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Full Length Research Paper

Evaluation of *in vitro* antioxidant and antibacterial activities of *Laminaria japonica* polysaccharides

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In the present study, *Laminaria japonica* polysaccharides was investigated for its free radical scavenging activity using three different *in vitro* assays and antibacterial activity. *Laminaria japonica* polysaccharides effectively scavenged the following free radicals: superoxide radical (O_2^-), hydroxyl radicals (OH^-) and 1,1-diphenyl-picrylhydrazyl radical ($DPPH^-$). The radical scavenging effect was found to be concentration-dependent. Also, *L. japonica* polysaccharides exhibited an inhibitory effect on several bacterial *in vitro*. Of several bacteria, *Escherichia coli* and *Staphylococcus aureus* are most sensitive to the polysaccharides. In conclusion, findings of the present study strongly suggest a free radical scavenging and antibacterial role for *L. japonica* polysaccharides.

Key words: *Laminaria japonica* polysaccharides, antioxidant, hydroxyl radicals.

INTRODUCTION

Numerous effective anticancer drugs have been developed from natural sources, but an important untapped resource in seaweeds remains to be exploited. Among them, a brown alga, *Laminaria japonica*, is commonly consumed in Korea, Japan, and China, and has been used for more than 1000 years as a drug in traditional Chinese medicine (Go et al., 2010). Fucoidan extracted from *L. japonica* is a heteropolysaccharide, mainly made of fucose, galactose and sulfate, with smaller amounts of mannoses, glucuronic acid, glucose, rhamnose, arabinose and xylose. Fucoidan could purify into several fractions from high-uronic-acid-, low-sulfate-, fucose-containing polymers to highly sulfated fucoidan. Evidence from different studies suggested that the antioxidant and anticoagulant activities of polysaccharides were strongly dependent on the degree of sulfation (DS), the molecular weight, the sulfation pattern and glycosidic branches (Han et al., 2002; Wang et al., 2008). It is now accepted that free radical-mediated oxidation of biological molecules such as lipids, proteins, and DNA is involved in a variety of disorders and diseases (Massaeli et al., 1999). Above all, lipids are very susceptible to free radical attack and lipid peroxidation induces alterations in integrity, disturbances in fine structure, and functional loss of biomembranes (Sun, 1990). Furthermore, lipid peroxidation mediated by free radicals proceeds by a chain mechanism, amplifying the damaging effect of free radicals (Indira et al., 2003). Lipid peroxidation products are potentially cytotoxic and modify proteins and DNA (Tsiapali et al., 2001). It is now accepted that lipid peroxidation is involved in the pathogenesis of various diseases (Massaeli et al., 1999; Indira et al., 2003) and consequently the role of free radical-scavenging antioxidants has received much attention. In addition to synthetic antioxidants, natural antioxidants have been prepared and their antioxidant capacity has been assessed for prevention of lipid peroxidation in various systems.

Since ancient times, antimicrobial properties of herbs and spices have been used for food preservation (Zaika, 1988; Conner, 1993). Naturally occurring antimicrobial

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agents reported date back to more than a century (Maruzzella and Sicurella, 1960). Antimicrobials in extracts from seaweeds have been explored since the 1950s (Glombitza, 1979; Nobile et al., 2009). A renewed interest in natural preservation appears to be stimulated by present food safety concerns, growing problems with microbial resistance, and a rise in production of minimally processed food together with "green image" policies of food industries (Suhr and Nielsen, 2003). In this paper, *in vitro* antioxidant activity of *L. japonica* polysaccharides was evaluated. In addition, antibacterial effects of *L. japonica* polysaccharides were also examined.

MATERIALS AND METHODS

Preparation of the polysaccharides

L. japonica sample (300 g) was extracted by boiling water. The water extraction solutions were separated from insoluble residue by centrifugation (10,000 rpm for 15 min), and then precipitated by the addition of ethanol. The precipitate was filtered and dried in an oven at 50°C for 24 h. The dried crude polysaccharides were refluxed three times to remove lipids with acetone and chloroform. The result product was extracted in hot water and then filtered, and the combined filtrate was precipitated using ethanol again. The content of the polysaccharides was measured by Phenol-Sulfuric acid methods (Dubois, 1956).

Scavenging effect on superoxide anion radicals

Superoxide anion radical scavenging activity was determined using a commercial kit (Jiancheng Bioengineering Institute, Nanjing, China). The superoxide anion radicals were generated by the xanthine/xanthine oxidase system and reacted with 2,4-iodiphenyl-3,4-nitrophenyl-5-phenyltetrazolium chloride to form formazan, a coloured compound which can be spectrophotometrically quantified at 550 nm. The production of formazan is inversely related to the superoxide anion radical scavenging activity of the samples tested. The final results were expressed as the inhibition degree of formazan production. The percentage inhibition of superoxide anion radicals was calculated using the same formula as:

((Abs. of control–Abs. of sample)/Abs. of control)) × 100%.

Determination of scavenging activity on hydroxyl radicals

The radical scavenging activity of the polysaccharides against hydroxyl radicals was measured using the method of Ohkawa et al. (1979) with some modifications. Inhibitory effects of the polysaccharides on deoxyribose degradation were determined by measuring the competition between deoxyribose and the polysaccharides for the hydroxyl radicals generated from the Fe^{3+}/ascorbate/EDTA/H_2O_2 system. The attack of the hydroxyl radical on deoxyribose leads to TBARS formation (Kunchandy and Rao, 1990). Solutions of the reagents were made up in deaerated water before use. The test sample with different concentrations was added to the reaction mixture containing 3.0 mM deoxyribose, 0.1 mM $FeCl_3$, 0.1 mM EDTA, 0.1 mM ascorbic acid, 1 mM H_2O_2, and 20 mM phosphate buffer (pH 7.4) and made up to a final volume of 1.2 mL.

The amount of TBARS formed following 1 h of incubation at 37°C was measured as follows: A 1.0 mL aliquot of thiobarbituric acid (TBA, 1%) and 1.0 mL of trichloroacetic acid (TCA, 2.8%) were mixed with the reaction mixtures in the tube, and the mixtures were then incubated at 100°C for 20 min. After the mixtures were cooled to room temperature, their absorbances at 532 nm were measured against a blank containing deoxyribose and buffer. Mixture without sample was used as control. Inhibition of deoxyribose degradation was calculated with the equation:

((Abs. of control–Abs. of sample)/Abs. of control)) × 100%.

DPPH radical-scavenging activity

DPPH radical-scavenging activity of the polysaccharides was evaluated according to the method of Shimada et al. (1992) with minor modifications. Each sample was allowed to react with a stable free radical, 1,1-diphenyl-2-picrylhydrazyl radical (DPPH). Briefly, each extract solution (0.1 ml) in 95 ml/100 ml aq. ethanol at different concentrations was added to solution (3.9 ml, 0.004 g/ml) of DPPH in 95 ml/100 ml aq. ethanol. The reaction mixture was incubated at 28°C. The scavenging activity on DPPH radical was determined by measuring the absorbance at 515 nm after 30 min. The antioxidant activity was expressed as a percentage of scavenging activity on DPPH radical:

$SC\% = [(A_{control} - A_{test})/A_{control}] \times 100\%$,

Where, $A_{control}$ is the absorbance of the control (DPPH solution without test sample) and A_{test} is the absorbance of the test sample (DPPH solution plus extract). The control contains all reagents except the extract. All tests were performed in triplicate and means were calculated.

Tests of antibacterial activity

The polysaccharides were tested for antibacterial activity according to a published methodology (Mongelli et al., 1995). Briefly, 4 mL of the polysaccharides were added to 16 mL of culture medium and homogenized thoroughly. Vegetative cells grown in MYPGP agar for 72 h of incubation at 37°C under microaerobic conditions were suspended in physiological solution and adjusted to 0.5 of Mac Farland scale. The microorganisms were streaked in radial patterns on the MYPGP agar plates, four streaks per plate. Finally, the plates were hatched at 37°C, during 72 h under microaerobic conditions. After the incubation period, the minimum inhibitory concentration (MIC) of growth inhibition of the bacterium was measured with a caliper. All the experiments were carried out in duplicate.

RESULTS AND DISCUSSION

Superoxide scavenging activity

Superoxide anions are the most common free radicals *in vivo* and are generated in a variety of biological systems and the concentration of superoxide anions increases under conditions of oxidative stress (Lee et al., 2002). Hence, an assay was carried out to test whether the polysaccharides scavenge superoxide anions. As shown in Figure 1, the superoxide scavenging activity of the polysaccharides was found to increase with increasing polysaccharides inhibited the production of hydroxyl

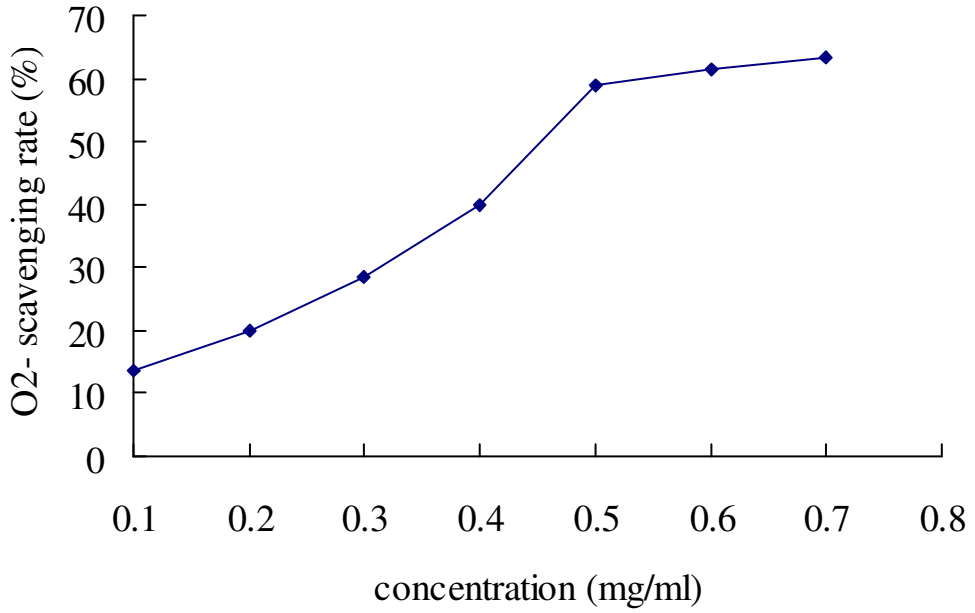

Figure 1. Superoxide anions scavenging activity of the polysaccharides.

polysaccharides inhibited the production of hydroxyl radicals by 59%. However, even if the concentration of extracts was increased, the inhibition curve was not largely changed. Similar *in vitro* studies by other researchers, have reported that the polysaccharides could also significantly scavenge superoxide anions and act as a dietary antioxidant *in vivo*.

Hydroxyl radicals scavenging activity

The hydroxyl radical, $OH^{\cdot}$, is the neutral form of the hydroxide ion (OH^{-}). Hydroxyl radicals are highly reactive and consequently short-lived (Gao et al., 2010); however, they form an important part of radical chemistry. Most notably hydroxyl radicals are produced from the decomposition of hydroperoxides (ROOH) or, in atmospheric chemistry, by the reaction of excited atomic oxygen with water (Liu et al., 2009). It is also an important radical formed in radiation chemistry, since it leads to the formation of hydrogen peroxide and oxygen, which can enhance corrosion and SCC in coolant systems subjected to radioactive environments (Deladino et al., 2008). Hydroxyl radicals are also produced during UV-light dissociation of H_2O_2 and likely in Fenton chemistry, where trace amounts of reduced transition metals catalyze peroxide-mediated oxidations of organic compounds. Hydroxyl radicals scavenging activity of the polysaccharides was shown in Figure 2. The polysaccharides, at concentrations from 0.1 to 0.8 mg/ml, markedly inhibited the production of hydroxyl radicals. Result showed 55% hydroxyl radicals scavenging activity at 0.8 mg/ml.

$DPPH^{\cdot}$ radicals scavenging activity

The results of this study, using the DPPH method, showed that the polysaccharides exhibited antiradical activity associated to its ability to scavenge free radicals (Figure 3). $DPPH^{\cdot}$ redical scavenging activity increased with increasing concentration of the polysaccharides. When concentration of the polysaccharides was 0.8 mg/ml, the scavenging rate was 85.2%. The DPPH assay involves a single electron transfer mechanism, rather than a hydrogen atom transfer characteristic in other antioxidant methods such as the oxygen radical absorbance capacity (ORAC) assay (Arts et al., 2003). Although, the DPPH method is not discriminative with respect to the radical species, and studies indicate that the TEAC value does not exactly correlate with the antioxidant capacity; it gives a general idea of the radical quenching ability of the extract samples (Nuengchamnong et al., 2009).

Antibacterial activity of the polysaccharides

It has been reported that some volatile compounds released from spices and herb extracts showed wide antimicrobial activities against fungi (Sindhu et al., 2009) and bacteria (Bandyopadhyay et al., 2007). Seaweeds are considered as source of bioactive compounds and produce a great variety of secondary metabolites characterized by a broad spectrum of biological activities. Compounds with

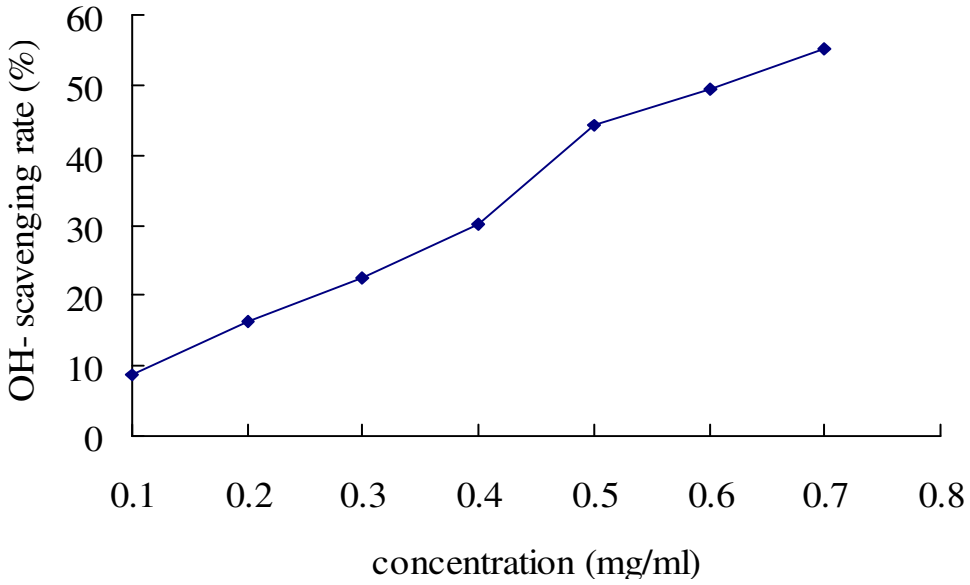

Figure 2. Hydroxyl radicals scavenging activity of the polysaccharides.

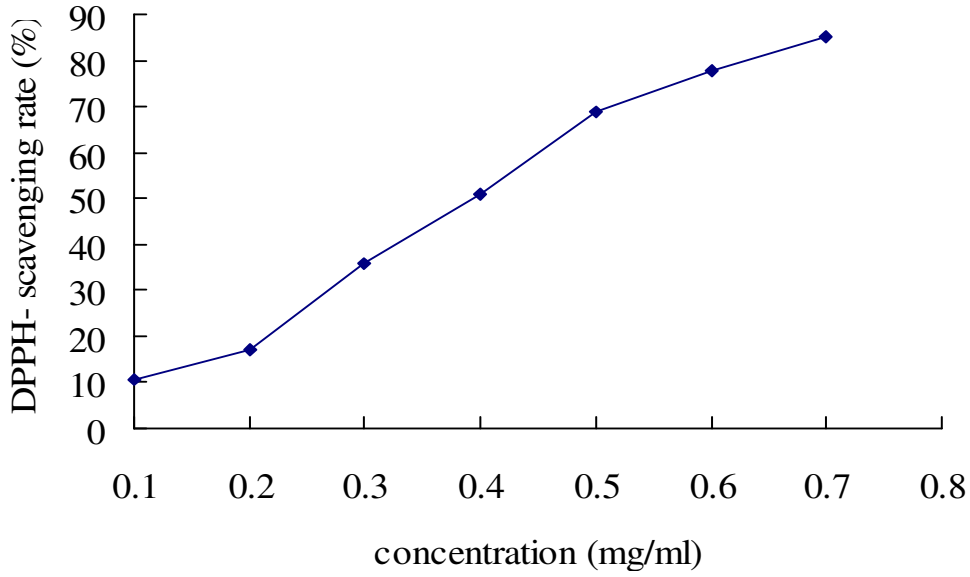

Figure 3. $DPPH^{\cdot}$ radicals scavenging activity of the polysaccharides.

cytostatic, antiviral, antihelminthic, antifungal and antibacterial activities have been detected in green, brown and red algae (Ganesan et al., 2008). As one of seaweeds, antibacterial activities of *L. japonica polysaccharides* were examined. Table 1 shows the antibacterial activity of the polysaccharides against Gram-positive and Gram-negative bacteria. The extracts were subjected to the determination of MIC values. The polysaccharides markedly inhibited staphylococcus, bacillus, proteus and *Escherichia coli*. In contrast, *Pseudomonas aeruginosa* and fusiform bacillus were more sensitive to the polysaccharides. The MIC concentrations ranged from 0.12 to 0.71 mg/ml. The very high values indicate only a very limited antibacterial efficacy. The polysaccharides did not exhibit antibacterial activity. Most species effective against *Staphylococcus aureus* are traditionally used to treat wound infection, throat infections, serious inflammations, or are post partum infections. Interestingly, the polysaccharides used in cleansing baths also showed high activity against this bacterium. The species effective against *E. coli* were

Table 1. Antibacterial activity of the polysaccharides.

Microorganism	Inhibition concentration (MIC, mg/ml)
staphylococcus aureus	0.21
Pseudomonas aeruginosa	0.66
Lactobacillus sp	-
Escherichia coli	0.34
bacillus proteus	0.12
fusiform bacillus	0.71

mostly employed in indications that traditional healers identified as “inflammation”.

Conclusion

This study demonstrated the antioxidant and antibacterial effects of *L. japonica* polysaccharides. The extract significantly scavenge superoxide radical (O_2^-), hydroxyl radicals (OH^-) and 1,1-diphenyl-picrylhydrazyl radical ($DPPH^-$) radicals in dose dependant manner. Furthermore, *L. japonica* polysaccharides markedly inhibited several selected bacteria growth. Thus, *L. japonica* polysaccharides could exert a beneficial action in food and medicine as antioxidant and antibacterial agent.

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