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Characterization and Antioxidant Properties of OJP2, a Polysaccharide Isolated from *Ophiopogon japonicus*

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Abstract

A water-soluble polysaccharide (OJP2) obtained from the roots of *Ophiopogon japonicas*, was precipitated with 95% ethanol and purified by DEAE-52 cellulose anion-exchange and Sephadex G-100 gel filtration chromatography. The characteristics of OJP2 were determined by chemical analysis, high performance gel permeation chromatography (HPGPC), and gas chromatography-mass spectrometry (GC-MS). The results showed that the average molecular weight (Mw) of OJP2 was 35.2 kDa, and five kinds of monosaccharides including rhamnose, arabinose, xylose, glucose and galactose in a molar ratio of 0.5:5:4:1:10. Furthermore, the antioxidant activity of OJP2 was evaluated in H_2O_2-treated HaCaT cells and glucose-treated LO2 cells. The results show that OJP2 can increase the activity of SOD and NO production, and decrease the level of MDA in these two kinds of injury cells. OJP2 should be explored as a novel and potential natural antioxidant agent for use in functional foods or medicine.

Keywords

Polysaccharide, *Ophiopogon japonicas*, Characterization, Antioxidant

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1. Introduction

Free radicals, the highly reactive molecules, play an important positive physiological role and, at the same time, they may exert toxic effects [1] [2]. There is increasing evidence that free radicals are able to damage cell membranes and numerous biological substances [3] [4], resulting in various diseases including cardiovascular diseases, cancer, aging, Parkinson's and Alzheimer's disease, atherosclerosis, and impairment of immune function [5]-[8]. Antioxidants can scavenge free radicals and help to reduce oxidative damage. Polysaccharides extracted from natural sources have been found to have variety of biological activities, such as antioxidant, immunobiological and antitumor activity, which have attracted lots of attention in the biochemical and medical area [9]-[13].

Ophiopogon japonicus (Thunb.) Ker-Gawl is a well known traditional Chinese medicine used to treat cardiovascular and chronic inflammatory diseases for thousands of years, widely distributed in south-east Asia, and has been confirmed in various experiments as having anti-inflammatory, anti-arrhythmia, and microcirculation improvement etc. [14] [15]. Chemical studies have shown that this plant includes saponins, polysaccharide and homoisoflavonoidal compounds [16]. In recent years, the polysaccharides isolated from the roots of *O. japonicus* have drawn the attention of researchers and consumers due to their nutritional and health protective value in hypoglycemic, anti-ischaemia, immunostimulation, inhibiting platelets aggregation, etc. [17]-[19]. However, the structure and function of these polysaccharides have not been well characterized.

In the present study, we isolated and purified a polysaccharide (designated OJP2 below) from *O. japonicas* using DEAE-cellulose anion-exchange and a Sephadex G-150 column chromatography. In addition, the characteristics and antioxidant activity of the polysaccharides are also investigated. It will be helpful to better find its functional properties for the wide application in food and pharmaceutical industries.

2. Material and Methods

2.1. Materials and Chemicals

The roots of *O. japonicus* were collected in Dongtou, Zhejiang province (China). Sephadex G-150, DEAE-cellulose, fucose, arabinose, rhamnose, xylose, glucose, galactose, mannose, trifluoroacetic acid (TFA) was purchased from Sigma. RPMI-1640 and DMEM medium was purchased from Gibco. The assay kits for superoxide dismutase (SOD), malondialdehyde (MDA) and nitric oxide (NO) were purchased from Jiancheng Biologic Project Company, Nanjing, Jiangsu Province. Cell line HaCaT and LO2 was purchased from Shanghai Institute of Cell Biology, Chinese Academy of Sciences. All the other chemicals used were of analytical grade.

2.2. General Methods

Gas chromatography (GC) and Gas chromatography-mass spectrometry (GC-MS) were described previously [20]. The products were dried by lyophilization. Gas chromatography (GC) (Shimadzu GC-2010) equipped with RTX-50 column (30.0 m × 0.25 mm × 0.25 μm) and flame-ionization detector (FID). Evaporation was performed at around 45°C under reduced pressure. The operation was performed using the following conditions: column temperature was programmed from 140˚C (maintained for 2 min) to 170˚C at a rate of 6˚C/min, and increased to 173˚C at a rate of 0.2˚C/min, then increased to 233˚C at a rate of 6˚C/min, held for 40 min at 233˚C; the rate of N_2 carrier gas was 1.0 ml/min; injection temperature was 250˚C; detector temperature was 300˚C. Gas chromatography-mass spectrometry (GC-MS) was run on the instrument Shimadzu GCMS-QP2010 (Shimadzu, Japan) and equipped with RTX-50 column (30 m × 0.25 mm × 0.25 μm), and at temperatures programmed from 140˚C (maintained for 2 min) to 250˚C (kept for 20 min) at a rate of 3˚C /min. Nitrogen was the carrier gas.

2.3. Cell Culture and MTT Assay

HaCaT cell were maintained in DMEM medium supplemented with heat-inactivated fetal bovine serum (10%) at 37˚C in humidified air containing 5% CO_2. The cells were plated into triplicate wells (100 μL/well) in 96-well ((5×10^5 cells/well) flat bottom tissue culture plates and co-cultured with samples at indicated concentrations and 250 μM H_2O_2. Serum free DMEM was used as control. Following 24 h incubation at 37˚C and 5% CO_2, the supernatants were collected for detection of SOD activity, NO and MDA levels using commercial assay kits.

LO2 Cell were maintained in DMEM medium supplemented with heat-inactivated fetal bovine serum (10%)

at 37˚C in humidified air containing 5% CO_2. The cells were plated into triplicate wells (200 μL/well) in 96-well flat bottom tissue culture plates and co-cultured with samples at indicated concentrations and 30 mmol/L glucose. Serum free DMEM was used as control. Following 24 h incubation at 37˚C and 5% CO_2, the cell was measured by MTT assay. The absorbance at 570 nm test wavelength was translated into inhibition ratio for comparison.

$$\text{Inhibition ratio} = \left(A_0 - A_s\right)/A_0 \times 100\%$$

where A_0 and A_s are absorbance of blank and sample respectively.

2.4. Isolation and Purification of the Polysaccharide

The polysaccharide was prepared as described previously [20]. The roots of *O. japonicus* were soaked with 95% ethanol to remove the pigments and small lipophilic molecules. The residue was then extracted with 10 vol. of distilled water at 90˚C for 3 h thrice. All water-extracts were combined, filtrated, concentrated, and precipitated with 95% EtOH (1:4, v/v) at 4˚C for overnight. The precipitate was collected by centrifugation and deproteinated by Sevag method [21]. Finally the supernatant was lyophilized to give crude polysaccharides.

The crude polysaccharides were dissolved in distilled water and filtered through a membrane (0.45 μm). Then the solution was applied to a DEAE-52 cellulose column. Fractions were eluted with increased concentration of NaCl (0.01 - 1.0 M). The polysaccharide fractions were collected, concentrate, dialysed and finally lyophilized. Then, the sample was further purified by Sephadex G-150, and the second fraction was collected and lyophilized to give a polysaccharide named OJP2.

The polysaccharide was monitored by the phenol-sulfuric acid method [22].

2.5. Homogeneity and Molecular Weight

The homogeneity and molecular weight of OJP2 was evaluated and determined by high performance gel permeation chromatography (HPGPC) as described previously [20]. The sample solution was applied to Waters High Performance Liquid Chromatography (HPLC) equipped with a TSK-GEL G5000 SWXL column (7.8 × 300 mm), eluted with 0.1 mol/L Na_2SO_4 solution at a flow rate of 0.4 ml/min and detected by a Waters 2414 Refractive Index Detector. The columns were calibrated with Dextran T-series standard of known molecular weight (200,000, 70,000, 40,000, 10,000, 5000 Da). The molecular weight of OJP2 was estimated by reference to the calibration curve made above.

2.6. Analysis of Monosaccharide Composition

The monosaccharide of OJP2 was analyzed by GC as described previously [20]. OJP2 was hydrolyzed with 2 M TFA (2 ml) at 120˚C for 2 h. After removing TFA with methanol, the hydrolyzed product was reduced with $NaBH_4$ (50 mg), followed by neutralization with dilute acetic acid and evaporated at 45˚C. The reduced products (alditols) were added with 1 ml pyridine and 1 ml acetic anhydride in a boiling water bath for 1 h. The acetylated products were analyzed by GC.

2.7. Methylation Analysis

OJP2 (20 mg) was methylated three times according to the method of Needs and Selvendran [23]. The methylated products were extracted by chloroform and examined by IR spectroscopy. The absence of the absorption peak corresponding to hydroxyl indicated the complete methylation. The product was hydrolyzed using 2 M TFA, followed by reduction using $NaBH_4$ and finally acetylated with acetic anhydride. The partially methylated alditol acetates were analyzed by GC-MS.

2.8. Biochemical Assays

Lipid peroxidation was determined by quantifying MDA concentrations, which was spectrophotometrically measured by the absorbance of a red-colored product with thiobarbituric acid [24]. SOD and NO were measured with commercial kits, and the test method was done according to the reagent protocol prepared by the manufacturing firm.

2.9. Statistical Analysis

Results were presented as mean ± standard deviation (S.D.). Data were analyzed by one-way ANOVA using Student's *t-test*. *P*-values less than 0.05 were considered significant.

3. Results

3.1. Isolation and Characterization of OJP2

The polysaccharides extracted from the root of *O. japonicus* were purified through with DEAE-cellulose column and Sephadex G-100 column and named OJP2. OJP2 has no absorption at 280 and 260 nm in the UV spectrum, indicating the absence of protein and nucleic acid.

The average molecular weight of OJP2 was determined as 88.1 kDa by HPGPC. Results from phenol-sulfuric acid assay showed that OJP2 contained 95.3% carbohydrate.

OJP2 was hydrolyzed by TFA into individual monosaccharides that were further reduced and acetylated for GC analysis. The results showed that OJP2 is composed of Rha, Ara, Xyl, Glc, Gal with a relative molar ratio of 0.5:5:4:1:10 (**Figure 1**).

The IR spectrum of OJP2 was shown in **Figure 2**. The IR spectrum revealed a typical major broad stretching peak around 3400 - 3500 cm^{-1} for the hydroxyl group, and the small band at around 2950.21 cm^{-1} was attributed to the C-H stretching and bending vibrations. The relatively strong absorption peak at around 1614.55 cm^{-1} reflects the absorption of the C=O group that is part of glycosides [20] [25]. The absorptions at 1020.91 and 1099.76 cm^{-1} indicated a pyranose form of sugars [26]. The region between 950 and 1200 cm^{-1} is dominated by ring vibrations overlapped with stretching vibrations of (C-OH) side groups and the (C-O-C) glycosidic band vibration [20] [27].

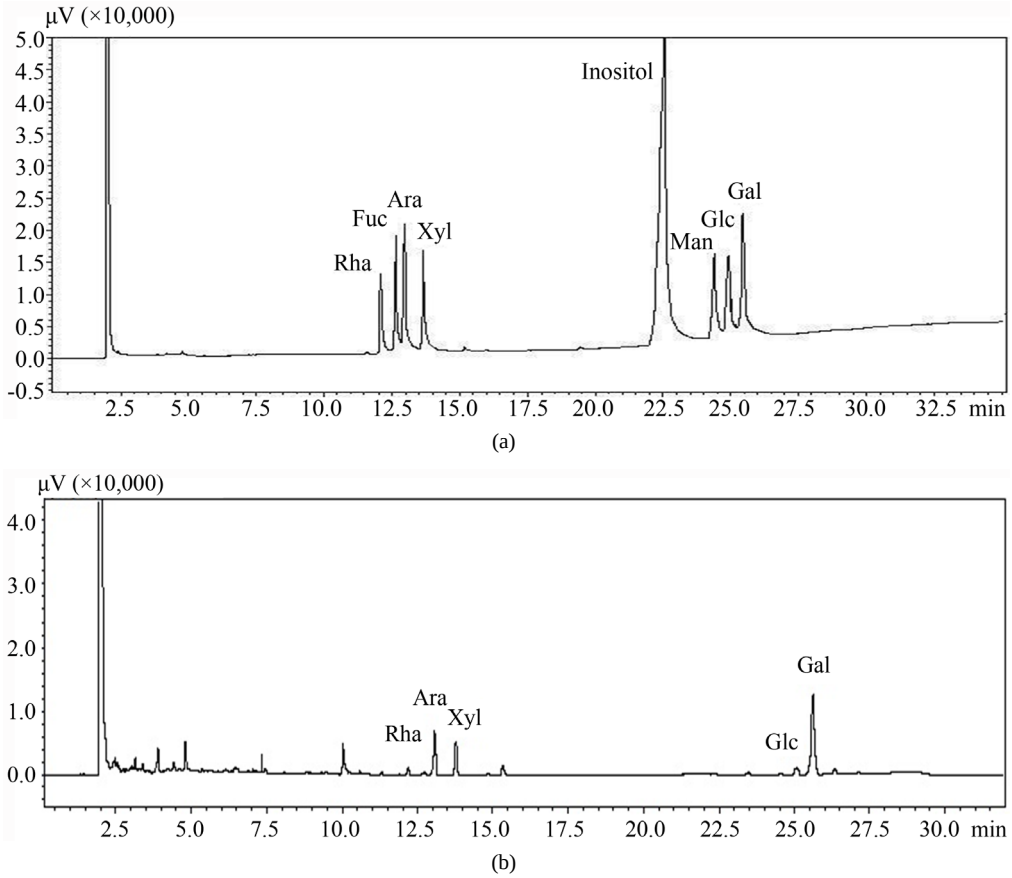

Figure 1. GC profile of OJP2. (a) Standard monosaccharides; (b) Monosaccharide composition of OJP2; Rha = Rhamnose, Fuc = Fucose, Ara = Arabinose, Xyl = Xylose, Man = Mannose, Glc = Glucose, Gal = Galactose.

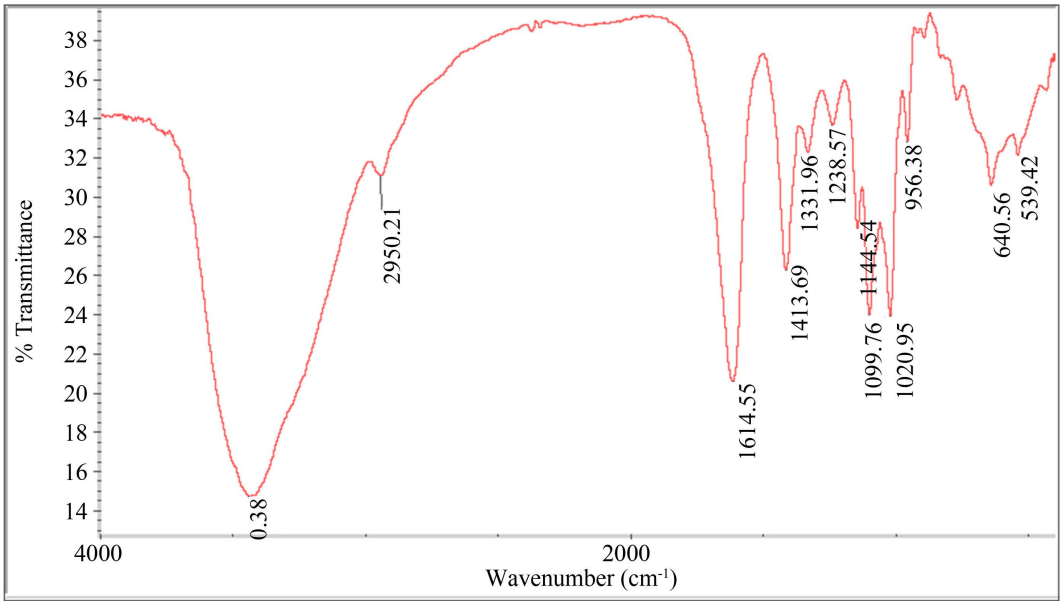

Figure 2. IR spectrum of the polysaccharide of OJP2 isolated from the roots of *O. japonicas*.

The fully methylated OJP2 was hydrolyzed with acid, converted into alditol acetates and analyzed by GC-MS. As shown in **Table 1**, the ratios of methylated fragments were calculated based on the areas of the methylated products and corrected using the effective-carbon response method [28]. The GC-MS results (**Table 1**) indicated that 2,5-Me-Xyl (1,3-linked Xyl), 2,3,4-Me-Gal (1,6-linked Gal) and part of Ara were major components of the backbone structure, part of Ara and Gal were distributed in branches, and residues of branches terminated with Rha, Glc and Gal.

3.2. Antioxidant Activities of OJP2 in H_2O_2-Treated HaCaT Cells

As shown in **Table 2**, the activity of SOD was significantly ($P < 0.01$) decreased in H_2O_2-treated group compared to the normal control group. OJP2-treated (250, 500, 1000 μg/mL) significantly ($P < 0.01$) increased the activities of SOD in H_2O_2-treated HaCaT cells with a dose-dependent manner. The treatment of HaCaT cells with H_2O_2 afforded the decrease in the NO production. However, incubation of the H_2O_2-treated cells with OJP2 polysaccharide markedly reduced in a dose-dependent manner the decrements in NO production of H_2O_2-treated cells ($P < 0.01$).

The levels of MDA in H_2O_2-treated groups was significantly ($P < 0.01$) increased (**Table 2**). Incubation of the H_2O_2-treated cells with OJP2 polysaccharide significantly decreased ($P < 0.01$ or $P < 0.05$) the levels of MDA, although it did not act as a dose-dependent manner.

3.3. Antioxidant Activities of OJP2 in Glucose-Treated LO2 Cells

As shown in **Table 3**, the treatment of LO2 cells with 30 mmol/L glucose caused inhibition of the cells growth and the inhibition ratio up to 52.8%. However, incubation of the glucose-treated cells with OJP2 polysaccharide markedly decreased in a dose-dependent manner the inhibition ratio of glucose-treated cells.

The treatment of LO2 cells with 30 mmol/L glucose afforded the decrease in the activity of SOD and NO production. However, incubation of the glucose-treated cells with OJP2 polysaccharide markedly reduced in a dose-dependent manner the decrements in SOD activity and NO production of glucose-treated cells ($P < 0.01$ or $P < 0.05$). The treatment of OJP2 not only recovered the glucose-decreased SOD activity and NO production, but also increased SOD activity and NO production, especially for NO production.

4. Discussion

Oxidation phenomena have been implicated in many illnesses, such as diabetes mellitus, arteriosclerosis,

Table 1. The results of methylation analysis of OJP2.

No.	Methylated sugar[a]	Molar ratio	Linkages types
1	2,3,4-Me-Rha	1	Rha-(1→
2	2,3-Me-Ara	3	→5)-Ara-(1→
3	2,3,4,6-Me-Glc	2	Glc-(1→
4	2,3,4,6-Me-Gal	3	Gal-(1→
5	3,5-Me-Ara	2	→2)-Ara-(1→
6	2,5-Me-Ara	3	→3)-Ara-(1→
7	2,5-Me-xyl	7	→3)-Xyl-(1→
8	2,3,4-Me-Gal	15	→6)-Gal-(1→
9	2-Me-Ara	1	→3,5)-Ara-(1→
10	2,4-Me-Gal	3	→3,6)-Gal-(1→

[a]2,3,4,6-Me-Glc = 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-glucose, etc.

Table 2. Effects of OJP2 on SOD activity, NO and MDA levels of H_2O_2-treated HaCaT cells[a].

	SOD (U/mL)	NO (μmol/L)	MDA (nmol/L)
Normal control	69.79 ± 2.68	35.24 ± 1.21	0.47 ± 0.04
H_2O_2-treated control	33.15 ± 3.25[b]	27.23 ± 1.03[b]	0.69 ± 0.03[b]
OJP2-250	46.73 ± 1.89[d]	32.01 ± 0.85[d]	0.58 ± 0.04[c]
OJP2-500	56.12 ± 3.17[d]	35.09 ± 0.89[d]	0.51 ± 0.03[d]
OJP2-1000	58.35 ± 3.08[d]	36.05 ± 1.09[d]	0.52 ± 0.04[d]

[a]Normal control: without H_2O_2 injury; H_2O_2-treated control: incubated with 250 μM H_2O_2; OJP2-250: incubated with 250 μg/mL OJP2 and 250 μM H_2O_2; OJP2-500: incubated with 500 μg/mL OJP2 and 250 μM H_2O_2; OJP2-1000: incubated with 1000 μg/mL OJP2 and 250 μM H_2O_2; OJP2 was added to the culture 24 h prior to H_2O_2 addition; Three independent experiments were carried out in triplicates. Data represent mean ± S.D. [b]$P < 0.01$ compared with the normal control. [c]$P < 0.05$ compared with the H_2O_2-treated control. [d]$P < 0.01$ compared with the H_2O_2-treated control.

Table 3. Effects of OJP2 on NO production and the activity of SOD in glucose-treated LO2 cells.

.	BC[a]	GC[a]	OJP2 (1.5 mg/mL)[a]	OJP2 (1 mg/mL)[a]	OJP2 (0.5 mg/mL)[a]	OJP2 (0.25 mg/mL)[a]
Inhibition (%)		52.8 ± 2.5	39.8 ± 3.7[b]	40.2 ± 2.6[c]	42.9 ± 3.2[c]	45.6 ± 2.5[c]
SOD (IU)	18.56 ± 1.23	15.21 ± 1.21[d]	20.25 ± 1.05[c]	19.18 ± 0.97[c]	19.06 ± 0.83[c]	17.52 ± 1.07[b]
NO (μmol/L)	5.89 ± 0.14	4.24 ± 0.08[d]	11.23 ± 0.43[c]	10.87 ± 0.39[c]	8.53 ± 0.56[c]	7.81 ± 0.32[c]

[a]BC: Blank control (without glucose treated); GC: Glucose (30 mmol/L) treated control; the OJP2 groups: LO2 cell co-cultured with samples at indicated concentrations and 30 mmol/L glucose; [b]$P < 0.05$, [c]$P < 0.01$ (compared with the Glucose treated); [d]$P < 0.01$ (compared with the Blank control).

nephritis, Alzheimer's disease and cancer [29] [30]. SOD, one of the major antioxidant enzymes, decompose superoxide peroxide, against reactive oxygen species generated *in vivo* during oxidative stress, and being involved in the cellular defense mechanisms [31]. Our study shows that OJP2 treatment markedly restores the activity of SOD in H_2O_2-treated cells, and also increases the activity of SOD in glucose-treated LO2 cells. These finding suggest that OJP2 could considerably improve cellular antioxidative defense against oxidative stress.

MDA, generated under high levels of un-scavenged free radicals, is regarded as an index of cellular damage and cytotoxicity [32]. The study showed that the levels of MDA in H_2O_2-treated HaCaT cells or glucose-treated LO2 cells were increased. On the contrary, OJP2 treatment could decrease the MDA content elevation in heart tissues of diabetic rats. It was quite possible that the free radicals were effectively neutralized or scavenged, resulting in antioxidant effect of OJP2.

Nitric oxide (NO), a biologically active unstable radical, is generated from the metabolism of L-arginine by the enzyme nitric oxide synthase (NOS), and may quench the superoxide anion [33]. NO is also known to regulate immune responses and plays an important role in the protection against the onset and progression of cardiovascular disease, and decreased NO bioavailability has been proposed as one of the determinants of vascular damage [34] [35]. Our results show that OJP2 treatment increased the NO production both in H_2O_2-treated HaCaT cells and glucose-treated LO2 cells.

5. Conclusions

In conclusion, according to our experiment, the polysaccharide fraction (OJP2), with the MW 88.1 KDa, isolated from the root of *O. japonicus*. We demonstrated that OJP2 was a heteropolysaccharide consisting of Rha, Ara, Xyl, Glc, and Gal with a relative molar ratio of 0.5:5:4:1:10; 2,5-Me-Xyl (1,3-linked Xyl), 2,3,4-Me-Gal (1,6-linked Gal) or part of Ara were major components of the backbone structure; part of Ara and Gal were distributed in branches; and residues of branches terminated with Rha, Glc and Gal.

Biologically this polysaccharide exhibits significant antioxidant activity *in vitro*, and protective effects on H_2O_2-induced injury in HaCaT cells or glucose-induced injury LO2 cells. It might be a great potential source for the development of antioxidant agent for use on functional foods or medicine.

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References

[1] Kozarski, M., Klaus, A., Niksic, M., Jakovljevic, D., Helsper, J.P.F.G. and Van Griensven, L.J.L.D. (2011) Antioxidative and Immunomodulating Activities of Polysaccharide Extracts of the Medicinal Mushrooms *Agaricus bisporus*, *Agaricus brasiliensis*, *Ganoderma lucidum* and *Phellinus linteus*. *Food Chemistry*, **129**, 1667-1675. http://dx.doi.org/10.1016/j.foodchem.2011.06.029

[2] Wu, X.J. and Hansen, C. (2008) Antioxidant Capacity, Phenol Content, and Polysaccharide Content of *Lentinus edodes* Grown in Whey Permeate-Based Submerged Culture. *Journal of Food Science*, **73**, 1-8. http://dx.doi.org/10.1111/j.1750-3841.2007.00595.x

[3] Tsai, M., Song, T., Shih, P. and Yen, G. (2007) Antioxidant Properties of Water-Soluble Polysaccharides from *Antrodia cinnamomea* in Submerged Culture. *Food Chemistry*, **104**, 1115-1122. http://dx.doi.org/10.1016/j.foodchem.2007.01.018

[4] Thetsrimuang, C., Khammuang, S., Chiablaem, K., Srisomsap, C. and Sarnthima, A. (2011) Antioxidant Properties and Cytotoxicity of Crude Polysaccharides from *Lentinus polychrous* Lév. *Food Chemistry*, **128**, 634-639. http://dx.doi.org/10.1016/j.foodchem.2011.03.077

[5] Wu, X.J. and Hansen, C. (2008) Antioxidant Capacity, Phenol Content, and Polysaccharide Content of *Lentinus edodes* Grown in Whey Permeate-Based Submerged Culture. *Journal of Food Science*, **73**, 1-8. http://dx.doi.org/10.1111/j.1750-3841.2007.00595.x

[6] Xu, W., Zhang, F., Luo, Y., Ma, L., Kou, X. and Huang, K. (2009) Antioxidant Activity of a Water-Soluble Polysaccharide Purified from *Pteridium aquilinum*. *Carbohydrate Research*, **344**, 217-222. http://dx.doi.org/10.1016/j.carres.2008.10.021

[7] Shin, K.H., Lim, S.S., Lee, S.H., Lee, Y.S. and Cho, S.Y. (2001) Antioxidant and Immunostimulating Activities of the Fruiting Bodies of *Paecilomyces japonica*, a New Type of Cordyceps sp. *Annals of the New York Academy of Sciences*, **928**, 261-273. http://dx.doi.org/10.1111/j.1749-6632.2001.tb05655.x

[8] Finkel, T. and Holbrook, N.J. (2000) Oxidants, Oxidative Stress and the Biology of Aging. *Nature*, **408**, 239-247. http://dx.doi.org/10.1038/35041687

[9] Xue, S.X., Chen, X.M., Lu, J.X. and Jin, L.Q. (2009) Protective Effect of Sulfated Achyranthes Bidentata Polysaccharides on Streptozotocin-Induced Oxidative Stress in Rats. *Carbohydrate Polymers*, **75**, 415-419. http://dx.doi.org/10.1016/j.carbpol.2008.08.003

[10] Chen, R.Z., Liu, Z.Q., Zhao, J.M., Chen, R.P., Meng, F.L., Zhang, M. and Ge, W.C. (2011) Antioxidant and Immunobiological Activity of Water-Soluble Polysaccharide Fractions Purified from *Acanthopanax senticosu*. *Food Chemistry*, **127**, 434-440. http://dx.doi.org/10.1016/j.foodchem.2010.12.143

[11] Chen, X.M., Nie, W.J., Fan, S.R., Zhang, J.F., Wang, Y.X., Lu, J.X. and Jin, L.Q. (2012) A Polysaccharide from *Sar-*

gassum fusiforme Protects against Immunosuppression in Cyclophosphamide-Treated Mice. *Carbohydrate Polymers*, **90**, 1114-1119. http://dx.doi.org/10.1016/j.carbpol.2012.06.052

[12] Chen, R.Z., Meng, F.L., Liu, Z.Q., Chen, R.P. and Zhang, M. (2010) Antitumor Activities of Different Fractions of Polysaccharide Purified from *Ornithogalum caudatum* Ait. *Carbohydrate Polymers*, **80**, 845-851. http://dx.doi.org/10.1016/j.carbpol.2009.12.042

[13] Chen, X.M., Nie, W.J., Yu, G.Q., Li, Y.L., Hu, Y.S., Lu, J.X. and Jin, L.Q. (2012) Antitumor and Immunomodulatory Activity of Polysaccharides from *Sargassum fusiforme*. *Food and Chemical Toxicology*, **50**, 696-700. http://dx.doi.org/10.1016/j.fct.2011.11.015

[14] Zhou, Y.H., Xu, D.S., Feng, Y., Fang, J.N., Xia, H.L. and Liu, J. (2003) Effects on Nutrition Blood Flow of Cardiac Muscle in Mice by Different Extracts in Radix Ophiopogonis. *China Journal of Experimental Traditional Medical Formulae*, **9**, 22-23.

[15] Huang, H.C. and Ni, Z. (2003) Effect of Ophiopogonis on the Auricular Microcirculation in Mice. *Shanghai Laboratory Animal Science*, **23**, 57-58.

[16] Kou, J., Sun, Y., Lin, Y., Cheng, Z., Zheng, W., Yu, B. and Xu, Q. (2005) Anti-Inflammatory Activities of Aqueous Extract from Radix *Ophiopogon japonicus* and Its Two Constituents. *Biological & Pharmaceutical Bulletin*, **28**, 1234-1238. http://dx.doi.org/10.1248/bpb.28.1234

[17] Zheng, Q., Feng, Y., Xu, D.S., Lin, X. and Chen, Y.Z. (2009) Influence of Sulfation on Anti-Myocardial Ischemic Activity of *Ophiopogon japonicus* Polysaccharide. *Journal of Asian Natural Products Research*, **11**, 306-321. http://dx.doi.org/10.1080/10286020902727363

[18] Chen, X.M., Tang, J., Xie, W.Y., Wang, J.J., Jin, J., Ren, J., Jin, L.Q. and Lu, J.X. (2013) Protective Effect of the Polysaccharide from *Ophiopogon japonicus* on Streptozotocin-Induced Diabetic Rats. *Carbohydrate Polymers*, **94**, 378-385. http://dx.doi.org/10.1016/j.carbpol.2013.01.037

[19] Fan, J. and Zhang, X. (2006) Research Progress in Pharmacology of *Ophiopogon japonicus* Polysaccharides. *Chinese Archives of Traditional Chinese Medicine*, **24**, 626-627.

[20] Chen, X.M., Jin, J., Tang, J., Wang, Z.F., Wang, J.J., Jin, L.Q. and Lu, J.X. (2011) Extraction, Purification, Characterization and Hypoglycemic Activity of a Polysaccharide Isolated from the Root of *Ophiopogon japonicus*. *Carbohydrate Polymers*, **83**, 749-754. http://dx.doi.org/10.1016/j.carbpol.2010.08.050

[21] Staub, A.M. (1965) Removal of Proteins from Polysaccharides. *Methods in Carbohydrate Chemistry*, **5**, 5-7.

[22] Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A. and Smith, F. (1956) Colorimetric Method for Determination of Sugars and Related Substance. *Analytical Chemistry*, **28**, 350-356. http://dx.doi.org/10.1021/ac60111a017

[23] Needs, P.W. and Selvendran, R.R. (1993) Avoiding Oxidative Degradation during Sodium Hydroxide/Methyl Iodide-Mediated Carbohydrate Methylation in Dimethyl Sulfoxide. *Carbohydrate Research*, **245**, 1-10. http://dx.doi.org/10.1016/0008-6215(93)80055-J

[24] Yagi, K. (1994) Lipid Peroxides and Related Radicals in Clinical Medicine. In: Armstrong, D., Ed., *Free Radicals in Diagnostic Medicine*, Plenum Press, New York, 1-14. http://dx.doi.org/10.1007/978-1-4615-1833-4_1

[25] Zhu, M.Y., Mo, J.G., He, C.S., Xie, H.P., Ma, N. and Wang, C.J. (2010) Extraction, Characterization of Polysaccharides from *Lycium barbarum* and Its Effect on Bone Gene Expression in Rats. *Carbohydrate Polymers*, **80**, 672-676. http://dx.doi.org/10.1016/j.carbpol.2009.11.038

[26] Zhao, G.H., Kan, J.Q., Li, Z.X. and Chen, Z.D. (2005) Structural Features and Immunological Activity of a Polysaccharide from *Dioscorea opposita* Thunb Roots. *Carbohydrate Polymers*, **61**, 125-131. http://dx.doi.org/10.1016/j.carbpol.2005.04.020

[27] Yang, L. and Zhang, L.M. (2009) Chemical Structural and Chain Conformational Characterization of Some Bioactive Polysaccharides Isolated from Natural Sources. *Carbohydrate Polymers*, **76**, 349-361. http://dx.doi.org/10.1016/j.carbpol.2008.12.015

[28] Sweet, D.P., Shapiro, R.H. and Albersheim, P. (1975) Quantitative Analysis by Various g.l.c. Response-Factor Theories for Partially Methylated and Partially Ethylated Alditol Acetates. *Carbohydrate Research*, **40**, 217-225. http://dx.doi.org/10.1016/S0008-6215(00)82604-X

[29] Misthos, P., Katsaragakis, S., Milingos, N., Kakaris, S., Sepsas, E., Athanassiadi, K., Theodorou, D. and Skottis, I. (2005) Postresectional Pulmonary Oxidative Stress in Lung Cancer Patients. The Role of One-Lung Ventilation. *European Journal of Cardiothoracic Surgery*, **27**, 379-383. http://dx.doi.org/10.1016/j.ejcts.2004.12.023

[30] Dadé, M.M., Schinella, G.R., Fioravanti, D.E. and Tournier, H.A. (2011) Antioxidant and Cytotoxic Properties of an Aqueous Extract from the Argentinean Plant *Hedeoma multiflorum*. *Pharmaceutical Biology*, **49**, 633-639. http://dx.doi.org/10.3109/13880209.2010.526949

[31] Yao, D.C., Shi, W.B., Gou, Y.L., Zhou, X.R., Tak, Y.A. and Zhou, Y.K. (2005) Fatty Acid-Mediated Intracellular Iron

Translocation: A Synergistic Mechanism of Oxidative Injury. *Free Radical Biology and Medicine*, **39**, 1385-1398. http://dx.doi.org/10.1016/j.freeradbiomed.2005.07.015

[32] Karthikeyan, K., Bai, B.R. and Devaraj, S.N. (2007) Cardioprotective Effect of Grape Seed Proanthocyanidins on Isoproterenol-Induced Myocardial Injury in Rats. *International Journal of Cardiology*, **115**, 326-333. http://dx.doi.org/10.1016/j.ijcard.2006.03.016

[33] Wattanapitayakul, S.K., Weinstein, D.M., Holycross, B.J. and Bauer, J.A. (2000) Endothelial Dysfunction and Peroxynitrite Formation Are Early Events in Angiotensin-Induced Cardiovascular Disorders. *The FASEB Journal*, **14**, 271-278.

[34] Hamed, S., Brenner, B., Aharon, A., Daoud, D. and Roguin, A. (2009) Nitric Oxide and Superoxide Dismutase Modulate Endothelial Progenitor Cell Function in Type 2 Diabetes Mellitus. *Cardiovascular Diabetology*, **8**, 56. http://dx.doi.org/10.1186/1475-2840-8-56

[35] Mendez, I.I., Chung, Y.H., Jun, H.S. and Yoon, J.W. (2004) Immunoregulatory Role of Nitric Oxide in Kilham Rat Virus-Induced Autoimmune Diabetes in DR-BB Rats. *The Journal of Immunology*, **173**, 1327-1335. http://dx.doi.org/10.4049/jimmunol.173.2.1327