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In vitro antioxidant and antitumor activities of polysaccharides extracted from *Asparagus officinalis*

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ABSTRACT

Ultrasonic circulating extraction technology was applied for the polysaccharide extraction from *Asparagus officinalis*. The crude polysaccharides were deproteinized by Sevag method and three main polysaccharide fractions, AOP-4, AOP-6 and AOP-8 were obtained by fractional precipitation with gradient concentrations of ethanol (40%, 60% and 80%). The *in vitro* antitumor and antioxidant activities of the polysaccharide fractions were evaluated by MTT assay and free radical-scavenging assay, respectively. Deproteinized AOPs showed higher antioxidant and antitumor activities than crude AOP. AOP-4 with molecular weight 5.75×10^4 Da showed significant function of scavenging hydroxyl radical. Three AOP fractions had significant antitumor activity against HeLa and BEL-7404 cells in a dose dependent manner. Furthermore, the inhibit activity of AOP-4 against HeLa cells was higher than those of other AOPs and the inhibition rate reached 83.96% at the concentration of 10 mg/mL. These results indicated that the AOP might be useful for developing natural safe antitumor drugs or health food.

1. Introduction

Asparagus (*Asparagus officinalis* L.) is an economically important vegetable crop cultivated in temperate zones all over the world. Asparagus contain various essential nutrients, including dietary fiber, oligosaccharides (Fukushi, Onodera, Yamamori, Shiomi, & Kawabata, 2000), amino acid derivatives (Kasai & Sakamura, 1981), vitamins and mineral (Kim et al., 2009). In addition to its high nutritional value and unique taste, the crop contains biologically active compounds, such as flavonoids (Kartnig, Gruber, & Stachel, 1985), lignans (Huang, Luo, & Zhang, 2007), steroidal saponin (Huang, Lin, & Kong, 2008) and so on. Therefore, in some countries, it has been used as an anti-inflammatory (Jang, Cuendet, Fong, Pezzuto, & Kinghorn, 2004) and antifungal activities (Shimoyamada, Suzuki, Sonta, Maruyama, & Okubo, 1990) as well as anticancer herbal medicine for a long time.

In recent decades, polysaccharides isolated from botanical sources, like mushrooms, algae, lichens and higher plants, have attracted a great deal of attention in the biomedical field because of their broad spectrum of therapeutic properties, relatively low toxicity and do not cause significant side effects (Schepetkin & Quinn, 2006). Some of plant polysaccharides have exhibited strong antioxidant properties and can be explored as novel potential antioxidants (Ramarathnam, Osawa, Ochi, & Kawakishi, 1995; Wang & Luo, 2007). *A. officinalis* polysaccharide (AOP) is another bioactive component in asparagus. Like other plant polysaccharides, AOP possess a wide range of pharmacological properties such as improving cellular immunity (Guo, Zhang, Meng, & Zhuang, 1995), anti-aging (Ming-san, Li-ya, Xiao-yan, & Yan-yan, 2004) and anti-tumor functions (Ji, Ji, & Chen, 2008; Ji, Ji, & Zou, 2008). Therefore, in this paper, the purification, characteristics and bioactivity of AOP were investigated.

2. Materials and methods

2.1. Materials

Green asparagus (*A. officinalis* L.) were provided by Qinhuangdao Chang Sheng Agricultural Science and Technology Co., Ltd. Nitroblue tetrazolium (NBT), 1,1-diphenyl-2-picryl-hydrazyl (DPPH), phenazine methosulfate (PMS), dimethyl sulfoxide (DMSO) and (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) MTT were purchased from Sigma Chemical Co. (St. Louis,

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Table 1
Average molecular weight and recovery rate of AOPs.

AOPs	Concentration of ethanol	Average molecular weight (Da)	Yield (%)	Protein content (%)
Graded precipitation	AOP-4 (40%)	5.75×10^4	13.5	0.36
	AOP-6 (60%)	5.23×10^4	40.5	0.83
	AOP-8 (80%)	5.11×10^4	39.7	0.12

MO, USA). RPMI 1640 medium and fetal bovine serum were purchased from Invitrogen, Inc. All other reagents were of analytical grade.

2.2. AOP extraction and treatment

AOP extraction procedure was described in our previously report (Zhao et al., 2011). The crude polysaccharides were deproteinized three times by using Sevag's method (Sevag, Lackman, & Smolens, 1938). The deproteinized polysaccharide (DP-AOP) was then successively sub-fractionated by graded precipitation at final ethanol concentrations of 40%, 60% and 80%, named AOP-4, AOP-6 and AOP-8, respectively. Protein content in AOPs was determined by Bradford method using bovine serum albumin as standard protein (Bradford, 1976). The average molecular weight of the AOPs was determined by gel permeation chromatography (GPC) method. Flow rate of the mobile phase (0.9% NaCl) was 0.5 mL min^{-1} at a column temperature of 25 °C. FT-IR of polysaccharides was carried out by the potassium bromide (KBr) pellet method on Fourier Transform-Infrared Spectrometer (FT/IR-660 Plus, JASCO) in the range of 400–4000 cm^{-1}.

2.3. Measurement of antioxidant activity

2.3.1. Assay for the scavenging effect on hydroxyl radicals

The hydroxyl radicals scavenging assay was evaluated using the hydroxyl radical system generated by the Fenton reaction (Sun, Li, & Liu, 2010). Briefly, Samples were dissolved in distilled water at 0 (control), 1–10 mg/mL. The reaction mixture contained 1 mL brilliant green (0.435 mM), 0.5 mL $FeSO_4$ (2 mM), 1.5 mL H_2O_2 (3.0%) and 1 mL samples with varying concentrations. After incubation at room temperature for 20 min, the absorbance of the mixture was measured at 624 nm. The hydroxyl radical-scavenging rate (%) was calculated as (1 − absorbance of sample/absorbance of control) × 100%.

2.3.2. Assay for the scavenging effect on superoxide radicals

The superoxide radical scavenging abilities of all samples were assessed by the method of Zhang et al. (2009). Superoxide anion radicals were generated in 4.5 mL Tris–HCl buffer (16 mM, pH 8.0) containing 0.5 mL NBT (300 μM) solution, 0.5 mL NADH (468 μM) solution and one sample (0.5–50.0 μg/mL). The reaction was started by adding 0.5 mL PMS (60 μM) solution to the mixture. The reaction mixture was incubated at room temperature for 5 min and measured at 560 nm. The capability of scavenging the superoxide anion radicals was calculated using the following equation:

$$\text{Scavenging effect}\,(\%) = 1 - \left(\frac{\text{absorbance of sample}}{\text{absorbance of control}}\right) \times 100$$

2.3.3. Assay for the scavenging effect on DPPH radicals

The assay for scavenging DPPH radical was carried out according to the method of Wang et al. (2010). One milliliter of the polysaccharides of different addition quantities (1–20 mg) in water was thoroughly mixed with 2 mL of freshly prepared DPPH (0.2 mM) and 2 mL methanol. The mixture was shaken well, allowed to stand for 30 min in the dark, and the absorbance was then measured at 517 nm against a blank. Vc was used as positive controls. The capability to scavenge DPPH radical was calculated using the following equation:

$$\text{Scavenging effect}\,(\%) = \left[A_0 - \left(\frac{A - A_b}{A_0}\right)\right] \times 100$$

where A_0 is the absorbance of DPPH solution without sample; A is the absorbance of the test sample mixed with DPPH solution and A_b is the absorbance of the sample without DPPH solution.

2.4. Measurement of antitumor activity

2.4.1. Cell lines and culture

HeLa cells and BEL-7404 human hepatoma cells were provided by the Model Animal Research Center of Nanjing University and maintained with RPMI 1640 medium containing 10% fetal bovine serum at 37 °C in a humidified atmosphere with 5% CO_2.

2.4.2. Growth inhibition assay

The inhibition effects of crude AOP, DP-AOP, AOP-4, AOP-6 and AOP-8 on the growths of HeLa cells and BEL-7404 cells were evaluated *in vitro* by MTT assay (Yan & Katz, 2010). Briefly, the HeLa cells

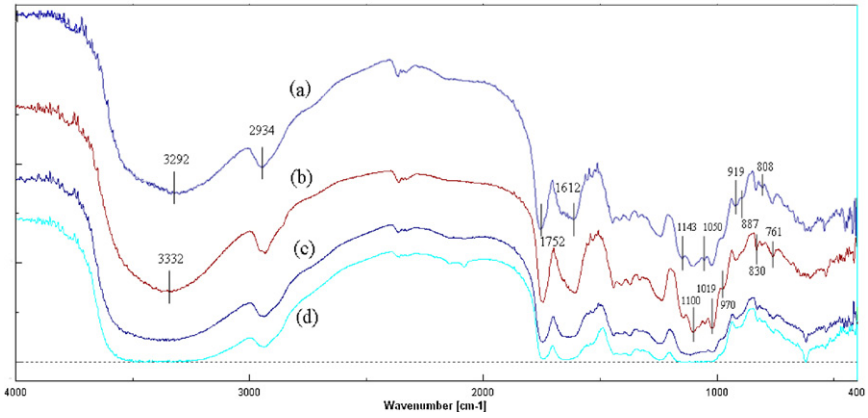

Fig. 1. IR spectrum of AOPs. (a) DP-AOP; (b) AOP-4; (c) AOP-6; and (d) AOP-8.

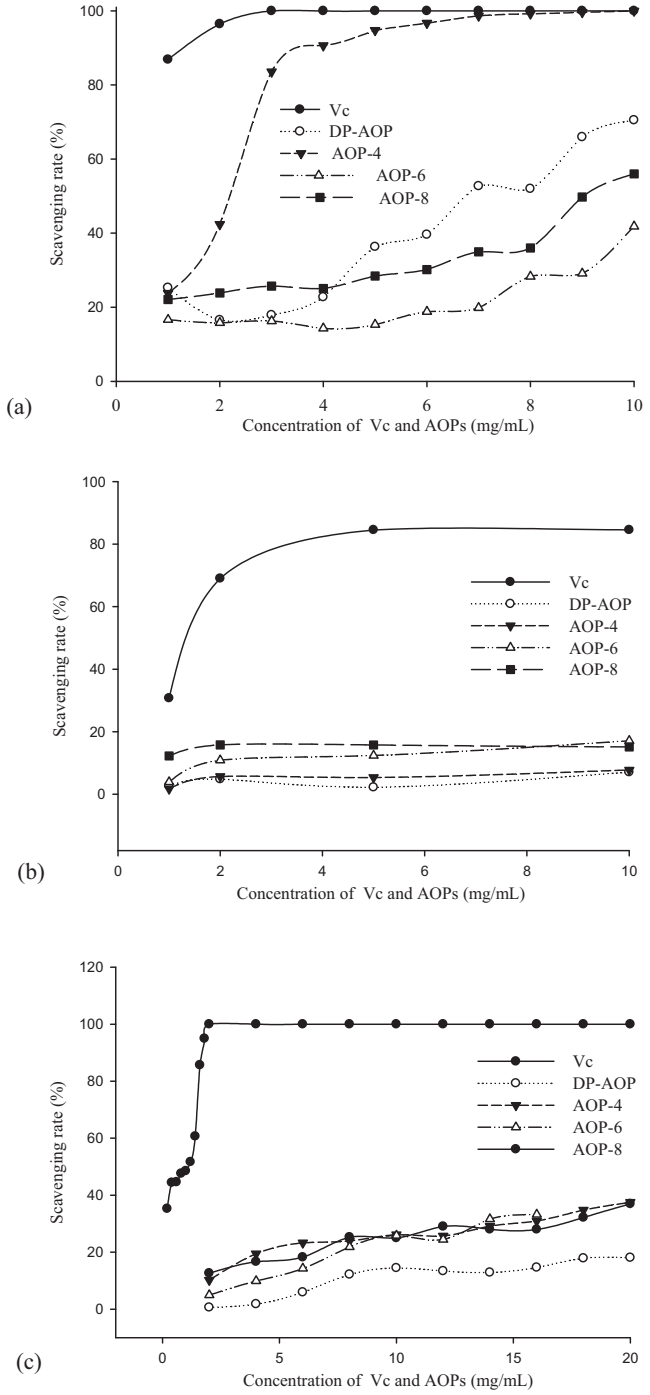

Fig. 2. Antioxidant activities of AOPs and Vc. (a) Hydroxyl radical. (b) Superoxide radical. (c) DPPH radical.

and BEL-7404 cells (5×10^4 cells/mL) were incubated in 96-well plates containing 0.1 mL the culture medium at 37 °C in a humidified atmosphere with 5% CO_2. The cells were permitted to adhere for 24 h. One hundred microlitres of polysaccharides solution with

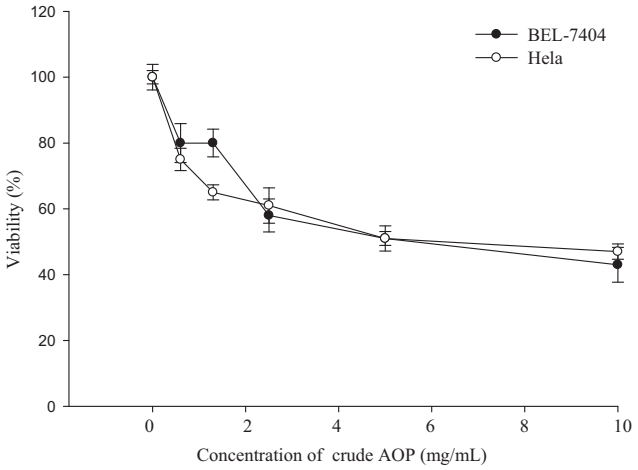

Fig. 3. Effects of the concentration of crude AOP on the cell viability of HeLa and BEL-7404 after 72 h co-culture.

different concentrations (0.125, 0.250, 0.500, 1.000 mg/mL), prepared in culture medium, were added to each well. After 48 h of exposure, the polysaccharide-containing medium was removed. The cells in each well were then incubated in culture medium with 0.10 mL MTT solution (5 mg/mL) for 4 h. After the media were removed, 0.100 mL DMSO was added to each well. Absorbance at 570 nm (maximum) was determined by an ELISA Reader. The viability rate was calculated according to the formula below: Viability rate (%) = (absorbance of experimental group/absorbance of blank control group) × 100%.

3. Results and discussion

3.1. Characteristics of AOPs

The average molecular weight (Mw), yield and protein content of the AOPs are given in Table 1. Clearly, an increase in ethanol concentration from 40% to 80% resulted in the decline of Mw from 57,500 to 51,100 Da. AOP-4, AOP-6 and AOP-8 had low protein content of 0.36%, 0.83% and 0.12%, respectively. The recovery yield of the three AOPs was 13.5%, 40.5% and 39.7% (Table 1).

FT-IR spectra of AOPs are shown in Fig. 1. The spectra are typical of polysaccharides in a strong and wide stretching peak around 3292 cm^{-1} and 3332 cm^{-1} for O–H stretching vibrations and a weak absorption peak of about 2934 cm^{-1} for C–H stretching vibrations, respectively. Absorption of 1752 cm^{-1}, 1612 cm^{-1} was attributed to carbonyl group bending vibration and 1237 cm^{-1}, 761 cm^{-1} was C–O–C vibration. In the spectra of DP-AOP and AOP-4, the strong absorption of 1100 cm^{-1} and 1050 cm^{-1} is the characteristic peaks of primary alcohol and secondary alcohol, but in AOP-6, AOP-8, these two peaks disappeared. There is a weak absorption peak at 808 cm^{-1} in DP-AOP and AOP-4, indicating that the two AOPs containing mannose except for glucose, fucose, arabinose, galactose and rhamnose (Zhao et al., 2011). The relatively strong absorption peaks at around 970 cm^{-1} and 1019 cm^{-1} of DP-AOP and AOP-4 reflect the absorption of the furan ring and pyran ring respectively, but these two peaks become weakening in AOP-6 and AOP-8.

Table 2
Inhibition rate of AOPs (10 mg/mL) on HeLa and BEL-7404 at 72 h.

Cell lines	Inhibition rate (%)				
	Crude AOP	DP-AOP	AOP-4	AOP-6	AOP-8
HeLa	54.64	67.86	83.96	81.03	72.05
BEL-7404	57.31	57.73	55.39	62.28	54.41

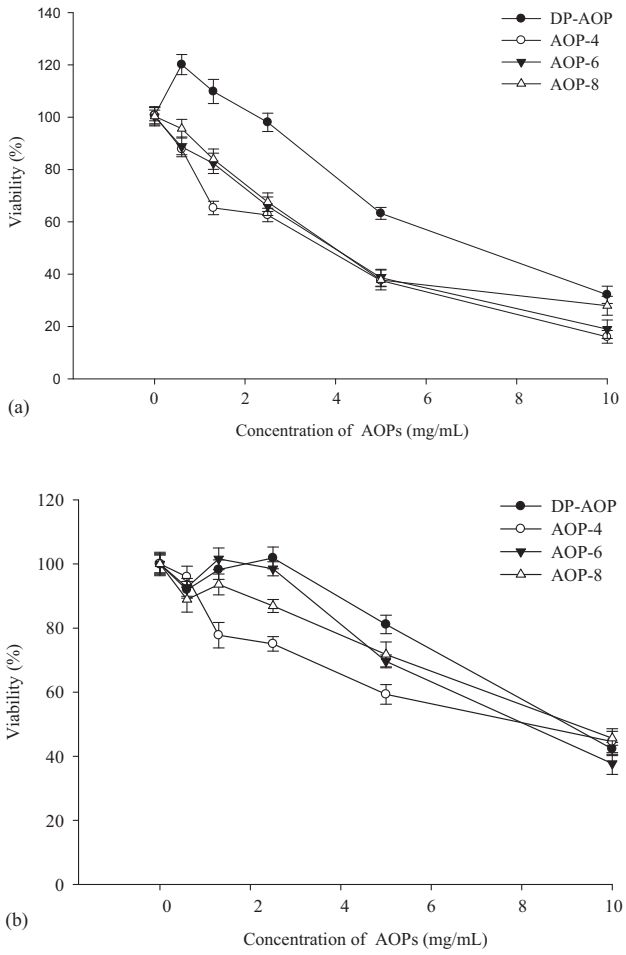

Fig. 4. Antitumor activity of AOPs with different concentrations. (a) Hela and (b) BEL-7404.

All AOPs spectra present two peaks at 830 and 919 cm^{-1}, which attribute to the α-glycosidic bond, DP-AOP has weak absorption at 887 cm^{-1}, indicating there is a small amount of β-glycosidic bond.

3.2. Antioxidant activity of AOPs

Antioxidant activities have been attributed to various reactions and mechanisms, such as radical scavenging, reductive capacity, prevention of chain initiation, and binding of transition metal ion catalysts (Frankel & Meyer, 2000). In this experiment, the *in vitro* antioxidant capacities of AOPs were evaluated using different biochemical methods including hydroxyl, superoxide anion and DPPH radical scavenging assay.

The results of hydroxyl radical scavenging assay are shown in Fig. 2a. The scavenging rate of AOP-4 at 9 mg/mL was 99.6%, which was closed to Vc. At 10 mg/mL, the scavenging rate of DP-AOP, AOP-6 and AOP-8 was 70.5%, 41.8% and 56.0%. AOP-4 exhibited higher hydroxyl radical-scavenging activity than other three AOPs (Fig. 2a). The superoxide radical and DPPH scavenging rates of AOPs were not very significant, the superoxide radical scavenging rate of AOP-6 was only 17.1% at 10 mg/mL (Fig. 2b) and the DPPH scavenging rate of AOP-6 at 20 mg/mL reached 37.5% (Fig. 2c). These results indicated that the AOPs have potential antioxidant capacities.

3.3. Growth inhibition on the HeLa cells and BEL-7404 cells

In this study, the antitumor activities of the five polysaccharide samples, crude AOP, DP-AOP, AOP-4, AOP-6 and AOP-8, were tested on HeLa cells and BEL-7404 cells. Both fractions of AOPs and DP-AOP presented significantly higher antitumor activities against HeLa cells and BEL-7404 *in vitro* than those of blank control groups, and the inhibition abilities were dose-dependent (Figs. 3 and 4). At 10.0 mg/mL, the inhibition rates of crude AOP, DP-AOP, AOP-4, AOP-6 and AOP-8 on the HeLa cells were 54.64%, 67.86%, 83.96%, 81.03% and 72.05%, respectively. AOP-4 presented significantly higher antitumor activity against the HeLa cells (83.96%) *in vitro* than a blank control at 10.00 mg/mL, dose-dependently. The inhibition rates of

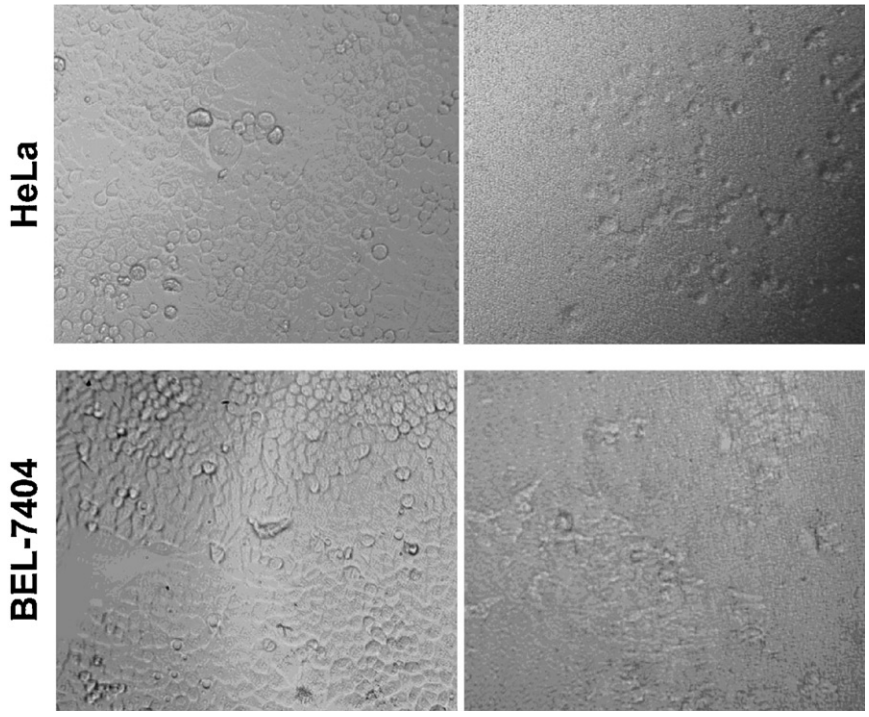

Fig. 5. Photos of HeLa and BEL-7404 cells cultured for 72 h with 10 mg/mL AOP (left: vehicle treated group; right: experimental group treated with AOP).

crude AOP, DP-AOP, AOP-4, AOP-6 and AOP-8 on the BEL-7404 cells were 57.31%, 57.73%, 55.39%, 62.28% and 54.41% (Table 2), respectively. These differences in antitumor activities may be attributed to their different molecular weights, charge characteristics and monosaccharide distributions (Dias et al., 2005). In addition, AOPs can significantly inhibit the proliferation and induce apoptosis of HeLa cells and BEL-7404 *in vitro* (Fig. 5), indicating AOP could be a potential anti-cancer drug.

4. Conclusions

AOP fractions were obtained by fractional precipitation with gradient concentrations of ethanol. AOP-4 with molecular weight 5.75×10^4 Da showed significantly higher effect on scavenging hydroxyl radical. AOP fractions had significant antitumor activity against HeLa and BEL-7404 in a dose dependent manner. AOP-4 presented highest antitumor activity against the HeLa cells (inhibition rate 83.96%) *in vitro* at 10.00 mg/mL.

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References

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, *72*(1–2), 248–254.

Dias, P. F., Siqueira, J. M., Vendruscolo, L. F., Neiva, T. J., Gagliardi, A. R., Maraschin, M., et al. (2005). Antiangiogenic and antitumoral properties of a polysaccharide isolated from the seaweed Sargassum stenophyllum. *Cancer Chemotherapy and Pharmacology*, *56*(4), 436–446.

Frankel, E. N., & Meyer, A. S. (2000). The problems of using one-dimensional methods to evaluate multifunctional food and biological antioxidants. *Journal of the Science of Food and Agriculture*, *80*(13), 1925–1941.

Fukushi, E., Onodera, S., Yamamori, A., Shiomi, N., & Kawabata, J. (2000). NMR analysis of tri- and tetrasaccharides from asparagus. *Magnetic Resonance in Chemistry*, *38*(12), 1005–1011.

Guo, B., Zhang, C. L., Meng, J. L., & Zhuang, Z. J. (1995). The effect of asparagus and its extract on phagocytic function of macrophages in Mice. *Journal of Guiyang Medical College, Chinese*, *20*(4), 285–287.

Huang, X. F., Lin, Y. Y., & Kong, L. Y. (2008). Steroids from the roots of *Asparagus officinalis* and their cytotoxic activity. *Journal of Integrative Plant Biology*, *50*(6), 717–722.

Huang, X., Luo, J., & Zhang, Y. (2007). Studies on lignan components of *Asparagus officinalis*. *Chinese Pharmaceutical Journal – Beijing*, *42*(5), 339.

Jang, D. S., Cuendet, M., Fong, H. H. S., Pezzuto, J. M., & Kinghorn, A. D. (2004). Constituents of *Asparagus officinalis* evaluated for inhibitory activity against cyclooxygenase-2. *Journal of Agricultural and Food Chemistry*, *52*(8), 2218–2222.

Ji, Y., Ji, C., & Chen, X. (2008). Effects of Asparagus polysaccharide on GPA and Band 3 from erythrocyte membrane of S 180 mice. *7th Asian-Pacific Conference on Medical and Biological Engineering*, *19*, 528–530.

Ji, C. F., Ji, Y. B., & Zou, X. (2008). Effect of Asparagus polysaccharides on migration time of erythrocytes in tumor-bearing organisms using high performance capillary electrophoresis. *International Seminar on Future BioMedical Information Engineering*, 199–201.

Kartnig, T., Gruber, A., & Stachel, J. (1985). Flavonoid pattern from *Asparagus officinalis*. *Planta Medica*, *51*, 288.

Kasai, T., & Sakamura, S. (1981). N-carboxymethyl-L-serine, a new acidic amino acid from Asparagus (*Asparagus officinalis*) shoots. *Agricultural and Biological Chemistry*, *45*(6), 1483–1485.

Kim, B. Y, Cui, Z. G., Lee, S. R., Kim, S. J., Kang, H. K., Lee, Y. K., et al. (2009). Effects of *Asparagus officinalis* extracts on liver cell toxicity and ethanol metabolism. *Journal of Food Science*, *74*(7), H204–H208.

Ming-san, M., Li-ya, G., Xiao-yan, F., & Yan-yan, M. (2004). Effect of Phragmites communis polysaccharide on the aged-model mice. *China Journal of Chinese Materia Medica*, *29*(7), 673–675.

Ramarathnam, N., Osawa, T., Ochi, H., & Kawakishi, S. (1995). The contribution of plant food antioxidants to human health. *Trends in Food Science & Technology*, *6*(3), 75–82.

Schepetkin, I. A., & Quinn, M. T. (2006). Botanical polysaccharides: Macrophage immunomodulation and therapeutic potential. *International Immunopharmacology*, *6*(3), 317–333.

Sevag, M. G., Lackman, D. B., & Smolens, J. (1938). The isolation of the components of streptococcal nucleoproteins in serologically active form. *Journal of Biological Chemistry*, *124*(2), 425–436.

Shimoyamada, M., Suzuki, M., Sonta, H., Maruyama, M., & Okubo, K. (1990). Antifungal activity of the saponin fraction obtained from *Asparagus officinalis* L. and its active principle. *Agricultural and Biological Chemistry*, *54*(10), 2553–2557.

Sun, Y., Li, T., & Liu, J. (2010). Structural characterization and hydroxyl radicals scavenging capacity of a polysaccharide from the fruiting bodies of Auricularia polytricha. *Carbohydrate Polymers*, *80*(2), 377–380.

Wang, J., Zhang, J., Zhao, B., Wang, X., Wu, Y., & Yao, J. (2010). A comparison study on microwave-assisted extraction of *Potentilla anserina* L. polysaccharides with conventional method: Molecule weight and antioxidant activities evaluation. *Carbohydrate Polymers*, *80*(1), 84–93.

Wang, Z., & Luo, D. (2007). Antioxidant activities of different fractions of polysaccharide purified from *Gynostemma pentaphyllum Makino*. *Carbohydrate Polymers*, *68*(1), 54–58.

Yan, J., & Katz, A. (2010). PectaSol-C modified citrus pectin induces apoptosis and inhibition of proliferation in human and mouse androgen-dependent and- independent prostate cancer cells. *Integrative Cancer Therapies*, *9*(2), 197–203.

Zhang, Z., Zhang, Q., Wang, J., Shi, X., Song, H., & Zhang, J. (2009). *In vitro* antioxidant activities of acetylated, phosphorylated and benzoylated derivatives of porphyran extracted from Porphyra haitanensis. *Carbohydrate Polymers*, *78*(3), 449–453.

Zhao, Q., Kennedy, J. F., Wang, X., Yuan, X., Zhao, B., Peng, Y., et al. (2011). Optimization of ultrasonic circulating extraction of polysaccharides from *Asparagus officinalis* using response surface methodology. *International Journal of Biological Macromolecules*, *49*, 181–187.