Comparative Studies on Fucoidan Yield, Monosaccharide and Hypolipidemic Activity from Complex Enzymes and Water Method

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***Abstract*—Fucoidan extraction yield, monosaccharide, hypolipidemic activity from new complex enzyme extraction method and water extraction of different temperature were compared in this paper. The results showed that fucoidan and total sugar extraction yield by composite enzyme extraction were significantly higher than those from hot-water method, which the crude fucoidan yield (2.04%) increased 58.13% and the first withdrawal rate reached more than 90%. The higher proportion of mannose (18.41%) and sulfate radical (42.42%) content, lower proportion of xylose (1.80%) and glucosamine (2.67%) content in the fucoidan from complex enzyme extraction was found by the monosaccharide composition analysis. Fucoidan obtained from composite enzyme extraction had higher hypolipidemic activity, which could reduce hyperlipidemia mice atherosclerosis index (AI) was higher than hot-water extraction (66.83% VS 53%, *P*<0.01). These results indicated that the complex enzyme extraction method was an effective method that could efficiently extract fucoidan from *Laminaria japonica*, and suitable for production of fucoidan with high hypolipidemic activity.**

***Index Terms*—fucoidan, *Laminaria japonica*, complex enzyme, water extraction method, monosaccharide, hypolipidemic activity**

I. Introduction

Fucoidan is a complex water soluble sulphated polysaccharide mainly derived from marine brown seaweed and some marine invertebrates. Usually, fucoidan contains large proportions of L-fructose and sulphate, in addition to this, also contains galactose, mannose, xylose, glucose, glucuronic acid and protein, etc. [1]. In previous research, extensive biological activities of fucoidan have been found, such as hypolipidemic, anti-tumor, anti-virus, anticoagulant, anti-oxidation, regulating immune function, etc. [2]-[6]. And these results have also shown that the biological activities of fucoidan are closely related to the sulfate radical content and location.

Hyperlipidemia is disease caused by a lipid metabolic disorder. As people speeding up the pace of life and dietary structure changed, atherosclerosis, coronary heart disease and other disease of heart head blood-vessel has become "the first killer" of global human health induced by the high blood fat. It has become a major health ailment and a serious social problem. Among various factors leading to atherosclerosis, high blood concentrations of total cholesterol (TC), triglycerides (TG) and low density lipoprotein cholesterol (LDL-C) have been considered to be the major risk factors in the pathogenesis of atherosclerosis [7]. It has been reported that natural polysaccharides extracted from plants and microorganisms have antioxidant and hypolipidemic activities and could be developed as novel potential hypolipidemic agents [8], [9]. Polysaccharides of *Laminaria japonica* (LP) have also been shown to be effective for lowering blood lipids in vivo [10], [11].

LP is the most important economic seaweed for edible-medicinal use which has high nutritional value and health functions [12]. Composition analysis showed that LP contains 0.14-4% (average 2.46%) of fucoidan and is deal raw materials for fucoidan extraction. Until now, the main extraction methods of fucoidan include traditional hot-water extraction process, acid extraction, ultrasonic method, microwave assisted method and enzymatic extraction method [13]-[18]. Research and application of the method of hot-water extraction method is the most methods, other ways are the new methods and need further research.

With the development of modern biological engineering and enzyme engineering, the advance of enzyme preparation industry is rapid so that constantly emerging high stability and high active enzyme preparation provides the basis for progress many industrial sectors of new process conditions, especially biological related industry. Fucoidan extracted using

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enzymatic method from LP has a small amount of research [17], [18], but at the exploration stage. This paper will compare extraction efficiency of crude polysaccharides and total sugar by different extraction methods which contain complex enzyme extraction and hot-water extraction of different temperature. The monosaccharide composition and the hypolipidemic activities of fucoidan had also been compared. Our target is to developing an efficient and easy industrialized production method which may product high physiological activity of fucoidan.

II. Materials and Methods

A. Materials and Chemicals

L. japonica (LP) was obtained from Rongcheng, Shandong province, China. L-Fucose (Fuc), D-Galactose (Gal), L-Rhamnose (Rha), D-Glucose (Glu), L-Mannose (Man), L-Xylose (Xyl), D-Glucuronic acid (GlcA) D-Glucosamine (Gln) and D-Galacturonic acid (Gaa) were purchased from Sigma–Aldrich (St. Louis, MO, USA). All other reagents were of analytical grade. Male Kunming mice (20 ± 2g) were purchased from the Experimental Animal Center, Shandong University of China at 6-8 weeks of age. The mice were housed under normal laboratory conditions, i.e., room temperature (RT), 12/12h light-dark cycles with free access to standard rodent chow and water.

B. Comparison of Extraction Efficiency of Crude Polysaccharides and Total Sugar by Different Extraction Methods

1) Materials pretreatment

All dried materials were ground in a blender to obtain a fine powder, and were then pre-extracted in a soxhlet system with acetone for 24 h and subsequently with methanol for another 24 h. The residue was dried at 40 ℃ to a constant weight and stored at room temperature for polysaccharide extraction. The effects of extraction conditions on extraction efficiency of crude polysaccharides and total sugar were investigated. The polysaccharide and total sugar content were measured by the phenol-sulphuric acid method, using D-glucose as a standard [19].

2) Water extraction

The dried powder (30 g) after pretreatment in soxhlet system, was mixed with 900 ml deionized water, and then heated in water each at 53, 60, 75 and 90 ℃ for 8h, followed centrifugation for 15 min at 5, 000 g, respectively. After centrifugation, supernatants were further concentrated to a certain volume with a rotary evaporator and added ethanol to a final concentration of 80% (v/v). Precipitation of polysaccharide proceeded overnight at room temperature and the precipitate was collected by centrifugation at 8, 000 g for 10 min. The precipitate was then washed with Sevag reagent and freeze-dried to obtain the crude polysaccharides of LP.

3) Complex enzyme extraction

The dried powder (30 g) after pretreatment in soxhlet system, was mixed with 900 ml deionized water, and was then heated at 53 ℃ in a water, adjusted pH to 4.5, added cellulase, pectinase, amylase and xylanase (4.6g/100g dried powder of LP, 25:10:10:1:, complex enzyme digested 2 h, then 90 ℃ for 30 min out the enzyme activity, followed a centrifugation for 15 min at 5, 000g. After centrifugation, added excess Calcium chloride aqueous solution (20%, w/v) to the supernatants and precipitated for 30 min, followed a centrifugation for 15 min at 5, 000 g to remove the calcium alginate; further concentrated to a certain volume with a rotary evaporator. Excess Na_2CO_3 aqueous solution (20%, w/v) was added to concentrated solution and precipitated for 30min, followed a centrifugation for 15 min at 5, 000 g to remove calcium ion; supernatants were neutralized by hydrochloric acid and added ethanol to a final concentration of 80% (v/v). Precipitation of polysaccharide proceeded overnight at room temperature and the precipitate was collected by centrifugation at 8, 000 g for 10 min. The precipitate was then washed with Sevag reagent and freeze-dried to obtain the crude polysaccharides of LP.

C. Purification of Crude Polysaccharide

After extraction of crude polysaccharides from the raw materials of LP by different method, the purification technology was further performed using ethanol precipitation method ref. [20]. Ethanol was added to different polysaccharide samples solution at a final concentration of 80% (v/v) and centrifuged (8000 g for 10min), and the precipitate was dissolved in appropriate distilled water. Repeat the above operation 2 times, and then the precipitation were freeze-dried to give the purified polysaccharides.

D. Monosaccharide Analysis

1) Hydrolysis of fucoidan

The purified polysaccharides samples (2-4 mg) were dissolved in 2M trifluoroacetic acid (TFA, 2.0 ml) and hydrolysed at 110 ℃ for 4 h. After cooling to room temperature, added 2.0 ml aqueous sodium hydroxide (2M) to neutralize until analysis.

2) Derivatization with PMP

We added 0.5M methanolic solution of PMP (100 μl) and 0.6M aqueous sodium hydroxide (50 μl) to the monosaccharide reference solution or a hydrolysed fucoidan solution (50 μl each). The mixture solution was incubated at 70 ℃ for 100 min. After cooling to room temperature, 0.3M hydrochloric acid (100 μl) was added to neutralization and deionized water (700 μl). We then added 1 ml of chloroform to the solution. The mixture was shaken well and centrifuged at 5, 000 g for 10 min. The chloroform layer was discarded and the aqueous layer was extracted twice with chloroform. The final aqueous layer was analyzed directly by HPLC.

3) HPLC determination

Ultimate 3000 HPLC (DIONEX China limited.) with auto sample injector and variable wavelength detector, Shimadzu ODS-3 HPLC column (250×4.6 mm, 5 μm), Chromatographic conditions were generally as follows: temperature, 28 ℃; solvent A, 0.05 mol/l phosphate buffer solution (pH 6.7); solvent B, acetonitrile; gradient: 85%A+15%B for 10 min, 82.5%A+17.5%B from 11min

to 25min, 80%A + 20%B from26 min to 30min, 75%A + 25%B during the next 17 min at 1 ml/min. The eluate was monitored at 245 nm.

E. Measurement Hypolipidemic Activity of Fucoidan in the Hyperlipidemic Mouse Model

110 mice were randomly caged and adaptively fed 1 week, feeding the normal feed, free drinking water. After 1 week, according to the quality equilibrium all mice were divided into 11 groups, each group of 10 mice: control group, hyperlipidemia model group, W-75, W-90 and E-53 groups (high, medium and low dose respectively 200, 100 and 30 mg/kg d). In addition to the control group, the other groups were fed with hyperlipidemia feedstuff from the experiment the first day. Starting from the second week, the different fucoidan samples were fed by pouring with corresponding dose in test groups in addition the control group and hyperlipidemia model group. Test mice free fed and drank during 4 weeks, all mice were fasted overnight, and blood was collected under anaesthesia by cardiac puncture. The serum was prepared and frozen at -80 ℃ until analysis.

Total TC, TG, HDL-C, and LDL-C were measured by enzymatic methods with an automatic analyzer (Hitachi High-Technologies, Tokyo, Japan). Atherosclerosis index (AI) was calculated according to the follow equation as in (1).

$$AI = [TC/ (mmol/l) - HDL/ (mmol/l)] \div HDL/ (mmol/l) \quad (1)$$

F. Statistical Analysis

Data are expressed as the mean ± S.E.M. and were analyzed by t-test using SPSS. The difference considered statistically significant was $P<0.05$.

III. Results and Discussion

A. Effects of Extraction Methods on Extraction Efficiency of Polysaccharides and Total Sugar

First, extraction of fucoidan from LP by different temperature hot-water extraction method and complex enzyme extraction method were carried out. The results of the polysaccharide extraction yield and total sugar were shown in Fig. 1. By different temperature hot-water extraction methods, with the increase of temperature, the crude fucoidan yield and total sugar were increased distinctly at the same time, which showed that high temperature could increase the permeability of the cell walls of LP and make water soluble fucoidan rapid dissolution. At the same time, the polysaccharide and total sugar extraction yield by composite enzyme extraction were significantly higher than those from hot-water method, which the crude fucoidan yield (2.04%) increased 58.13% and the first withdrawal rate reached more than 90% compared with hot-water method. This might be caused by the composite enzyme that could effectively destroy cell wall structure of LP to produce more water soluble sugars. Meanwhile, the complex enzyme extraction method had shorter extraction time. According to polysaccharides of extraction yield, W-90 and W-75 from hot-water method and E-53 obtained by composite enzyme method were selected as samples in the process of follow-up studies.

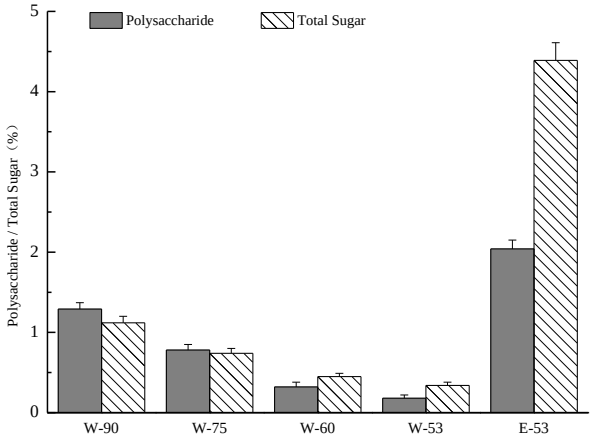


Figure 1. Effects of different extraction methods on polysaccharides and total sugar extraction efficiency

TABLE I. Monosaccharide Composition of Different Polysaccharides

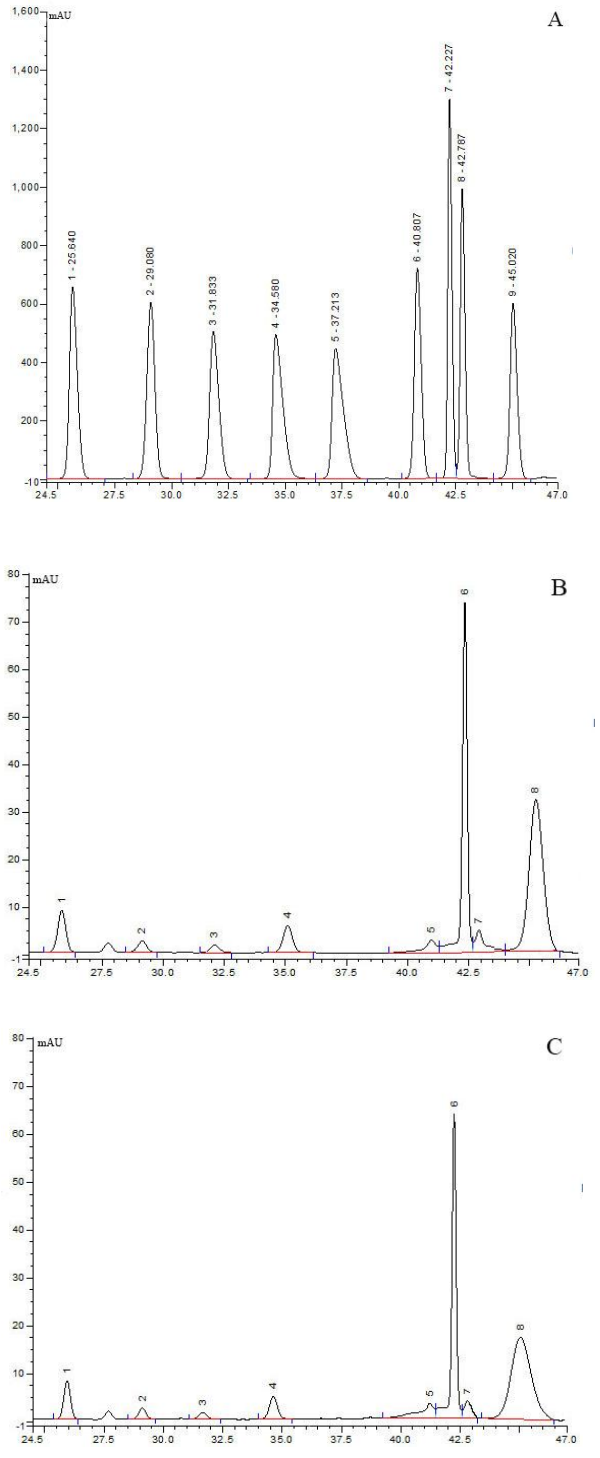
	W-90	W-75	E-53
	%	%	%
Polysaccharide	65.3	63.8	62.6
L-Fucose	37.02	40.57	32.92
D-Glucose	8.34	6.86	5.18
D-Mannose	3.49	7.92	18.41
L-Rhamnose	2.15	1.77	1.24
D-Galactose	31.89	28.78	29.93
D-Xylose	4.15	3.42	1.80
D-Glucuronic acid	7.88	6.49	7.75
D-Glucosamine	5.10	4.20	2.67
Sulfate content*	28.66	33.56	42.42

*By polysaccharide content meter

B. Monosaccharide Analysis

As shown in Fig. 2A, the monosaccharide standards of Man (25.640), Gln (29.080), Rha (32.833), GlcA (34.580), Gaa (38.673), Glu (40.807), Gal (42.227), Xyl (42.787), Fuc (45.020) were well separated on HPLC with ODS-3 column. The HPLC chromatogram (Fig. 2) revealed that there were many differences in monosaccharide composition of the three polysaccharides from different extraction method and the details have been shown in Table I. First of all, it was no significant different that all samples of the polysaccharide content obtained different methods, and galacturonic acid was not contained in all fucoidan samples. Secondly, the ratio of fucose in the polysaccharide E-53 was lower than the samples from hot-water method, but extremely high ratio mannose (18.41%) was contained in E-53. And the

xylose and glucosamine of E-53 were lower than those of W-90 and W-75 produced by hot-water method, which could be due to the effect of enzyme (xylanase, pectin enzyme). At last, sulfate radical contained in E-53 was 42.42% (by polysaccharide content meter) and significantly higher than the samples from hot-water method (W-90 28.66%, W-75 33.56%). Thus, the benign and quick conditions of the complex enzyme extraction could improve fucoidan sulfate radical content and could greatly improve its physiological activity.



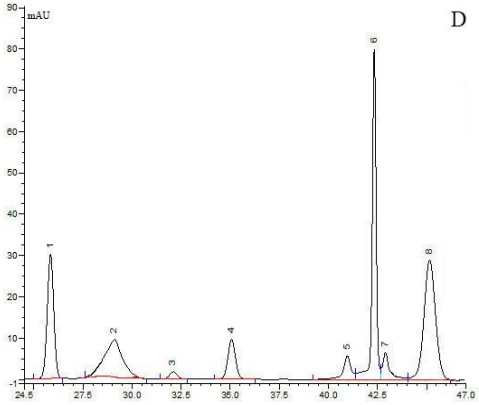


Figure 2. HPLC chromatogram of monosaccharides
A: monosaccharide standards; B: W-90; C: W-75; D: E-53.
Peaks: (1) D-Mannose, (2) D-Glucosamine, (3) D-Rhamnose, (4) D- Glucuronic acid, (5) D-Galacturonic acid, (6) D-Glucose, (7) D-Galactose, (8) D-Xylose, (9) L-Fucose

C. The Tests of Hypolipidemic Activity from Different Fucoidan

After five weeks feeding, blooded and measured, the TC, TG, HDL-C and LDL-C content from each group of mice are shown in Table II. In the hyperlipidemia model mice (Test1) serum, TC, TG and LDL-C content increased by 49.4%, 42.2% and 42.2%, respectively, HDL-C content lowered by 28.4%, and AI increased to 4.3 times. Thus, the hyperlipidemia mouse model to build was successful (P<0.01). After supplementary feeding different doses of fucoidan from different extraction method (Test 2, 3, 4), all experimental group could significantly reduce the content of TC (P<0.05) except W-90 and W-75 low dose group (30 mg/kg·d). In addition to the W-90 low dose group, other experimental groups could significantly increase HDL-C content (P<0.05). Only E-53 high-dose group (200 mg/kg·d) can significantly lower the content of TG, LDL-C of the hyperlipemia mice (P<0.05). All experimental groups can significantly reduce the AI of the hyperlipemia mice. In addition to W-90 low dose group, other each test groups all could decrease the AI very significantly (P<0.01). These results indicated that the fucoidan samples obtained different extraction method completely have hypolipidemic activity, and have the obvious dose efficiency which are similar results from Ref. [20] and Ref. [2] relevant studies.

Compared hypolipidemic activity of fucoidan obtained from different methods, all indicators from fucoidan E-53 were significantly better than the other samples in the same dose, which indicated that hypolipidemic activity of fucoidan obtained by composite enzyme method was significantly higher than hot-water extraction process. According to related study reports about fucoidan biological activities, the effect of sulfate radical content in fucoidan was very significant, and its content was as high as 42.42% (by polysaccharide content meter) by composite enzymatic extracting, which had been significantly higher than hot-water extraction of two trials (W-90 28.66%, W-75 33.56%), hence it might have a relationship with high sulfate radical content that E-53 showed prominent hypolipidemic activity. By AI

decreased rate evaluating the effect of different fucoidan on hypolipidemic activity, AI from E-53 obtained composite enzyme extraction could be decreased by 66.83% compared with the group of hyperlipidemic mouse model, which was higher than one in four to the highest reducing rate by hot-water method (53.18%), so visibly, the composite enzymatic extraction of fucoidan has higher hypolipidemic activity

TABLE II. EFFECT OF DIFFERENT FUCOIDAN ON HYPOLIPIDEMIC ACTIVITY IN THE HYPERLIPIDEMIC MOUSE MODEL

	Fucoidan	Dose (mg/kg d)	TC (mmol/l)	TG (mmol/l)	HDL-C (mmol/l)	LDL-C (mmol/l)	AI
Control		0	2.243±0.114	1.120±0.085	1.678±0.097	1.012±0.078	0.337±0.045
Test 1		0	3.356±0.180	1.603±0.145	1.202±0.049	1.576±0.104	1.788±0.099
Test 2	W-90	30	3.187±0.109	1.583 ±0.111	1.278±0.059	1.521±0.107	1.495±0.117
		100	2.944±0.107	1.483±0.160	1.345±0.082	1.468±0.090	1.219±0.084
		200	2.851±0.096	1.433±0.087	1.402±0.057	1.379±0.078	1.056±0.093
Test 3	W-75	30	3.070±0.167	1.568±0.117	1.296±0.049	1.513±0.084	1.393±0.099
		100	2.846±0.159	1.468±0.109	1.398±0.076	1.423±0.076	1.059±0.064
		200	2.627±0.112	1.403±0.097	1.456±0.077	1.324±0.081	0.837±0.068
Test 4	E-53	30	3.017±0.154	1.552±0.118	1.302±0.051	1.486±0.100	1.313±0.099
		100	2.711±0.125	1.435±0.090	1.489±0.071	1.389±0.081	0.856±0.082
		200	2.498±0.151	1.352±0.061	1.598±0.095	1.225±0.078	0.593±0.047

IV. CONCLUSION

Compared with the traditional hot-water extraction method, the crude fucoidan yield (2.04%) increased 58.13% and the first withdrawal rate reached more than 90% by the fucoidan extraction method based on the composite enzyme. This result suggested that the complex enzyme extraction method was more efficient for production of fucoidan from LP with a good prospect of industrialization. Different fucoidan monosaccharide composition analysis by PMP-HPLC showed that the most significant difference were higher proportion of mannose (18.41%) and sulfate radical (42.42%) content, lower proportion of xylose (1.80%) and glucosamine (2.67%) content in the fucoidan from complex enzyme extraction compared with hot-water extraction method. Due to significantly the proportion of mannose increased by composite enzyme extraction, the content of fucose has been decreased dramatically, which indicated that the effect on monosaccharide composition of fucoidan from different extraction method was very significant. By testing effect of hypolipidemic activity on the hyperlipidemia mouse model from different fucoidan, Fucoidan E-53 obtained from composite enzyme extraction has higher hypolipidemic activity, which can reduce hyperlipidemia mice AI by 66.83% and is higher than hot-water extraction 53% by a quarter. In addition, only high dose of fucoidan (200 mg/kg d) obtained from composite enzyme extraction could significantly reduce TC, TC, LDL-C and improve HDL-C at the same time (P<0.05). According to the results of previous related studies, more content sulfate radical should be the main dominant factor that has higher hypolipidemic activity from composite enzymatic income.

In conclusion, the composite enzyme extraction method is an effective method that can efficiently extract high hypolipidemic activity of fucoidan from LP, and suitable for the industrialization promotion. On Follow-up studies, the other biological activities fucoidan from composite enzyme extraction will be continued to be in-depth study, such as anti-tumor, anti-cancer, anti-inflammatory, antiviral, etc., in order to comprehensively interpreting the superiority of fucoidan product from the new composite enzyme extraction.

REFERENCES

[1] E. Percival, "The polysaccharides of green, red and brown seaweeds: Their basic structure, biosythesis and fuction," *Br. Phycol. J*., vol. 14, no. 2, pp. 103-117, 1979.

[2] S. Chen, W. Wang, C. Lu, S. Zhong, S. Zhong, and E. Xie, "Regulatory effects of *Sargassum wightii* Fucoidan on lipid metabolism of hyperlipidemic mice," *Food Science*, vol. 36, no. 19, 244-249, 2015.

[3] T. Alekseyenko, S. Zhanayeva, and A. Venediktova, et al, "Antitumor and antimetastatic activity of fucoidan, a sulfated polysaccharide isolated from the Okhotsk sea Fucus evanescens brown alga," *Bulletin of Experimental Biology and Medicine*, vol. 143, no. 6, pp. 730–732, 2007.

[4] N. P. Chand á and B. Matsuhiro, "Characterization of a fucoidan from *Lessonia vadosa (Phaeophyta)* and its anticoagulant and elicitor properties," *International Journal of Biological Macromolecules*, vol. 42, no. 3, pp. 235–240, 2008.

[5] A. Cumashi, N. A. Ushakova, and M. E. Preobrazhenskaya et al, "A comparative study of the anti-inflammatory, anticoagulant, antiangiogenic, and antiadhesive activities of nine different fucoidans from brown seaweeds," *Glycobiology*, vol. 17, no. 5, pp. 541–552, 2007.

[6] K. Hayashi, T. Nakano, M. Hashimoto, K. Kanekiyo, and T. Hayashi, "Defensive effects of a fucoidan from brown alga Undaria pinnatifida against herpes simplex virus infection," *International Immunopharmacology*, vol. 8, no. 1, pp. 109-116, 2008.
[7] K. K. Koh, S. H. Han, P. C. Oh, E. K. Shin, and M. J. Quon, "Combination therapy for treatment or prevention of atherosclerosis: Focus on the lipid-RAAS interaction," *Atherosclerosis*, vol. 209, pp. 307–313, 2010.
[8] R. Chen, Z. Liu, and J. Zhao et al., "Antioxidant and immunobiological activity of water-soluble polysaccharide fractions purified from Acanthopanax senticosu," *Food Chemistry*, vol. 127, pp. 434–440, 2011.
[9] H. Li, M. Zhang, and G. Ma, "Hypolipidemic effect of the polysaccharide from Pholiota nameko," *Nutrition*, vol. 16, pp. 556–562, 2010.
[10] T. Wang, T. Wang, and J. Pang, "Study on the hypoglycemic and hypolipidemic effect of *Laminaria japonica* polysaccharide," *Acta Nutrimenta Sinica*, vol. 29, no. 1, pp. 99–100, 2007.
[11] X. Zhao, C. H. Xue, and B. F. Li, "Study of antioxidant activities of sulfated polysaccharides from *Laminaria japonica*," *Journal of Applied Phycology*, vol. 20, pp. 431–436, 2008.
[12] J. Wang, Q. Zhang, Z. Zhang, and Z. Li, "Antioxidant activity of sulfated polysaccharide fractions extracted from Laminaria japonica," *International Journal of Biological Macromolecules*, vol. 42, pp. 127–132, 2008.
[13] X. Jie, S. Liu, J. Gao, and G. Zhao, "Studies on extraction of polysaccharides from Laminaria Japonica and isolation purification and identification of polysaccharide L1," *Journal of Liaoning University of Petroleum & Chemical Technology*, vol. 18, no. 1, pp. 8-9, 1998.
[14] Y. You and S. Xu, "The extraction procedure of Fucoidan from *Sargassum* fusiforme," *Journal of Wuxi University of Light Industry*, vol. 21, no. 3, May 2002.
[15] J. Liao, J. Zhang, and Z. Shen, "Extraction of Laminaran by Supersonic Method," *Pharmaceutical Biotechnology*, vol. 9, no. 3, pp. 157-160, 2002.
[16] Z. Wang, F. Sun, and R. Liu, "Research process of microwave-assisted extraction of pine seed polysaccharides," *Science and Technology of Food Industry*, vol. 27, no. 9, pp. 133-135, 2006.
[17] Y. He, Q. Wang, and H. Liu, "Extracting technology of fucoidan from kelp Laminaria japonica by enzymatic hydrolysis," *Journal of Dalian Fisheries University*, vol. 21, no. 1, pp. 55-58, Mar 2006.
[18] Y. Guo and Z. Wang, "Extraction and purification of fucoidan from *Laminaria japonica*," *Journal of Shanghai Fisheries University*, vol. 9, no. 3, pp. 276-279, Sep 2000.
[19] M. Dubois, K. A. Gilles, J. K. Hamilton, P. A. Rebers, and F. Smith, "Colorimentric method for determination of sugars and related substances," *Analytical Chemistry*, vol. 28, pp. 350–356, 1956.
[20] X. Zha, J. Xiao, and H. Zhang et al, "Polysaccharides in Laminaria japonica (LP): Extraction, physicochemical properties and their hypolipidemic activities in diet-induced mouse model of atherosclerosis," *Food Chemistry*, vol. 134, pp. 244-252, 2012.

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