of 1-naphthylamine (5%). Scavengers of nitric oxide compete with oxygen and reduce the production of nitric oxide. The reaction mixture (3 mL) containing 2 mL of 10 mM sodium nitroprusside, 0.5 mL of phosphate buffer saline (pH 7.4, 0.01 M) and 0.5 mL of polysaccharide was incubated at 25°C for 150 min. Thereafter, 0.5 mL of the reaction mixture containing nitrite was pipetted and mixed with 1 mL of sulfanilic acid reagent (0.33% in 20% glacial acetic acid) and allowed to stand for 5 min to complete diazotisation. Then, 1 mL of naphthylethylenediamine dihydrochloride (0.1%) was added, and allowed to stand for 30 min in diffused light. An absorbance of the pink coloured chromophore was measured at 540 nm.

Hydrogen peroxide scavenging activity in V79-4 cells

The Chinese hamster lung fibroblast cell line (V79-4) was maintained in DMEM medium containing 10% heat-inactivated fetal calf serum, streptomycin (100 μg/mL) and penicillin (100 μg/mL) under humidified atmosphere of 5% CO_2 at 37°C. For the assay, the cells at a concentration of 1×10^4 cells/mL were seeded in a 24 micro-plate and incubated for 16 hr in a cell growth chamber. Then, the cells were treated with different concentrations of algal polysaccharides and kept in a humidified atmosphere for 30 min. After treatment with 1 mM of H_2O_2 (incubated for 30 min), 20 μL of 5 μg/mL of DCFH-DA was introduced into the medium and absorbance was detected at 535 nm using a PerkinElmer LS-5B spectrofluorometer.

Total phenolic content assay

Total phenolic content was determined according to the protocol described by Chandler and Dodds (16). One milliliter of the polysaccharide sample was mixed in a test tube containing 1 mL of 95% ethanol, 5 mL of distilled water and 0.5 mL of 50% Foiln-Ciocalteu reagent. The resultant mixture was allowed to react for 5 min and 1 mL of 5% Na_2CO_3 was added. It was thoroughly mixed and placed in dark for 1 hr and absorbance was recorded at 725 nm in a UV-VIS spectrophotometer. A gallic acid standard curve was obtained for the calculation of phenolic content.

Monosugar analysis

The monosugar composition of the samples was estimated as it has been reported previously (17). The purified polysaccharide was hydrolyzed in a sealed glass tube with 2 M of trifluoroacetic acid for 4 hr at 100°C to analyze neutral sugars. Then, 10 μL of sample was applied to CarboPac PA1 (4.5×250 mm, Dionex, Sunnyvale, CA, USA) with CarboPac PA1 cartridge (4.5×50 mm) column to analyze the neutral sugar composition. The column was eluted using 18 mM of NaOH at 1.0 mL/min flow rate. Each sugar of the sample was detected by using ED50 Dionex electrochemical detector and data were analyzed by Peack Net on-line software. Quantitative data for the sugar composition was calculated by comparing their peak areas on an HPLC chromatogram with those of the commercial sugar standards (fucose, rhamnose, arabinose, galactose, glucose, mannose and xylose).

Determination of the molecular weight of the sample

In order to determine the molecular weight of the sample, the freeze-dried sample was introduced into a PL-Aquaz OH 40 column and eluted with de-ionized water at 0.8 mL/min flow rate (23°C). Dextran standards (48.6, 148, 273, 410, 830, and 2,000 kDa) were also introduced into the column under the same experimental condition for comparison purposes. The retention time was plotted against the average molecular weight of the dextrans and used to calculate the molecular weight of the sample.

RESULTS

DPPH is extensively utilized stable free radical mediator used to evaluate the radical scavenging efficacy of plant extracts. In the presence of hydrogen donating antioxidant, the stable DPPH radical is converted into a non-radical component (DPPH-H), due to this reaction that the color of the DPPH solution is converted from purple to yellow (18-20). In this assay, all the tested polysaccharide samples showed high DPPH radical scavenging capacities (Fig. 1). Of the algal polysaccharides, the polysaccharide fraction separated from *S. fulvellum* exhibited the highest activity with a value of over 90% at 10 mg. The commercial fucoidan fraction showed almost similar activity to that of *U. pinnatifida* sample. However, none of the polysaccharide samples in this

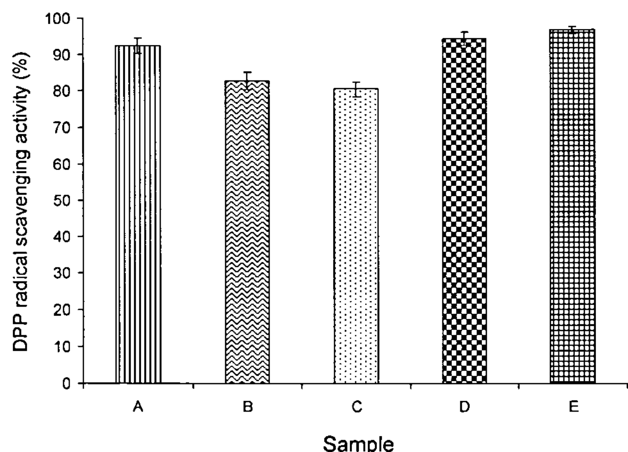

Fig. 1. The DPPH radical scavenging activity of algal polysaccharides and conventional antioxidants. (A) *S. fulvellum* polysaccharide sample, (B) commercial fucoidan, (C) *U. pinnatifida* polysaccharide sample, (D) BHT and (E) α-tocopherol. All the data points are means of three determinations. The sample concentration of polysaccharide was 10 mg/mL and that of commercial counter parts was 2 mg/mL.

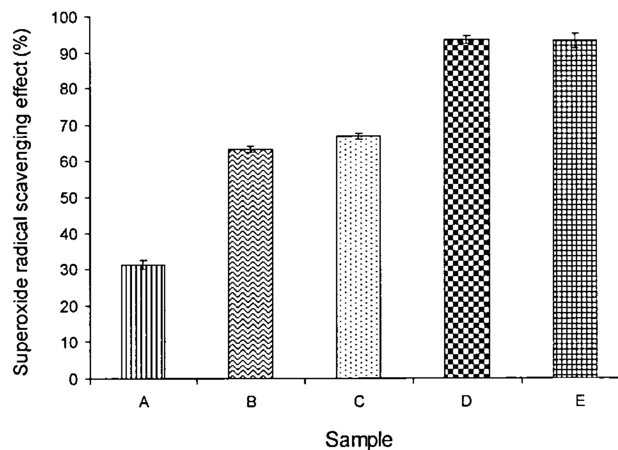

Fig. 2. The superoxide radical scavenging activity of algal polysaccharides and conventional antioxidants. (A) *S. fulvellum* polysaccharide sample, (B) commercial fucoidan, (C) *U. pinnatifida* polysaccharide sample, (D) BHT and (E) α-tocopherol. All the data points are means of three determinations. The sample concentration of polysaccharide was 10 mg/mL and that of commercial counter parts was 2 mg/mL.

study exceeded the activity of the commercial antioxidant counterparts. The hydrogen ion donation ability of polysaccharides is believed be correlate closely with their DPPH radical scavenging ability. The polysaccharide purified from *Lygodium japonicum* showed a dose-dependant DPPH radical scavenging effect; however, the investigators observed a remarkable effect at high concentrations (19).

Superoxide anions are week oxidants, but they can create several kinds of very harmful radicals which can create/initiate lipid peroxidation. In this assay, the commercial fucoidan sample and *U. pinnatifida* samples showed fairly strong activity (>50%). Among the tested samples, the polysaccharide separated from *S. fulvellum* showed the least activity (Fig. 2). The BHT and the α-tocopherol samples showed similar activity towards superoxide radical inhibition. Sulfated polysaccharides isolated from marine algae have been previously to reported quench superoxide radicals. The polysaccharide fractions (F_1, F_2 and F_3) of *Porphyra haitanesis* exhibit high superoxide radical inhibition at very low concentrations (IC_{50} from 0.06 to 1.6 mg/mL) (20). Superoxide radical is one of the main causes of oxygen cytotoxicity; it is the first oxygen radical produced *in vivo*, and lasts for a longer time than other radicals. A low molecular weight sulfated polysaccharide (LMWF) prepared from *Laminaria japonica* exhibited ~75% superoxide radical ameliorating effect at 0.4 mg/mL (21). Polysaccharides extracted from *Ulva pertusa* (Chlorophyta) showed an IC_{50} value of 22.1 μg/mL for superoxide radical inhibition (22).

Hydrogen peroxide itself is not very reactive, but sometimes it is toxic to cells because it may give rise to hydroxyl radicals in the cells (23). Hence, compounds with good hydrogen peroxide scavenging ability are considered physiologically important. The commercial fucoidan sample exhibited the most potent hydrogen peroxide scavenging activity (>90); in contrast, the other polysaccharide samples showed 70~80% inhibition (Fig. 3). At the tested sample concentration (2 mg/mL), BHT was less effective than was the commercial fucoidan. Obviously, in this assay, the tested commercial anti-

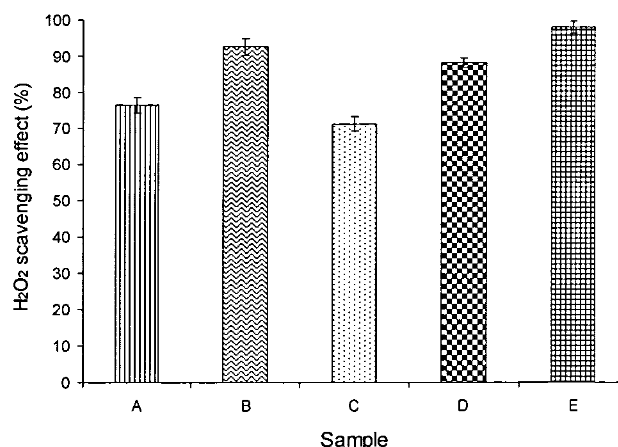

Fig. 3. The hydrogen peroxide scavenging activity of algal polysaccharides and conventional antioxidants. (A) *S. fulvellum* polysaccharide sample, (B) commercial fucoidan, (C) *U. pinnatifida* polysaccharide sample, (D) BHT, (E) α-tocopherol. All the data points are means of three determinations. The sample concentration of polysaccharide was 10 mg/mL and that of commercial counter parts was 2 mg/mL.

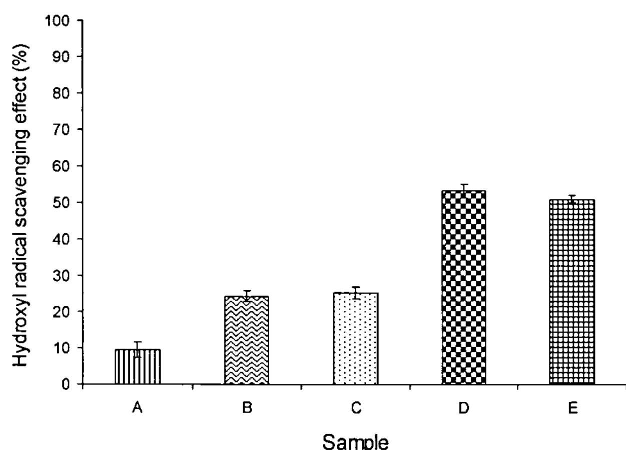

Fig. 4. The hydroxyl radical scavenging activity of algal polysaccharides and conventional antioxidants. (A) *S. fulvellum* polysaccharide sample, (B) commercial fucoidan, (C) *U. pinnatifida* polysaccharide sample, (D) BHT and (E) α-tocopherol. All the data points are means of three determinations. The sample concentration of polysaccharide was 10 mg/mL and that of commercial counter parts was 2 mg/mL.

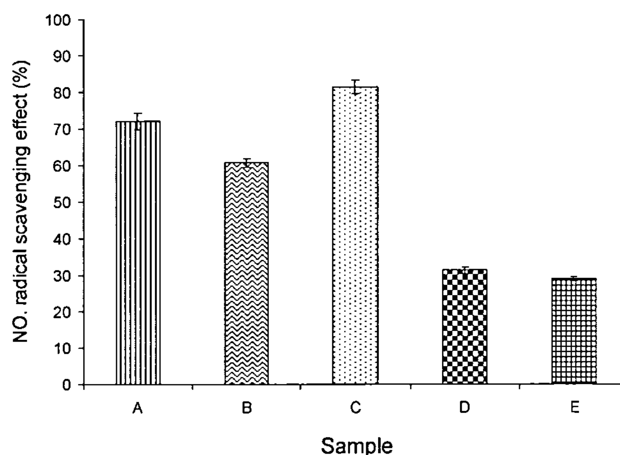

Fig. 5. The NO radical scavenging activity of algal polysaccharides and conventional antioxidants. (A) *S. fulvellum* polysaccharide sample, (B) commercial fucoidan, (C) *U. pinnatifida* polysaccharide sample, (D) BHT and (E) α-tocopherol. All the data points are means of three determinations. The sample concentration of polysaccharide was 10 mg/mL and that of the commercial counterparts was 2 mg/mL.

oxidant counterparts had ~10% activity difference. Few studies are have evaluated polysaccharides for their radical scavenging ability, however red algal polysaccharide isolated from *Porphyra haitanesis* showed ~30% inhibition at ~7 mg/mL (20).

Fig. 4 shows the hydroxyl radical scavenging activity of polysaccharides and the commercials samples. The samples showed different capacities for scavenging hydroxyl radicals under the same experimental conditions. The polysaccharide samples had less hydroxyl radical scavenging ability than commercial antioxidants, with effectiveness as follows: BHT>α-tocopherol>*U. pinnatifida* polysaccharide>commercial fucoidan>*S. fulvellum*. As it is shown, the commercial antioxidant samples also exhibited low activity on HO˙ assay. These results are in the line with our previous experiment results, and normally BHT has been shown relatively low activity on hydroxyl radical scavenging assay (24). The polysaccharide isolated from *Tinospora cordifoloa* showed good protection over ion mediated lipid peroxidation of rat brain homogenate, and the observed activity of that polysaccharide was explained by its high radical scavenging activity, especially hydroxyl radical (25).

Nitric oxide radical is an important signaling molecule in human body, however the accumulation of this radical creates adverse side effects. Therefore, compounds with high nitric oxide radical-scavenging activities are important, but fewer compounds have been documented for their NO˙ scavenging ability. The nitric oxide radical scavenging activities of the tested samples are shown in Fig. 5. All the tested polysaccharide samples showed much better NO˙ scavenging activity than those of the commercial antioxidants. *U. pinnatifida* and *S. fulvellum* samples showed superior activities to commercial fucoidan. Senevirathne et al. (26) reported IC_{50} values of 1.5 and 2.1 (mg/mL) for BHT and α-tocopherol on nitrogen oxide radical scavenging, respectively, and *Ecklonia cava*, a marine brown alga, showed pronounced activity on NO˙ scavenging. The present results of low NO˙ scavenging ability for the tested commercial samples agreed with the previous study (26).

Taken together, the tested polysaccharides showed good radical scavenging activity *in vitro*, therefore they are expected to be important in controlling the propagation of radicals in an oxidized medium. The introduction of H_2O_2 into a cultured cell medium enhances the production of harmful radical species in a number of ways resulting in oxidative stress. By reducing the amount of antioxidant enzymes and releasing LDH (lactate dehydrogenase), the presence of H_2O_2 deteriorates the intracellular homeostasis of the cells. Therefore, hydrogen peroxide scavenging activity of polysaccharides in V79-4 cells was investigated to clarify their antioxidant efficacy as an indicator of their total antioxidant capacity (Fig. 6). Interestingly, both commercial fucoidans and *S. fulvellum* produced similar results in this assay, however *U. pinnatifida* samples showed slightly higher activity (data not shown). Hence, it can be assumed that the presence of the algal polysaccharides may alleviate H_2O_2-induced oxidative stress. Even well-known (BHA and BHT), antioxidant compounds have shown variable low/high potentiality for

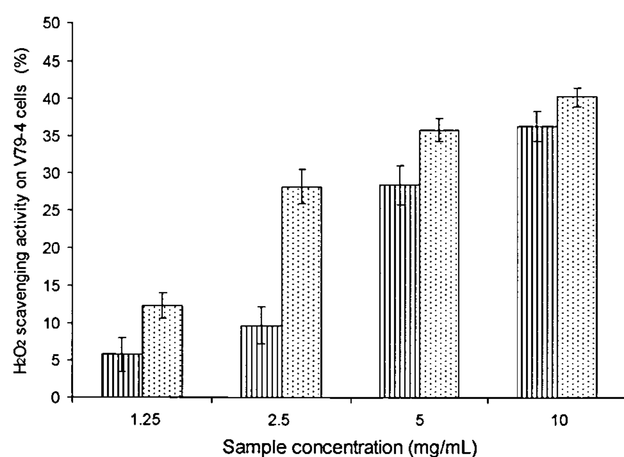

Fig. 6. Hydrogen peroxide scavenging activity for the algal polysaccharide samples on the V79-4 cell line. Experiments were performed in triplicate and the data are expressed as the average difference from control.

H_2O_2 scavenging compared with their total antioxidant capacity. However, the structural requirements for efficient quenching of hydrogen peroxide are rather more complicated than those required for radical scavenging activities (27). As has been pointed out, both M-blocks (mannuronate blocks) and esterified carboxyl residue of polysaccharide were determinant structures in preventing lymphocytes from being oxidized by H_2O_2, indicating that the existence of M-blocks is more important for scavenging free radicals (28).

To determine the sugar composition of the polysaccharide samples, we analyzed the samples by HPLC and compared the absorption spectra with known sugar standards (Fig. 7A). Moreover, the monosugar composition of the polysaccharide isolated from *S. fulvellum* was compared with those of *U. pinnatifida* and the commercial fucoidan (Table 1). The fucoidan sample was mostly composed of fucose (~88%) and small amount of glucose and xylose (5~6%). *S. fulvellum* showed considerably more xylose (~16%), mannose (~6) and rhamnose (~2%) than those of the other polysaccharides (Fig. 7B). Moreover, the isolated polysaccharide showed a 529.11 kDa molecular weight (Fig. 8) and contained a considerable amount of sulfate (0.6 sulfate/total sugar). Therefore, the sample can be designated as a sulfated polysaccharide. The *U. pinnatifida* which is known as a galacto-fucan is composed mostly of galactose and fucose (Fig. 7C), and high amounts of sulfate. In this study, potent antioxidant potential was reported from the commercial fucoidan, similarly, the F2 fraction of *P. haitanesis*, composed mostly of galactose, fucose and xylose, showed very high hydroxyl radical scavenging and lipid peroxidation scavenging activities (20).

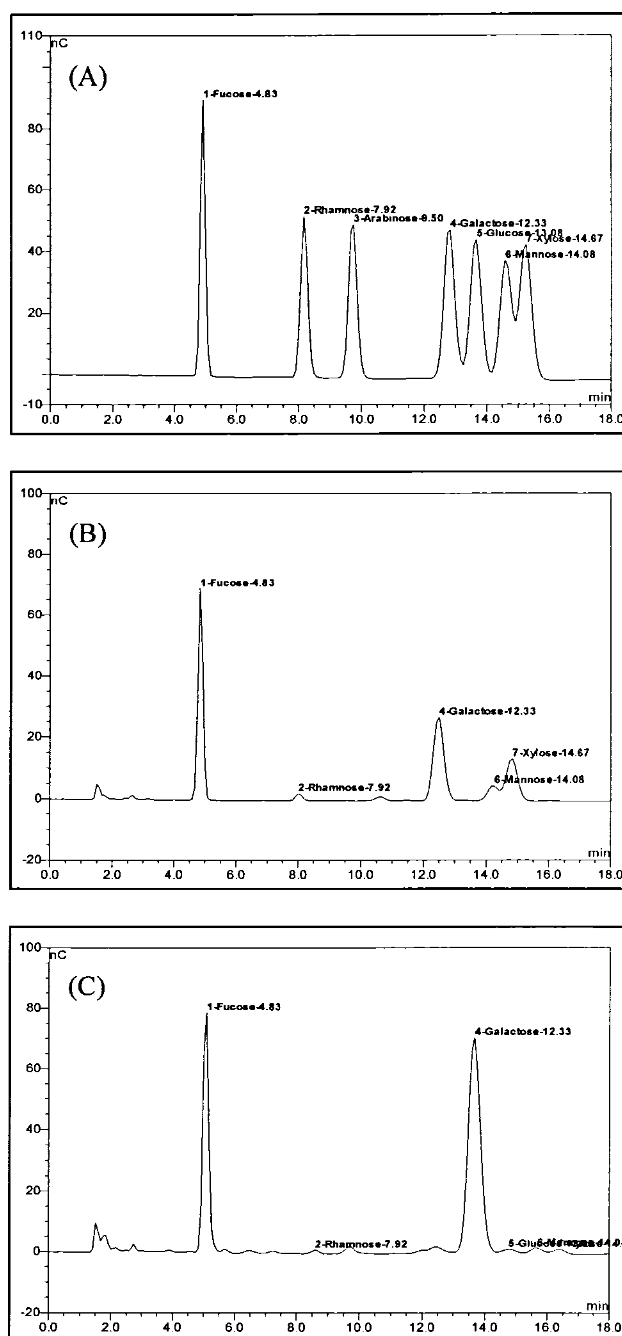

Fig. 7. The HPLC chromatograms for the monosugar composition of the algal polysaccharide. Chromatograms for (A) the sugar standards, (B) the *S. fulvellum* polysaccharide sample, and (C) the *U. pinnatifida* sample.

The tested polysaccharides were subjected to total phenolic assay to estimate their associated phenolic content (Table 2). Of the tested samples, the commercial fucoidan sample had the highest amount of phenolic compounds (696 mg/100 g). The content of phenolic compounds in *S. fuluvalum* and *U. pinnatifida* samples were almost half that of the commercial fucoidan. Phenolic compounds are known for their high antioxidant activity, therefore the

Table 1. Sugar composition of the polysaccharide samples

Sugar	A	B	C
Fucose	88.73	44.32	38.11
Rhamnose	0	2.57	1.03
Galactose	6.13	30.35	57.81
Glucose	0	0	0.87
Mannose	0	6.46	1.28
Xylose	5.12	16.27	0.85
Sulfate / total sugar	0.98	0.61	0.99

A: commercial fucoidan, B: *Sargassum fulvellum*, C: *Undaria Pinnatifida.*

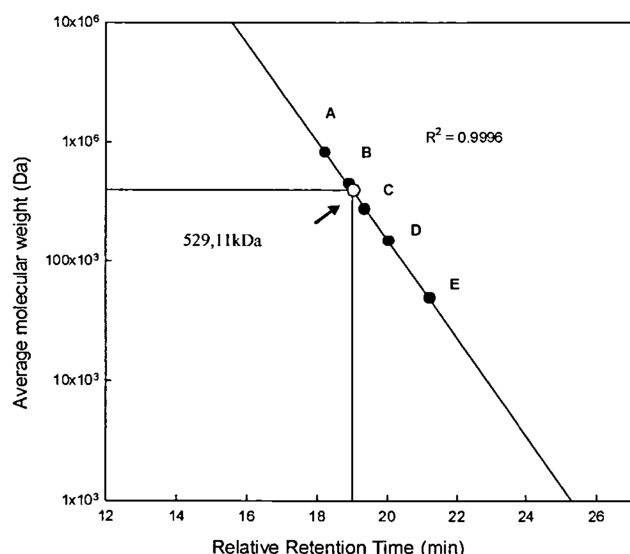

Fig. 8. Calibration curve of dextran standards for the determination of the average molecular weight of the *S. fulvellum* polysaccharides. The retention time is plotted against the molecular weight of the dextrans (A) 830 kDa, (B) 450 kDa, (C) 273 kDa, (D) 148 kDa, and (E) 48.6 kDa.

Table 2. Total phenolic content of the polysaccharide samples

Sample	Content[1)]
Commercial fucoidan	696.22
Sargassum fulluvalum	378.94
Undaria pinnatifida	315.67

[1)]mg/100 g of the sample.

observed antioxidant activity in this study is most probably due to a combined effect of polysaccharides and phenolic compounds.

DISCUSSION

Seaweeds are a renewable natural source with diverse biological activities. The edible algae are rich in a wide variety of nutrients which are essential for a healthy life. Hence, the edible algae are not only in the market of Asian countries but also highly available in some European countries due to their nutraceutical value. In this study, polysaccharides separated from the low prized edible seaweed, *S. fulvellum*, were investigated for their antioxidant activities and the results compared with those of fucoidan and commercially available synthetic counterparts (BHT and α-tocopherol).

Taken together, the results suggest that *S. fulvellum* crude polysaccharide is a bioactive complex with potential bioactivity which is important in food industry. In a previous study, a polysaccharide (59%) combined with ~4% of polyphenolic compounds was the main active compound of *Cochlospermum tinctorium*, an herb collected from the West African country, Mali. The sample showed high radical, and immunomodulatory activity *in-vitro*, and polysaccharide (pectic arabinogalactans type II) and polyphenolic (gallotannins and ferulic acids) compounds were the main active principals of the tested herb (29). Porphyran, a polysaccharide isolated from marine red alga, has been reported to exert antioxidant activity in aging mice, the polysaccharide reduced the onset of lipid perixidation and thereby successfully controlled lipid peroxidation (20). Polysaccharides are believed to promote antioxidant activity because they exhibit a greater ease of abstraction of the anomeric hydrogen from the internal monosaccharides (24). The available dietary fiber content of *Laminaria* sp. is reportedly around 36 g/100 g (30). In a previous study, an antioxidant polysaccharide purified from *Lycium barbarum* composed of D-rhamnose, D-xylose, D-arabinose, D-fucose, D-glucose, and D-galactose showed a pronounced activity on the β-carotene-linoleate model and DPPH radical scavenging assays (31). Also, polysaccharide produced by *Rhizobium meliloti* (RPS), xanthan, curdlan, and carboxymethylcellulose (CMC) were comparable to tertiary butylhydroxyquinone (TBHQ) for their inhibition of autoxidation in sunflower oil-in water emulsions. The antioxidant activity of xanthan, curdlan, and RPS at concentration of 40 mg/100 g emulsion was equal to that of TBHQ in a 20 mg/100 g emulsion (32). Polysaccharides purified from mushroom and *Keissleriella* sp. YS 4108 exopolysaccharide were also reported to have free radical scavenging effects related to its affinity to the radical in the specific site (33,34).

The amorphous, slimy fraction of brown algae fiber consists mainly of water-soluble alginates and/or fucoidan (35,36). The consumption of dietary fiber processes multifunctional health benefits, especially dietary fiber regulates proper function of the intestinal flora, reduces the glycemic response, enhances the stool volume and greatly reduces the risk of colon cancer risk (30). Their possible effects on lipid and cholesterol digestion and

absorption have been reviewed by taking into account their gel formation, faecal bulking and binding capacities and fermentability (37).

However, few studies have focused on the antioxidant potential of algal polysaccharides. In this study, though the tested polysaccharide concentrations are high for their antioxidant activity, the addition of antioxidant activity of the polysaccharides may expand their pharmaceutical value. In addition, the effects were shown at concentrations readily achievable at a reasonable intake of *S. fulvellum*, provided that bio-availability is not a problem.

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