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Ex vivo Anti-Oxidation Activity of Polysaccharides from Red Alga *Porphyra yezoensis*

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Abstract: In this study, the *ex vivo* anti-oxidation activity of polysaccharide extracted from the red seaweed *Porphyra yezoensis* was studied. The scavenging efficiencies for free radical O^{2-}, •OH and •DPPH, the inhibitory effects on mice erythrocytes hemolysis induced by H_2O_2 as well as on the lipid peroxidation in mice liver homogenate, were systematically measured and analyzed. Results suggested that Porphyra polysaccharide, in the experimental concentration range, possessed anti-oxidation activity. The scavenging efficiency for O^{2-} was found to be remarkably high and the maximum scavenging rate was 82.77%. The Scavenging efficiency for •OH was even higher than O^{2-} with a maximum removing rate of 85.63% and the scavenging for •DPPH was 13.97%. The inhibitory effects on mice erythrocytes hemolysis and MDA formation in mice liver were significant and the maximum inhibition rates were 82.90 and 58.48%, respectively. The combined data indicated that the polysaccharide extracted from Porphyra had strong anti-oxidation activity.

Key words: *Porphyra yezoensis*, polysaccharide, anti-oxidation, free radical, DPPH, MDA

INTRODUCTION

Oxygen-derived free radicals are common byproducts in the metabolism process. However, these compounds are highly active and can cause apparent damages, commonly known as the oxidative damages, to the cell membranes and other cellular structures (Cuzzocrea *et al*., 2001). The free-radical damage has been reported to directly associate with diseases such as atherosclerosis, cataract formation, ageing and carcinogenesis (Cuzzocrea *et al*., 2001). To counteract the destructiveness of the free radicals, human body possesses a complex antioxidant defense system that utilizes various vitamins, minerals and other naturally producing substances (Cuzzocrea *et al*., 2001). It is possible, therefore, to prevent the radical damage by supplementing the diet with certain food, nutrients and herbs that have antioxidant activity.

In recent years, sulfated polysaccharides from the marine brown algae, Fucus vesiculosus, have been demonstrated to have antioxidant activities (Ruperez *et al*., 2002). Xue *et al*. (2001) also confirmed this activity by the antioxidant functional assay through ferric reduction. Sulfated polysaccharides from Laminaria japonica and Ecklonia kurome Okam have also been reported to possess free radical scavenging activities (Ruperez *et al*., 2002; Hu *et al*., 2001; Han *et al*., 2006). More recently, data from various research laboratories have suggested that the polysaccharide from the red alga, Porphyra haitanensis or *P. yezoensis*, had multiple biological functions, such as anti-coagulant, anti-senescence (Zhang *et al*., 2002), anti-fatigue (Guo *et al*., 2005), anti-cancer (Zhang *et al*., 2001), anti-virus (Zhou and Chen, 1990). Effects, proliferation promotion effects for lymphocytes and sertoli cells (Xiao *et al*., 2003; Guo *et al*., 2006), immune-modulation and free radical scavenging (Zhou and Chen, 1989; Yashizawa *et al*., 1993).

The red alga, Porphyra, has been an important food source in the Asian diet, as well as a drug used in the traditional Chinese medicine. Previously, three polysaccharide fractions with different sulfate content from *P. haitanesis* have been identified to have anti-oxidation activity (Wang *et al*., 2004) and two polysaccharide fractions from *P. yezoensis* have been discovered to possess anti-fatigue, enhancement of immunity and anti-cancer properties (Guo *et al*., 2005, 2006; Zhang *et al*., 2007). In this study, the anti-oxidation activity of polysaccharides from *Porphyra yezoensis* was studied and the mechanism of the polysaccharide bioactivity from *Porphyra yezoensis* was proposed.

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MATERIALS AND METHODS

Materials: The polysaccharides, extracted from *Porphyra yezoensis*, were provided by the Lab of Seaweed Biotechnology, Shanghai Fisheries University, Shanghai, China. They were purified by DEAE-52 and SephadexG-200 columns following the protocol as previously described (Liu *et al*., 2005; Gu *et al*., 2007).

Measurement of the superoxide radical scavenging activity: The method for the measurement of superoxide radical scavenging activity is similar to the protocol reported previously (Ponti *et al*., 1978). Briefly, superoxide radicals were generated by the PMS-NADH-NBT system. The reaction solution (3 mL) contained 0.5MTris-HCl (pH 8.0), 80 μM deoxy-coenzyme I (NADH), 50 μM Nitroblue Tetrazolium (NBT) and 16 μM Phenazin Methosulfate (PMS). Polysaccharides were added to the reaction buffer to the final concentrations of 2.5, 5, 10, 20, 40, 80 and 160 μg mL^{-1} and absorbencies at 517 nm after 30 min reaction were recorded. For the control, NADH was substituted with Tris-HCl buffer.

Measurement of the hydroxyl radical scavenging activity: The measurement of hydroxyl radical scavenging activity was following the protocol described by Smirnoff and Cumbes (1989). The reaction solution contained 150 mM sodium phosphate (pH 7.4), 0.15 mM $FeSO_4$-EDTA, 2 mM sodium salicylate and 6mM H_2O_2. Different polysaccharides were added to the final concentrations of 0.5-400 μg mL^{-1}. For the control, H_2O_2 was replaced with sodium phosphate buffer (150 mM, pH 7.4). The mixtures were incubated at 37°C for 1 h and the absorbencies at 510 nm were recorded.

Measurement of the •DPPH radical scavenging activity: Measurement of the •DPPH radical scavenging activity was following the protocol described by Zhang *et al.* (2003). Free radicals were generated by 1,1-Diphenyl-Picryl-Hydrazil (DPPH). Briefly, DPPH was dissolved in minimal toluene and mixed with 50% ethanol to a final concentration of 127 μ mol L^{-1}. The reaction system (2 mL) contained 1.9 mL DPPH and different concentrations (15-250 μg mL^{-1}) of polysaccharides. The absorbances of the mixtures were measured with a spectrophotometer at 525 nm after 20 min.

Measurement of the red cell hemolysis induced by H_2O_2 scavenging: Scavenging activities of polysaccharides on the rat erythrocytes hemolysis induced by H_2O_2 were measured according to the protocol described by Li *et al.* (2000). In brief, heparin-natrium was added to the rat blood as the anticoagulant and the erythrocytes were separated from the rat blood by centrifugation and resuspended in 1% physiological saline. The reaction solution (3 mL) contained 1mL erythrocyte suspension, different concentrations (2.5-200 μg mL^{-1}) of polysaccharides and 1 mL 400 μM L^{-1} H_2O_2. The mixture was incubated at 37°C for 1 h followed by addition of six volumes of physiological saline and centrifugation at 3000 rpm for 6 min. The absorbance of the supernatant was then measured with a spectrophotometer at 415 nm. For the control, no polysaccharide was added.

Measurement of the lipid peroxide inhibition activity in the rat liver homogenate: The rat liver homogenate was prepared following the protocol described by Li *et al.* (2000). Immediately after the mice were sacrificed, the liver tissues were separated and suspended in cold 0.5% physiological saline. The suspended solution was then centrifuged at 500 rpm at 4°C for 10 min to obtain the supernatant. To measure the inhibition effects of polysaccharides on the lipid peroxide in mice liver homogenate, the supernatant (1 mL) was incubated with 1mL polysaccharide (2-280 μg mL^{-1}), 100 μL 6 m moL $FeSO_4$ and 40 μL 20 mmoL H_2O_2 at 37°C for 1 h. The reaction was stopped by 15% trichloroacetic acid (1 mL), followed by 0.7% 2-thiobarbituric acid (1 mL). After the protein precipitate was removed by centrifugation at 1000 rpm and 10 min, the absorbance at 532 nm was recorded. The color formation is due to the oxidation product of lipid peroxide, the malondialdehyde-thiobarbituric acid complex. For the control samples, $FeSO_4$ and H_2O_2 were omitted.

Calculation of the scavenging rate: The percentage of scavenging activity was evaluated according to the following equation, where A stands for the absorbance of the free radical generation system, Ax stands for the absorbance of samples and Ao stands for the absorbance of the control.

$$\text{Scavenging rate (\%)} = (A-Ax)/(A-Ao)\times 100\%$$

Statistical analysis: Data were processed with the SSPS software (SPSS Inc., IL, USA) and analyzed with the student's t-test.

RESULTS AND DISCUSSION

Effects of the polysaccharide concentration on the scavenging rate for the superoxide radical (O^{2-}): Superoxide radicals were generated by the NADH-PMS-NBT system. The superoxide radical scavenging rates

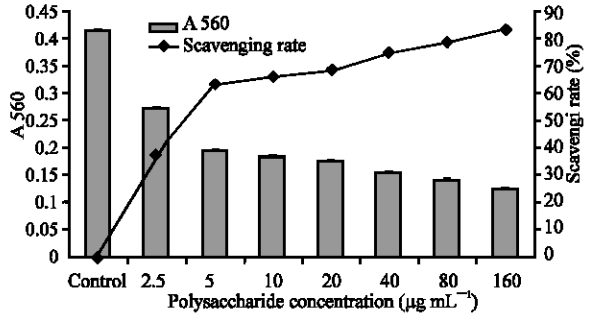

Fig. 1: Effects of the Porphyra polysaccharide concentration on O^{2-} scavenging (Mean±SD, n =6) ($p<0.01$)

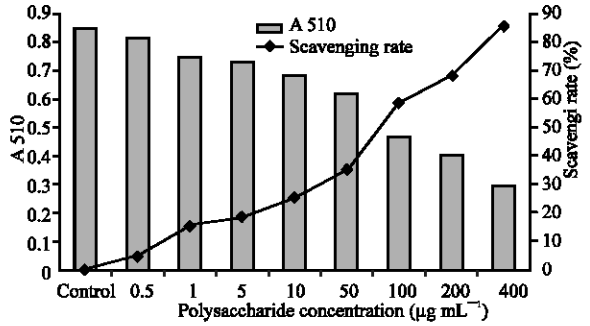

Fig. 2: Scavenging rates for •OH with the Porphyra polysaccharide (Mean±SD, n = 6) ($p<0.01$)

with different concentrations of Porphyra polysaccharide were summarized in Fig. 1. As shown, the IC_{50} of Porphyra polysaccharide was below 5 μg mL^{-1}, indicating that the superoxide radical scavenging activity of polysaccharides was significant. The scavenging rate reached 82.77% when the polysaccharide concentration was 160 μg mL^{-1}. Superoxide radicals were generated by the PMS-NADH-NBT system. Polysaccharides were added to the reaction buffer (3mL) to the final concentrations of 2.5, 5, 10, 20, 40, 80 and 160 μg mL^{-1} and absorbencies at 517 nm after 30 min reaction were recorded. For the control, NADH was substituted with Tris-HCl buffer

Effects of the polysaccharide concentration on the scavenging rate for hydroxyl free radicals: The effects of the polysaccharide concentration on the hydroxyl radical scavenging rate were summarized in Fig. 2. As shown, Porphyra polysaccharide also possessed hydroxyl radical scavenging activity. The scavenging efficiency reached 85.63% when the polysaccharide concentration was 400 g mL^{-1}. Different polysaccharides were added in the reaction solution to the final concentrations of 0.5-400 μg mL^{-1}. For the control, H_2O_2 was replaced with sodium

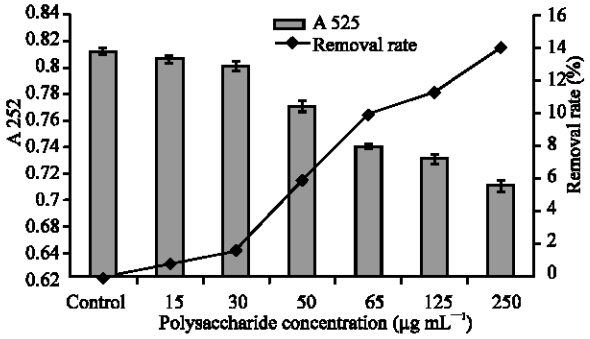

Fig. 3: The Porphyra polysaccharide on the removal rate of •DPPH (Mean±SD, n = 6) ($p<0.01$)

phosphate buffer (150 mM, pH 7.4). The mixtures were incubated at 37°C for 1 h and the absorbencies at 510 nm were recorded.

Scavenging effects of the polysaccharide on •DPPH radical: Figure 3 demonstrated the ability of Porphyra polysaccharide in quenching •DPPH radicals. Compared with the superoxide and the hydroxyl radicals, the scavenging activity of •DPPH radicals was much weaker. Only when the polysaccharide concentration was higher than 125 g mL^{-1}, was the scavenging efficiency able to reach higher than 10% (Fig. 3). Free radicals were generated by 1,1-diphenyl-picryl-hydrazil (DPPH). DPPH was dissolved in minimal toluene and mixed with 50% ethanol to a final concentration of 127 mol L^{-1}. The reaction system (2 mL) contained 1.9 mL DPPH and different concentrations (15-250 μg mL^{-1}) of polysaccharides. The absorbencies of the mixtures were measured with a spectrophotometer at 525 nm after 20 min.

Effects of the polysaccharide on the oxymatic hemolysis of erythrocytes: The effects of the Porphyra polysaccharide on the hemolysis of the rat erythrocytes induced by H_2O_2 were summarized in Fig. 4. As shown, at higher concentrations, Porphyra polysaccharide exhibited strong protective effects against hemolysis. The IC_{50} of Porphyra polysaccharide on hemolysis of erythrocytes induced H_2O_2 was around 26 g mL^{-1}. The scavenging efficiency of Porphyra polysaccharide on hemolysis of erythrocytes reached up to 82.90% at the polysaccharide concentration of 208 g mL^{-1}. Heparin-natrium was added to the rat blood as the anticoagulant and the erythrocytes were separated from the rat blood by centrifugation and resuspended in 1% physiological saline. The reaction solution (3 mL) contained 1 mL erythrocyte suspension, different concentrations (2.5-200 μg mL^{-1}) of polysaccharides and 1 mL 400 μM L^{-1} H_2O_2. The mixture was incubated at 37°C for 1 h followed by addition of six

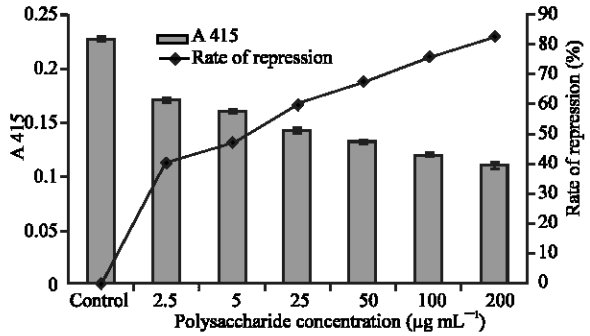

Fig. 4: The inhibitory effects of Porphyra polysaccharide on the oxymatic hemolysis of red blood cells (Mean±SD, n = 6) p<0.01

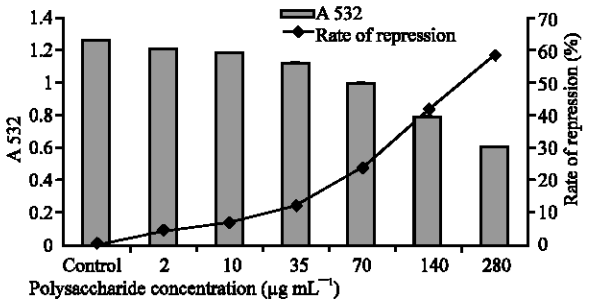

Fig. 5: The inhibitory effects of Porphyra polysaccharide on the MDA levels (Mean±SD, n = 6) (p<0.01)

volumes of physiological saline and centrifugation at 3000 rpm for 6 min. The absorbance of the supernatant was then measured with a spectrophotometer at 415 nm. For the control, no polysaccharide was added.

Effects of the polysaccharide on MDA levels: Malondialdehyde, formed from the breakdown of polyunsaturated fatty acids, is a convenient indicator for determining the extent of lipid peroxidation. According to our data, he Porphyra polysaccharide fractions from P. yezoensis significantly inhibited the microsomal lipid peroxidation (Fig. 5). The IC_{50} of Porphyra polysaccharide on lipid peroxidation was about 299.07 μg mL^{-1}. The liver tissues were separated from mice and suspended in cold 0.5% physiological saline and then centrifuged at 500 rpm at 4°C for 10 min to obtain the supernatant. The supernatant (1 mL) was incubated with 1 mL polysaccharide (2-280 μg mL^{-1}), 100 μL 6 mmoL $FeSO_4$ and 40 μL 20 mmoL H_2O_2 at 37°C for 1 h. The reaction was stopped by 15% trichloroacetic acid (1 mL), followed by 0.7% 2-thiobarbituric acid (1 mL). After the protein precipitate was removed by centrifugation at 1000 rpm for 10min, the absorbance at 532 nm was recorded. For the control samples, $FeSO_4$ and H_2O_2 were omitted.

Superoxide anion, hydroxyl and •DPPH free radicals are hazardous to living organisms and they directly associate with many diseases, such as tumor and inflammation. Hence, it is of high importance for the living organisms to get rid of or balance these harmful free radicals (Xin and Liu, 2000). Researches in recent years indicated that polysaccharides from alga possess remarkable anti-oxidation activity. For example, *Sargassum thunbergii* polysaccharide scavenges Reactive Oxygen Species (ROS) effectively with an IC_{50} of 0.5 mg, as measured by the Py-rogalb Huminol luminescent system (Zhang and Yu, 1997), Laminaria japonica fucoidan (IC_{50} = 20.3 μg mL^{-1}) could scavenge superoxide anion and to a less extent, the hydroxyl radical and the •DPPH radical (Zhang *et al*., 2003). For the higher plants, the superoxide anion scavenging ability is generally better. The highest reported values are from the peel extract of the red pomegranate and the extract of Quercus aliena acorn, with the IC_{50} values being 4.01 μg mL^{-1} and 4.92 μg mL^{-1} (Jin *et al*., 2005; Guo *et al*., 2007). The Porphyra polysaccharides used in our investigation was found to have even stronger scavenging effect on the superoxide radical with an IC_{50} value of 2.5-5.0 μg mL^{-1} and scavenging rate of up to 82.77%. The removal efficiencies for •OH and •DPPH were even better and the maximum removal rates were 85.63 and 13.97%, respectively.

The erythrocytes are in an oxygen-fully-loaded environment *in vivo*, with abundant polyunsaturated fatty acids around. The metal-chelating emoglobin around the erythrocytes could easily catalyze lipid peroxidation, thus making erythrocytes susceptible to oxidation injury. Researches on erythrocytes structure and function have provided valuable information for preventing the cellular oxidation injury(Zhang and Yu, 1997; Yang *et al*., 1999). It has been reported that certain polysaccharide from the alga, e.g., Laminaria polysaccharide, possessed protecting roles on the erythrocytes oxidation hemolysis induced by H_2O_2 (Zhang *et al*., 2003; Luo *et al*., 2004). Present data suggested that Porphyra polysaccharide effectively inhibited oxymatic hemolysis of erythrocytes induced by H_2O_2. The IC_{50} value was 25 μg mL^{-1} and the inhibition rate reached up to 82.90% at the polysaccharide concentration of 200 μg mL^{-1}.

The MDA level increases when the immune system of the organism is down. The redundant lipid peroxides damage the cell membrane and accelerate aging and cell death. Therefore, the MDA level is an important indicator for lipid oxidation (Zhang *et al*., 2003; Luo *et al*., 2004; Han *et al*., 2006) According to present data, at high concentrations, Porphyra polysaccharide inhibited the

increase of MDA levels induced by Fe^{2+} and H_2O_2 in the homogenate of rat liver tissues, indicating that Porphyra polysaccharide could hinder the lipid peroxidation process *in vitro*.

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REFERENCES

Cuzzocrea, S., D. Riley, A.P. Caputi and D. Selvemini, 2001. Antioxidant therapy: A new pharmacological approach in shock, inflammation and ischemia reperfusion injury. Pharmacol. Rev., 53: 135-159.

Guo, S., Q. Deng, J. Xiao, B. Xie and Z. Sun, 2007. Evaluation of antioxidant activity and preventing DNA damage effect of Pomegranate extracts by chemiluminescence method. Agric. Food Chem., 55: 3134-40.

Guo, T.T., J.W. Gu, L.X. Zhang, F. Liu and P.M. He, 2005. Mice's anti-fatigue experiment with crude polysaccharide from *Porphyra yezoensis*. Proceedings of 10th International Symposium on the Efficient Application and Preservation of Marine Biological Resources. Yeosu University, Korea, pp: 81-87.

Guo, T.T., L.X. Zhang, J.W. Gu, F. Liu and P.M. He, 2006. Effect of crude polysaccharide from *Porphyra yezoensis* on the lymphocytes and the sertoli cells. Lett. Biotech., 17: 359-361.

Guo, T.T., H.L. Xu, L.X. Zhang, J.P. Zhang, Y.F. Guo, J.W. Gu and P.M. He, 2007. *In vivo* protective effect of *Porphyra yezoensis* polysaccharide against carbon tetrachloride induced Hepatotoxicity in mice. Regulatory Toxicology and Pharmacology (In Press).

Gu, J.W., L.X. Zhang, H.L. Xu, T.T. Guo and P.M. He, 2007. Studies on the isolation and purification of polysaccharide from *Porphyra yezoensis* and its anti-cancer activity. J. Bioeng. China, 27: 50-54.

Han, H., H.Y. Zhou, C.C. Liu and C.B. Wang, 2006. Studies on the extraction of polysaccharides sulfate from three algae and their scavenging activity on free radicals. Chinese J. Marine Drug, 25: 33-36.

Hu, J.F., M.Y. Geng, J.T. Zhang and H.D. Jiang, 2001. An *in vitro* study of the structure-activity relations of sulfated polysaccharide from brown algae to its antioxidant effect. J. Asian Nat. Prod. Reports, 3: 353-358.

Jin, Y.S., S.I. Heo, M.J. Lee, H.I. Rhee and M.H. Wang, 2005. Free radical scavenging and hepatoprotective actions of *Quercus aliena* acorn extract against CCl_4-induced liver. Free Radic. Res., 39: 1351-1358.

Li, Z.X., C.G. Huang and Y.J. Cai, 2000. The chemical structure and antioxidative activity of polysaccharide from Asparagus cochinchinensis. Acta Pharma. Sinica, 35: 358-362.

Liu, F., Z.K. Gu and P.M. He 2005. Effect of factors on extraction of polysaccharide from Porphyra. J. Shanghai Fish. Univ., 14: 26-29.

Luo, J.H., Y.B. Yang and Y.C. Lin, 2004. Antioxdative activities of two metabolites of cultured marine fungus, *Halorosellinia oceanicum* 323 *in vitro*. J. Chinese Med. Materials, 27: 188-192.

Ponti, V., M.V. Dianzani and K.J. Cheeseman, 1978. Studies on the reduction of nitroblue tetrazolium chloride mediated through the action of NADH and phenazine methosulate. Chem. Biol. Interact., 23: 281-285.

Ruperez, P., O. Ahrazem and J.A. Leal, 2002. Potential antioxidant capacity of sulfated polysaccharides from the edible marine brown seaweed *Fucus vesiculosus*. J. Agric. Food Chem., 50: 840-845.

Smirnoff, N. and Q.J. Cumbes, 1989. Hydroxyl radical scavenging activity of compatible solutes. Phytochemistry, 28: 1057-1060.

Wang, Y.J., R.Y. Yao, H.P. Zhang and X.J. Guo, 2004. Studies on the response to oxidation of rat body induced by 60Co radiation with s sulfate polysaccharides from *Porphyra haitanensis*. Chinese J. Marine Drugs, 23: 32-35.

Xiao, M.T., J.L. Yang and H.Y. Lin, 2003. Extraction of *Porphyra haitanensis* polysaccharides and anti-influenza virus activity. J. Fuzhou Univ. Nat. Sci., 31: 631-635.

Xin, X.L. and C.H. Liu, 2000. Progress in the study of antioxidant effects of Chinese herbal polysaccharides. J. Beijing University of TCM, 23: 54-55.

Xue, C., Y. Fang and H. Lin, 2001. Chemical characters and antioxidative properties of sulfated polysaccharides from *Laminaria japonica*. J. Applied Phycol., 13: 67-70.

Yang, G.M., S.Y. Ye and J.L. Li, 1999. Effect of anisodamine on auto-oxidation of erythrocytes. Chinese Pharmacol. Bull., 15: 460-462.

Yashizawa, Y., A. Ametani, J. Tsunehiro, K. Numura, M. Itoh and F. Fukui, 1995. Macrophage stimulation activity of the polysaccharide fraction from a marine alga (*Porphyra yezoensis*): Structure-function relationships and improved solubility. Biosci. Biotech. Biochem., 59: 1933-1937.

Yashizawa, Y., A. Enomoto, H. Todoh, A. Ametani and S. Kaminogawa, 1993. Activation of marine macrophages by polysaccharide fractions from marine alga (*Porphyra yezoensis*). Biosci. Biotech. Biochem., 57: 1862-1866.

Zhang, W.Y., Y.F. Liu and H. Chen, 2001. The effect of polysaccharide from *Porphyra yezoensis* Ueda on proliferation of lymphocytes. J. China Pharm. Univ., 32: 57-59.

Zhang, L.X., H.L. Xu, J.W. Gu, T.T. Guo and P.M. He, 2007. Effects of polysaccharide from *Porphyra yezoensis* on the growth of four cancer lines. Lett. Biotech., 18: 608-611.

Zhang, Q.B., P.Z. Yu and G.F. Zhou, 2003. Studies on antioxidant activities of fucoidan from *Laninaria japonica*. Chinese Trad. Herbal Drugs, 4: 824-826.

Zhang, E.X., L.J. Yu, 1997. Studies on polysaccharide from *Sargassum thunbergh* for its ability to scavenge active oxygen species. Chin. J. Mar. Drugs, 16: 1-4.

Zhang, W.Y., J.F. Zhou and H. Chen, 2002. Effects of a polysaccharide from *Porphyra yezoensis* on murine immunocyte and human leukemia K562 cells. Life Sci. Res., 6: 167-170.

Zhou, H.P. and Q.H. Chen, 1989. Studies on anti-senescence of polysaccharide from *Porphyra yezoensis*. J. China Pharm. Univ., 20: 231-234.

Zhou, H.P. and Q.H. Chen, 1990. Anticoagulant and antihyperlipedemic effects of polysaccharide from *Porphyra yezoensis*. J. China Pharm. Univ., 21: 358-360.