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Preparation of lacquer polysaccharide sulfates and their antioxidant activity *in vitro*

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Abstract

Lacquer polysaccharide (LP) was isolated and purified from the sap of the lac tree (*Rhus vernicifera*). Five sulfated lacquer polysaccharide (LPS), with various molecular weights (M_w) and degrees of sulfation (DS) were prepared by the reaction of LP with sulfur trioxide–pyridine complex (SO_3·Py) in DMSO. The structure of LPS was analyzed by GPC, UV–vis, FT-IR and ^{13}C NMR spectroscopy; the M_w of LPS was in the range of $0.78–1.58 \times 10^4$, DS varied from 0.22 to 0.58, and unsaturated bond presence was observed by FT-IR. Antioxidant assays showed that LPS antioxidant activities were related to M_w, DS and unsaturated bond presence. One LPS, with moderate M_w and DS, showed the best antioxidant capacities, its reducing capacity was 0.61 at 500 μg/mL, scavenging ability for superoxide and hydroxyl radical were 56.4% at 500 μg/mL, 55.6% at 1000 μg/mL, respectively. The data obtained in in vitro models establish the antioxidant potential of LPS for application in pharmaceuticals. The LPS may be a promising antioxidant in vitro.

Keywords: Lacquer polysaccharide; Sulfation; Antioxidant activity; Reducing power; Radical scavenging activity

1. Introduction

Oxidative stress, induced by oxygen radicals, is believed to be a primary factor in various diseases such as cancer, rheumatoid arthritis, Alzheimer's disease and atherosclerosis as well as in degenerative processes of aging (Finkel & Holbrook, 2000; Halliwell, Gutteridge, & Cross, 1992; Mau, Lin, & Song, 2002; Zhu et al., 2004). Reactive oxygen species (ROS), in the forms of superoxide anion ($\cdot O_2^-$), hydroxyl radical (·OH), and hydrogen peroxide (H_2O_2), are generated by normal metabolic processes or from exogenous factors and agents, and they can easily initiate the peroxidation of membrane lipids, leading to the accumulation of lipid peroxides (Xing et al., 2005). ROS can cause damage to a wide range of essential biomolecules, such as DNA, and they have been associated with carcinogenesis, coronary heart disease, and many other health problems related to advancing age (Cadenas & Davies, 2000; Uchida, 2000). Although most organisms possess antioxidant defence and repair systems that have evolved to protect them against oxidative damage, these systems are insufficient to prevent the damage entirely. So it is essential to develop and utilize effective antioxidants so that they can scavenge free radicals in the human body. Antioxidants are substances that delay or prevent the oxidation of cellular oxidizable substrates. They exert their effects by scavenging and preventing the generation of ROS (Halliwell et al., 1992). In order to reduce damage to the human body, synthetic antioxidants are currently used for industrial processing. However, the most common antioxidants such as butylated hydroxyanisole and butylated hydroxytoluene have been suspected of being responsible for liver damage and carcinogenesis (Grice, 1988; Qi et al., 2005).

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Thus, in recent years, there has been increasing interest in finding natural antioxidants, since they can protect the human body from free radical damage and retard the progress of many chronic diseases (Kinsella, Frankel, German, & Kanner, 1993; Nandita & Rajini, 2004). For example, the water-soluble polysaccharide extracted and purified from *Gynostemma pentaphyllum* (Makino) and its three fractions GMA, GMB, and GMC, obtained from the *G. pentaphyllum* Makino, showed strong scavenging ability for superoxide radical (Wang & Luo, 2007). Two sulfated derivatives of a polysaccharide from the mycelium of a marine filamentous fungus *Phoma herbarum YS4108*, and its YCP-S1 and YCP-S2 fractions possessed good antioxidant abilities, the scavenging ability of both for superoxide anion was >50% at 400 μg/mL (Yang, Gao, Han, & Tan, 2005). Some polysaccharides (e.g. exopolysaccharide produced by the marine filamentous fungus *Keissleriella (=Phoma)* sp. *YS4108* (Sun, Wang, Fang, Gao, & Tan, 2004), and their derivatives have been accepted as potential candidates for the development of effective and non-toxic medicines with strong ability of scavenging free radicals and antioxidant actions.

Lac tree (*Rhus vernicifera*) is widely distributed in Asian countries, such as China, Japan, and Vietnam, etc., and is an abundant natural resource. Lacquer is obtained by the enzymatic polymerization of the sap of the lac tree in the presence of oxygen. It has been used in China and Japan for thousands of years as not only a highly durable coating material but also a traditional Chinese medicine. The sap of the lac tree is composed of urushiol (60–65%), glycoprotein (1.8–2.1%), gummy substances (6–7%), stellacyanin (7–8%) and some mono-, oligo- and polysaccharide (3–4%), and water (20–30%).

Chinese lacquer polysaccharide (LP) isolated from the sap of *R. vernicifera* is an acidic polysaccharide and its backbone is composed of 1,3-linked β-D-galactopyranose, which has complex branches with 4-*O*-methyl-β-D-glucuronic acid at the non-reducing terminals (Du, Yang, Kong, & Xiao, 1999; Oshima & Kumanotani, 1984). Studies on the molecular weight and solution properties of LP suggested that the polysaccharide exists in a random coil in aqueous solution (Zhang et al., 1992). Recently, lacquer polysaccharide has been found to have anticoagulant (Yang, Du, Huang, Wan, & Li, 2002; Yang, Du, Huang, Wan, & Wen, 2005), and antitumor (Yang et al., 2005) bioactivities, and activity against leucopenia induced by cyclophosphamide (Yang & Du, 2003), etc. The bioactivities are strongly dependent on the molecular weight, the degree of sulfation (DS), the sulfation pattern and the branches (Yang et al., 2002).

Plant polysaccharides in general have strong antioxidant activities (Hu, Xu, & Hu, 2003; Jiang, Jiang, Wang, & Hu, 2005; Ramarahnam, Osawa, Ochi, & Kawaishi, 1995). Though the lacquer is a highly durable coating material against oxygen, the relationship between antioxidant activity and the structure of lacquer polysaccharide is still not clear. And there are few reports on the antioxidant activities of lacquer polysaccharide and its sulfate derivative, so in this paper, antioxidant activities of lacquer polysaccharide and sulfated LP (LPS) have been investigated).

2. Materials and methods

2.1. Materials and chemicals

The sap of lacquer tree from Maoba in Hubei province was provided by Wuhan Chinese lacquer factory (Wuhan, China). The isolation process of lacquer polysaccharide (LP) was based on the method reported by Du, Kong, and Li (1994). The sulfation reagent, sulfur trioxide–pyridine complex (SO_3·Py), was from Fluka (Milan Italy), trichloroacetic acid was from Tianjin Kermel Chemical Reagents Development Centre (Tianjin, China), nitroblue tetrazolium (NBT) was from Sigma Chemicals Co (St. Louis, USA). DMSO (Shanghai Reagent Co Ltd., Shanghai, China) was treated with 5 Å molecular sieve to remove water, then distilled under reduced pressure. Dialysis tube (cut-off Da 3500) was from Wuhan Huashun Biotech Co. Ltd (Wuhan, China). All other chemicals and reagents were of analytical grade.

2.2. Sulfation of lacquer polysaccharide

LP powder (162 mg) was suspended in the dry DMSO (20 mL) and the mixture was stirred at different temperatures for 30 min, then SO_3·Py was added (Yang, Du, Wen, Li, & Hu, 2003). Various molar ratios of SO_3·Py to sugar units, temperature of reaction, and reaction time were used. After each reaction, the mixture was quickly cooled to room temperature, neutralized with 15% w/v aqueous NaOH, and then dialyzed for 120 h against distilled water. The dialysate was concentrated under reduced pressure below 40 °C and then precipitated with anhydrous ethanol. The precipitate was collected after drying over phosphorous pentoxide in vacuum.

2.3. Characterization

Weight average molecular weights (M_w) of samples were measured by a gel permeation chromatography (GPC) on a TSK G3000-pw column at 30 °C. The eluent (flow rate 1.0 mL/min) was 0.01 M sodium phosphate buffer, pH 7.0, containing 0.2 M Na_2SO_4. The eluate was monitored for refractive index. TOSHOH pullulan standards were used to calibrate the column (M_w's 2.7–11.2 kDa). The data provided by the GPC system were analyzed by the Jiangshen Workstation software package (Dalian, China).

Sulfate content was determined colorimetrically by precipitation of the sulfate with benzidine and then dissolution of the precipitate in hydrochloric acid to determine the amount of benzidine–sulfate spectrophotometrically) using sodium sulfate as standard (Antonopoulos, 1962).

UV–vis spectra of solutions of the crude and purified lacquer polysaccharides) were determined by a 1601 Shi-

madzu UV–vis spectrophotometer (Shimadzu, Kyoto, Japan). FT-IR spectra were measured as KBr pellets by a Nicolet FT-IR 5700 spectrophotometer (Thermo, Madison, USA). ^{13}C NMR spectra were recorded on an INOVA-600 NMR 600 MHz spectrometer (Varian, Palo Alto, USA).

2.4. Reducing power

The reducing power of LPS was determined based on the method of Oyaizu (1986). Different concentrations of LPS were mixed with 0.2 M sodium phosphate buffer, pH 6.6 (2.5 mL), and 1% w/v aqueous potassium ferricyanide (2.5 mL). The mixture was incubated at 50 °C for 20 min. 10% w/v Trichloroacetic acid (2.5 mL) was added to the mixture, which was then centrifuged at 1485*g* for 10 min. The upper layer of solution (2.5 mL) was diluted with distilled water (2.5 mL) and 0.1% w/v ferric chloride (0.5 mL) added. In this reaction, $K_3Fe(CN)_6$ was reduced by the sample, and $K_4Fe(CN)_6$ was formed, which in turn was reacted with Fe^{3+}; prussian blue was formed, the abundance of which was determined from the absorbance at 700 nm, higher absorbance indicating greater reducing power.

2.5. Superoxide radical (O_2^-) scavenging assay

The assay was based on the capacity of samples to inhibit the photochemical reduction of nitroblue tetrazolium (NBT) in the riboflavin-light-NBT system (Beauchamp & Fridovich, 1971). The method used by Martinez, Marcelo, Marco, and Moacyr (2001) for the determination of superoxide dismutase was followed after modification. Reagent containing 50 mM sodium phosphate buffer, pH 7.8, 13 mM methionine, 2 μM riboflavin, 100 μM EDTA, 75 μM NBT (3 ml) was mixed with sample solution (1 mL) The production of blue formazan was followed by monitoring the increase in absorbance at 560 nm after a 10 min illumination from a fluorescent lamp. The entire reaction assembly was enclosed in a box lined with aluminium foil. Identical tubes containing the reaction mixture and water (1 mL) were kept in the dark and served as blanks. (Calculations were based on the equation:

$$\%\,\text{Inhibition of superoxide radical} = [(A_0 - A_1)/A_0] \times 100,$$

where A_0 was the absorbance of the control and A_1 was the absorbance of samples.)

2.6. Hydroxyl radical (·OH) scavenging assay

The assay was based on a benzoic acid hydroxylation method, as described by Chung, Osawa, and Kawakishi (1997). Hydroxyl radicals are generated by the addition of ferrous ion (Fe^{2+}) to a reaction mixture which contains phosphate buffer (Gutteridge, 1984). Benzoate is hydroxylated to hydroxybenzoates. Benzoate possesses weak fluorescent intensity, while after monohydroxylation, products with strong fluorescent intensity are formed (Gutteridge, 1987). Spectrofluorometric changes have been measured to detect the damage caused by hydroxyl radicals.

Samples in 0.1 M sodium phosphate buffer, pH 7.4 (1.2 mL), were added to screw-capped tubes containing 10 mM sodium benzoate (0.2 mL), 10 mM $FeSO_4$ (0.2 mL), and 10 mM EDTA (0.2 mL) (total volume 1.8 mL). Finally, 10 mM hydrogen peroxide solution (0.2 mL) was added to the reaction mixture which was then incubated at 37 °C for 2 h. After this, the fluorescence was measured at 407 nm emission with excitation at 305 nm and calculations were based on the equation:

$$\text{OH-scavenging activity } (\%) = [1 - (FIs - FIo)/(FIc - FIo)] \times 100,$$

where FIo is fluorescence intensity at Ex 305 and Em 407 nm with no treatment, FIc is fluorescence intensity at Ex 305 and Em 407 nm of treated control, FIs is fluorescence intensity at Ex 305 and Em 407 nm of treated sample.

3. Results and discussion

3.1. The isolation and sulfation of lacquer polysaccharide

As shown in Fig. 1, the crude lacquer polysaccharide had absorbance in the region 250–270 nm; the peaks may be attributed (see: Du et al. (1994)) to water-soluble glycoprotein, suggesting that the glycoprotein was not removed completely by precipitation with aqueous ammonium sulfate. After purification by chromatography on Sephadex G-100 gel, the peaks at 250–270 nm had disappeared, and it was concluded that the glycoprotein was removed completely.

The reaction conditions and molecular structure parameters of LP and LPS are given in Table 1; higher reaction temperature and longer reaction time led to lower M_w and DS. Comparing LPS1 and LPS5, the higher molar

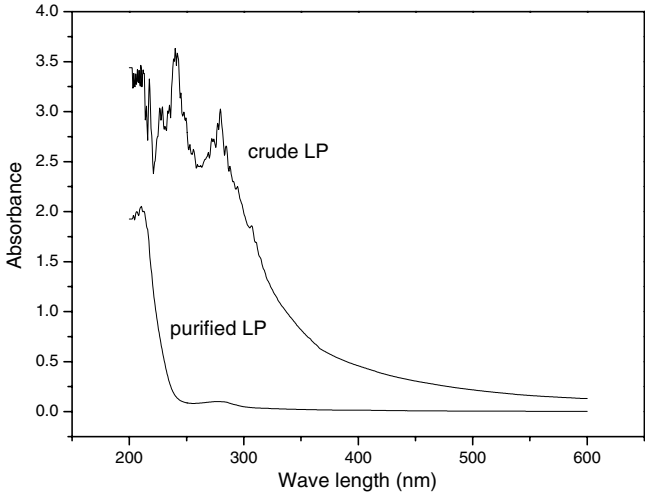

Fig. 1. Partial UV–vis spectra of crude and purified lacquer polysaccharide (LP).

Table 1
Reaction conditions, molecular weight and DS of lacquer polysaccharide and sulfated lacquer polysaccharide

Samples	n(SR)/n(SU)[a]: Molar rate of sulfation reagent to sugar unit	Reaction time (h)	Temperature (°C)	$M_w \times 10^{-4}$	DS
LP	–	–	–	6.30	–
LPS1	2:1	4	70	0.85	0.46
LPS2	2:1	3	80	0.78	0.40
LPS3	2:1	3	60	1.58	0.58
LPS4	1:1	4	80	0.87	0.22
LPS5	1:1	4	70	1.27	0.34

ratio of sulfating reagent to sugar unit resulted in a higher DS. According to the work of Yang et al. (2003)), DS increased with reaction time, up to 3 h, and with reaction temperature, up to 60 °C in DMSO. Longer reaction time and higher reaction temperature reduced the DS value, and DS was apparently proportional to the amount of SO_3·Py complex in DMSO (Yang, Du, Wen, Li, & Hu, 2003).

Based on the results of M_w and DS in various reaction conditions, it could be concluded that sulfation and degradation occur simultaneously in the sulfation process. The high temperature of reaction enhanced the degradation. The mechanism of degradation was regarded as hydrolysis, so the removal of residual water became an essential step in the sulfation of lacquer polysaccharide.

The FT-IR spectra of sulfated lacquer polysaccharides (Fig. 2) showed two characteristic bands, one at 1256 cm^{-1} which was attributed to an asymmetrical S=O stretching vibration and the other at 816 cm^{-1} with a shoulder at 857 cm^{-1} indicating a symmetrical C—O—S vibration assigned to a C—O—SO_3 group. The broad band at 816 cm^{-1} suggested that equatorial 6-sulfate groups on the galactosyl units had been formed (Yang et al., 2002).

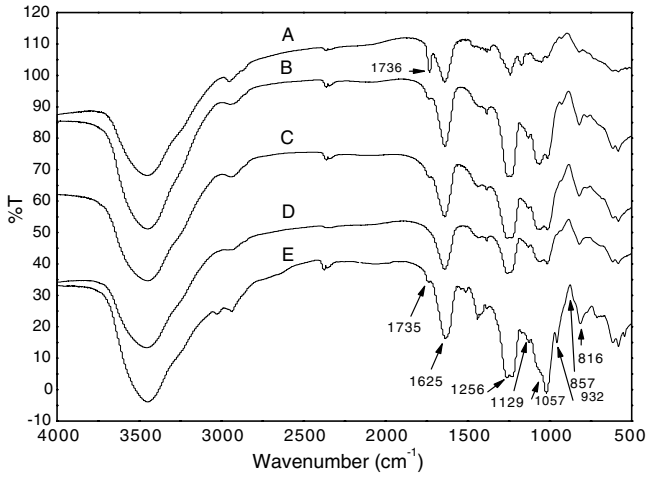

Fig. 2. FT-IR of sulfated lacquer polysaccharides A, lacquer polysaccharide sulfate LPS1; B, lacquer polysaccharide sulfate LPS2; C, lacquer polysaccharide sulfate LPS3; D, lacquer polysaccharide sulfate LPS4; E, lacquer polysaccharide sulfate LPS5.

The shoulder peak at 857 cm^{-1} indicated the presence of axial sulfate ester at O-4 of some β-D-galactose residues (Chiovitti et al., 2002; Liao et al., 1996). These data also suggested that equatorial primary hydroxyl group at O-6 and axial secondary hydroxyl group at O-4 of Gal residues are substituted easily.

In the FT-IR spectra, the strong band at 1057 cm^{-1} is probably attributable to the symmetrical S=O stretching vibration, and the band at 1129 cm^{-1} can be attributed to the asymmetrical C—O—S stretching vibration (Servaty, Schiller, Binder, & Arnold, 2001)). Furthermore, a new band appeared at 932 cm^{-1} indicating the presence of anhydro-galactose residues. Since SO_3 has strong properties of dehydration, it could be concluded that the anhydro-galactose residues were formed during the process of the sulfation. Another new band appeared at 1735 cm^{-1} originating from C=O stretching vibration (Conley, 1996), but this band disappeared when sulfated lacquer polysaccharides were treated with dilute NaOH solution, indicating the C=O stretching vibration from carboxylic acid. Interestingly, a new band appeared at 1625 cm^{-1}, and could be related to the unsaturated band formed in sulfation, or the deformation vibration of H_2O as suggested previously (Yang, Du, Huang, Wan et al., 2005).

The ^{13}C NMR analysis showed the structure of lacquer and the position of sulfate substitution. In the ^{13}C NMR spectrum (Fig. 3), the signals at 111.8, 107.1, 106.3, 105.2, 103.3, and 102.8 ppm are attributed to the anomeric carbons of α-L-arabinofuranose, β-D-glucopyranosyluronic acid, β-D-galactopyranose, 4-*O*-methyl-glucopyranosyluronic acid, α-L-rhamnopyranose and α-D-galactopyranose, respectively (Lu et al., 1999). The signal at 61 ppm is assigned to unlinked C-6 of the backbone. The movement of this signal of C-6 at 61 ppm on sulfation to 66 ppm indicated that the C-6 hydroxyl group was sulfated based on the α-effects [α-effects: when the electronegative substituent such as sulfate group connects with the carbon atom, the signal of this carbon atom will be shifted to lower field. (Gamzazade et al., 1997)]. The sulfation was assumed mainly to have occurred at C-6 (O-6) of the backbone; whether the sulfation also occurred at other positions was not easy to confirm; signals at 70–80 ppm were overlapped and difficult to assign.

3.2. Reducing power

From the measurements of the reductive ability of LP and LPS (Fig. 4), Fe^{3+}–Fe^{2+} transformation, the reducing power of all the five sulfated lacquer polysaccharides particularly the reducing power of LPS4 and LPS5 increased with increasing concentration; the reducing powers of LPS1 to LPS5 were 0.003, 0.003, 0.004, 0.005, 0.07, and 1.2 at 1.0 mg/mL, respectively, while the original lacquer polysaccharide did not show any reducing power. LPS with M_w 1.27×10^4 and DS of 0.34 possessed the greatest reducing power among the five LPS's at the same concentration,

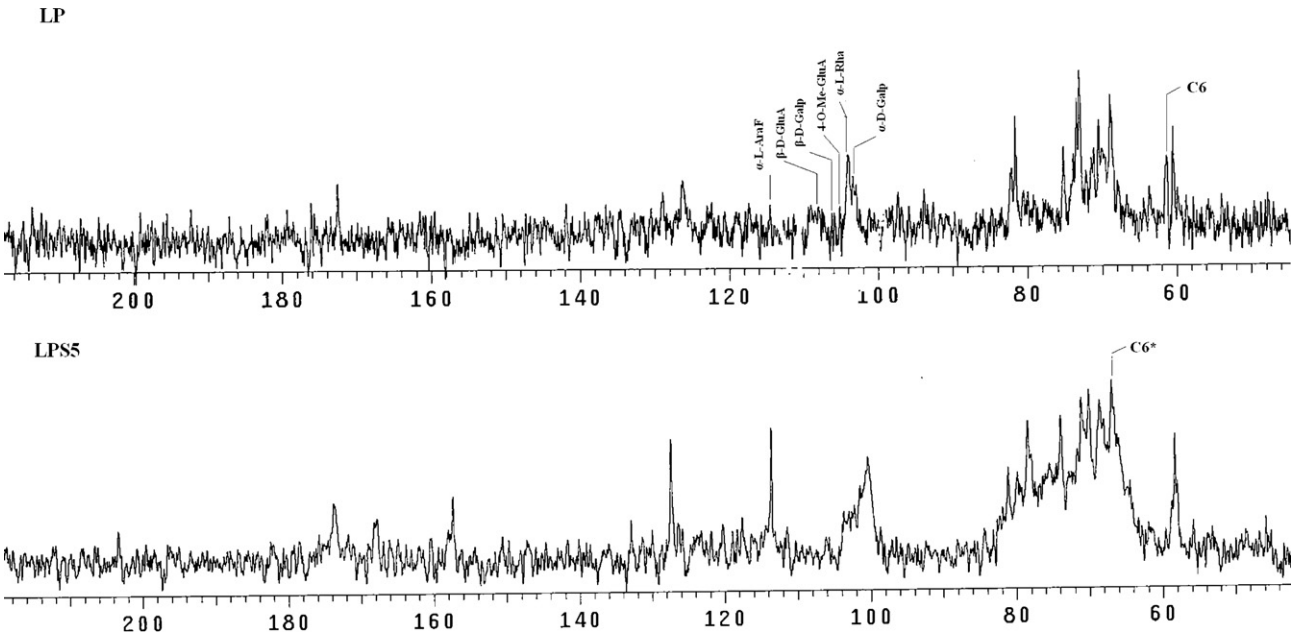

Fig. 3. ^{13}C NMR of lacquer polysaccharide (LP) and lacquer polysaccharide sulfate LPS5.

indicating that M_w and DS took an important role in the antioxidant activity level.

Mau, Chang, Huang, and Chen (2004) reported that the reducing power of ascorbic acid, α-tocopherol and butylated hydroxyanisole were 0.80, 0.89, and 0.92, respectively, at 1.0 mg/mL; compared to this, the reducing power of LPS5 was 1.2 at 1.0 mg/mL, so LPS5 had a better reducing power than these compounds at the same concentration. The reducing power of LPS5 had better reducing power than that of the compounds mentioned above at the same concentration, and the reductive capacity of LPS5 (0.61 at 500 μg/mL) was stronger than that of high-sulfate-content chitosans (0.17 at 0.75 mg/mL), (Xing et al., 2005). The antioxidant activities of antioxidants have been attributed to various mechanisms, among which are prevention of chain initiation, binding of transition metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction, reductive capacity and radical scavenging (Diplock, 1997; Yildirm et al., 2000). The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity (Meir, Kanner, Akiri, & Hadas, 1995).

3.3. Superoxide radical scavenging activity of LP and LPS

Superoxide anion is one of the precursors of the singlet oxygen and hydroxyl radicals, therefore, it indirectly initiates lipid peroxidation. Apart from that, the presence of superoxide anion can magnify cellular damage because it produces other kinds of free radicals and oxidizing agents (Athukorala, Kim, & Jeon, 2006). Photochemical reduction of flavins generates O_2^- which reduces NBT, resulting in the formation of blue formazan (Beauchamp & Fridovich, 1971). The % inhibition of superoxide radical generation by sulfated lacquer polysaccharides with different DS (Fig. 5), indicates from Fig. 5 that scavenging ability of the five LPS at 500 μg/mL was 33%, 38%, 41%, 48%, and 56%, respectively, while the original LP did not show scavenging capacity for superoxide radicals. The formation of blue formazan was inhibited by sulfated lacquer polysaccharides, and % inhibition of all samples was dose-dependent upon concentrations; the scavenging activity increased with the increase of concentrations of all samples. LPS5 with M_w of 1.27×10^{-4} and DS of 0.34 possessed the best scavenging capacity among the five LPS. It was concluded that moderate DS and M_w of LPS have a positive effect on the scavenging ability for superoxide radicals. Qi et al. (2006) reported that scavenging capacity of vitamin C for superoxide radical was about from 30% to 40% at 0.5–0.75 mg/mL. Compared to this result, the sulfated lacquer polysaccharides had stronger scavenging activity for superoxide radical than vitamin C. Athukorala et al. (2006) studied an enzymically extracted crude polysaccharide of *Ecklonia cava*(OE) and a slightly purified version of this (CpoF). For superoxide radical, the OE and CpoF at 500 μg/mL showed 40% and 30% scavenging effect, respectively. LPS possessed a better scavenging ability for superoxide radical than OE at the same concentration. While LPS1, LPS2 showed weaker ability than that of CpoF at 500 μg/mL, the rest of the three sulfated lacquer polysaccharides showed stronger scavenging capacity than that of CpoF.

Interestingly, Yang et al. (2005) reported a polysaccharide named YCP, from the mycelium of a marine filamentous fungus *Phoma herbarum* and its two chemical sulfated derivatives YCP-S1 and YCP-S2 with DS values of 1.01 and 1.36. The two derivatives could scavenge superoxide radical in a concentration-dependent fashion, the scavenging capacity of YCP-S1 and YCP-S2 at 200 μg/mL was

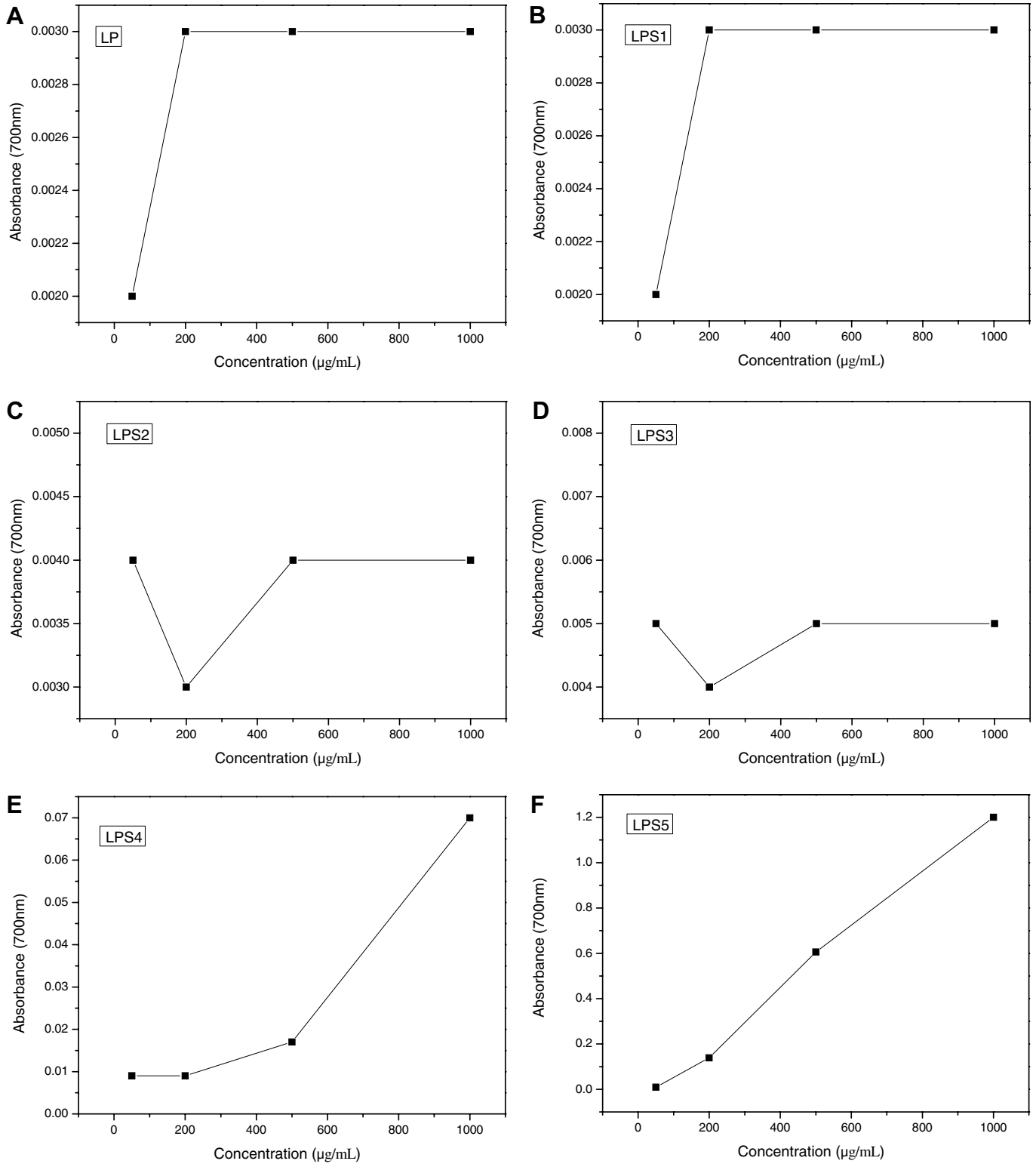

Fig. 4. Reducing power of different concentrations of (A), lacquer polysaccharide (LP); (B), lacquer polysaccharide sulfate LPS1; (C), lacquer polysaccharide sulfate LPS2; (D), lacquer polysaccharide sulfate LPS3; (E), lacquer polysaccharide sulfate LPS4; and (F), lacquer polysaccharide sulfate LPS5. Values are means ± *SD* of three times.

lower than 50%, while the ability of LPS5 was 52% at 200 μg/mL. It is obvious that LPS5 had the stronger ability than either of YCP-S1 and YCP-S2. It seems that the DS has a significant effect on the scavenging ability, and that higher DS is not necessary for the scavenging of free radicals. This is in agreement with the conclusions of Deng,

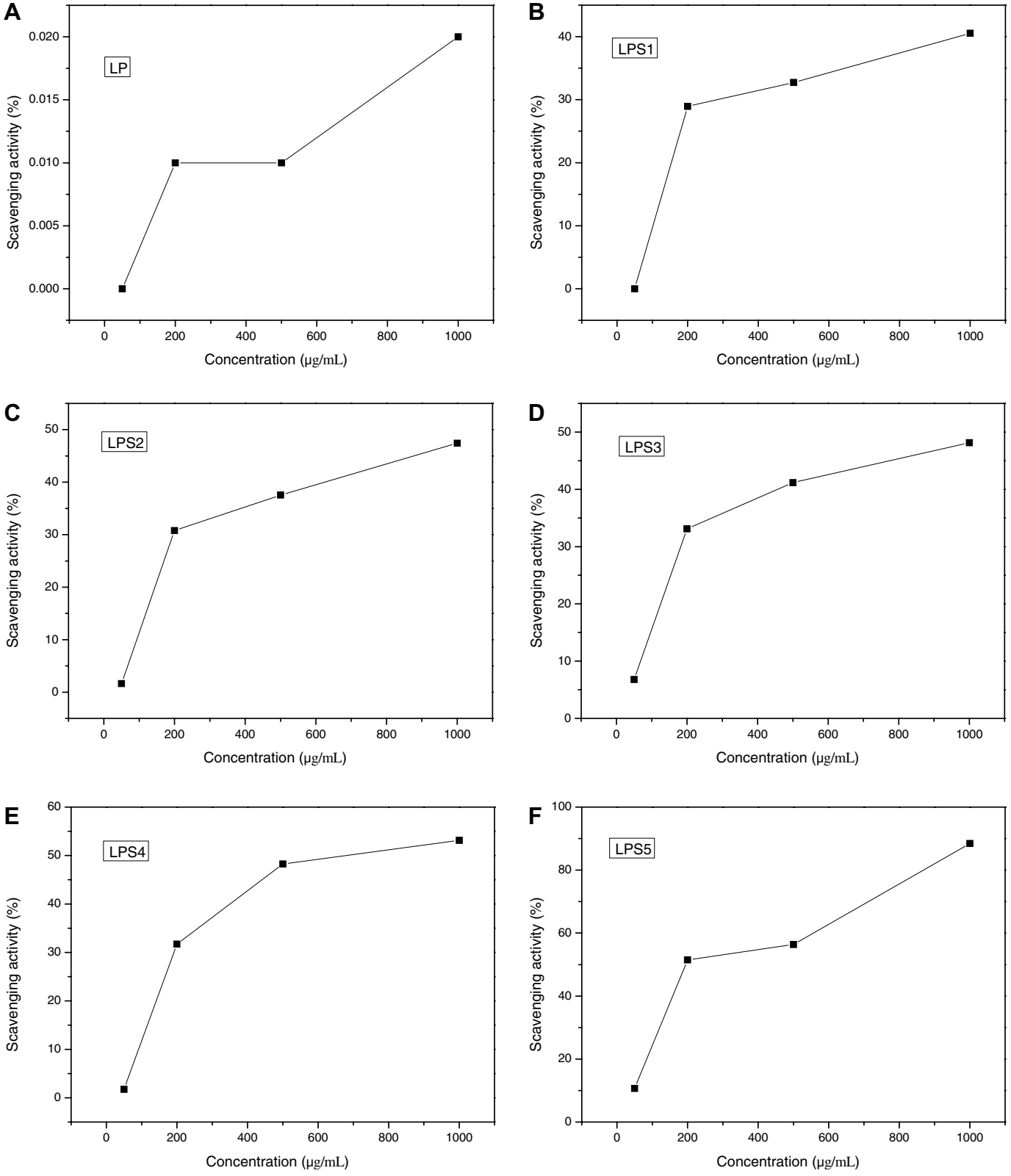

Fig. 5. Superoxide radical scavenging activity of different concentrations of (A), lacquer polysaccharide (LP); (B), lacquer polysaccharide sulfate LPS1; (C), lacquer polysaccharide sulfate LPS2; (D), lacquer polysaccharide sulfate LPS3; (E), lacquer polysaccharide sulfate LPS4; (F), lacquer polysaccharide sulfate LPS5. Values are means ± *SD* of three times.

Yang, Wang, and Xu (2000), who found that the sulfated Hunai polysaccharide had a good antioxidant capacity, and the DS of the sulfated Hunai polysaccharide was less than 0.5. The antioxidative capacity did not have a linear

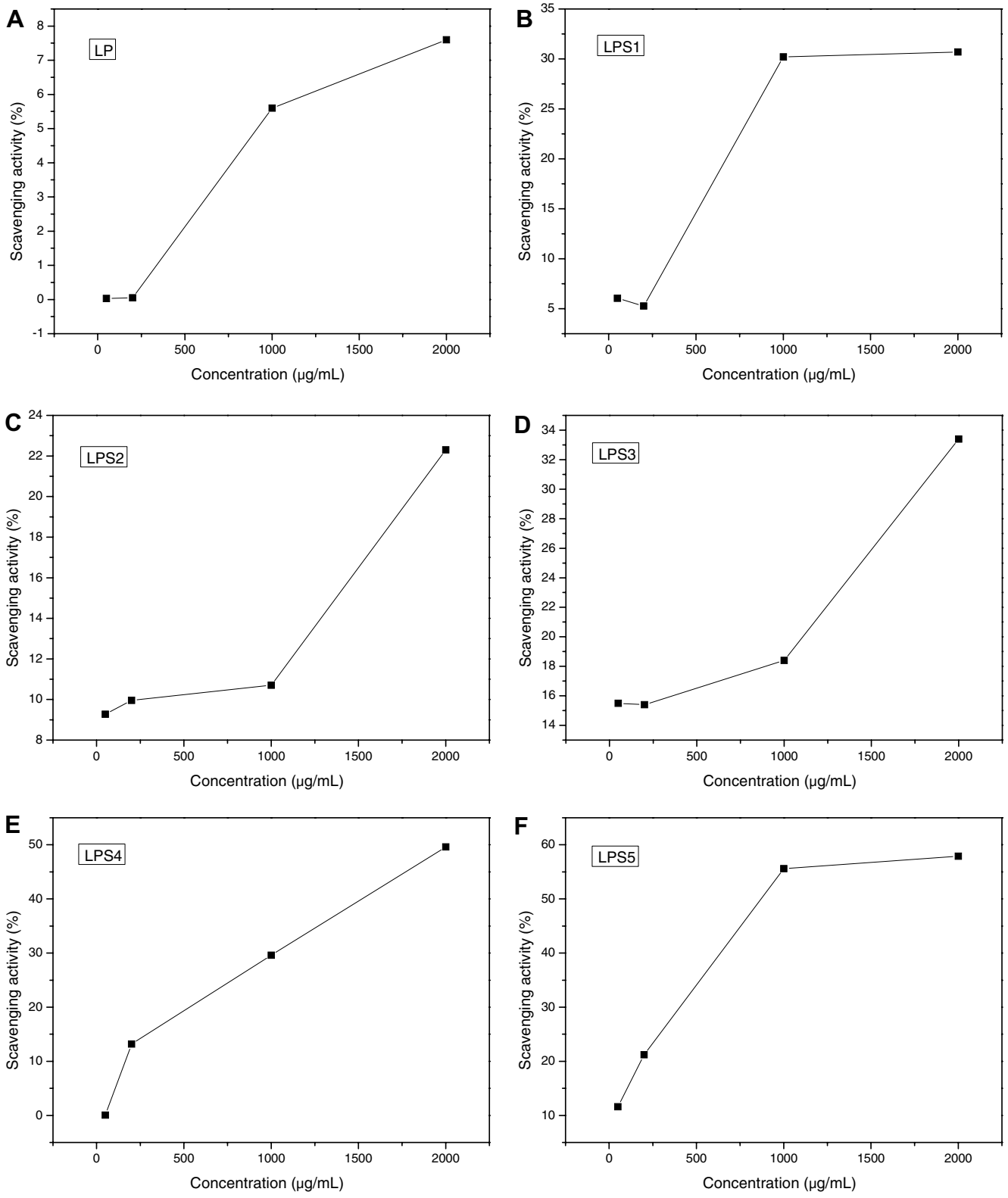

Fig. 6. Hydroxyl radical scavenging activity of different concentrations of (A), lacquer polysaccharide (LP); (B), lacquer polysaccharide sulfate LPS1; (C), lacquer polysaccharide sulfate LPS2; (D), lacquer polysaccharide sulfate LPS3; (E), lacquer polysaccharide sulfate LPS4; (F), lacquer polysaccharide sulfate LPS5. Values are means ± *SD* of three times.

relationship with the DS. In fact, as a natural source, the capacity of sulfated lacquer polysaccharide to scavenge O_2^- radical ($O_2^{\cdot -}$) is predicted to be useful in phytotherapy to prevent the pathological events in arthritis and aging.

3.4. Hydroxyl radical scavenging activity of LP and LPS

As shown in Fig. 6, the five sulfated lacquer polysaccharides were found to have the ability to scavenge hydroxyl radicals at concentrations between 200 and 2000 μg/mL. LPS5 had the best scavenging ability, while the original LP showed a very low scavenging ability. This indicated that the sulfate group played an important role in the scavenging of hydroxyl radicals. It has been reported that scavenging effect on hydroxyl radical of vitamin C was about 20% at 1.0 mg/mL (Qi et al., 2006), this result proved that the LPS1, LPS4, and LPS5 had more pronounced ability than vitamin C. Xing and his colleagues (2005) showed the antioxidant properties of a sulfated chitosan; its scavenging ability on hydroxyl radicals was 60% at 3.2 mg/mL. Compared with this, LPS5 had stronger scavenging capacity on hydroxyl radicals.

Some researchers reported that, for hydroxyl radical, the scavenging activity was not due to the direct scavenging but inhibition of hydroxyl radical generation by chelating ions such as Fe^{2+} and Cu^{2+} (Qi et al., 2006). Hydroxyl radicals can be generated by the reaction of Fe^{2+} and H_2O_2, and since the sulfate group of the polysaccharide had chelating ability for Fe^{2+}, this sulfated polysaccharide could reduce the generation of hydroxyl radicals by chelating the Fe^{2+}.

In the present study, the hydroxyl radical scavenging ability of sulfated lacquer polysaccharides is probably related to the specific chelating group (sulfate) in the molecule due to their high nucleophilic character (Yuan et al. (2005) prepared an oversulfated k-carrageenan oligosaccharide, and it showed a better scavenging capacity of hydroxyl radicals than did carrageenan oligosaccharide. The mechanism of scavenging behavior of sulfated lacquer polysaccharides on hydroxyl radicals needs to be further investigated.

4. Conclusions

In this first report of the antioxidant activity of sulfated lacquer polysaccharide, antioxidant tests in vitro showed that LPS had antioxidant capacities, especially the LPS5, with DS of 0.34, M_w of 1.27×10^{-4}. The scavenging ability for superoxide and hydroxyl radicals was 56% at 500 μg/mL and 56% at 1 mg/mL, respectively. The reducing power of LPS5 was stronger than that of vitamin C, α-tocopherol and butylated hydroxyanisole at 1.0 mg/mL. The LP and LPS have a unique structure, which is composed of 1,3-linked β-D-galactopyranose as a backbone with complex branches; the strong scavenging ability for free radicals may be attributed to these side chains. It was concluded that moderate M_w and DS could promote the antioxidant activities of LPS, and therefore that sulfated lacquer polysaccharide could be a potential candidate as an antioxidant – it is believed to prevent many degenerative diseases, such as cancer and atherosclerosis.

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References

Antonopoulos, C. A. (1962). A modification for the determination of sulfate in mucopolysaccharides by the benzidine method. *Acta Chimica Scandinavica, 16*, 1521–1522.

Athukorala, Y., Kim, K. N., & Jeon, Y. J. (2006). Antiproliferative and antioxidant properties of an enzymatic hydrolysate from brown alga, Ecklonia cava. *Food and Chemical Toxicology, 44*, 1065–1074.

Beauchamp, C., & Fridovich, I. (1971). Superoxide dismutase: Improved assays and an assay applicable to acrylamide gels. *Analytical Biochemistry, 44*, 276–287.

Cadenas, E., & Davies, K. J. A. (2000). Mitochondrial free radical generation, oxidative stress, and aging. *Free Radical Biology and Medicine, 29*, 222–230.

Chiovitti, A., Bacic, A., Craik, D. J., Kraft, G. T., Liao, M. L., Falshaw, R., et al. (2002). A pyruvated carrageenan from Australian specimens of the red alga *Sarconema filiforme*. *Carbohydrate Research, 310*, 77–83.

Conley, R. T. (1996). In *Infrared spectroscopy* (pp. 136–165). Boston: Allyn and Bacon.

Chung, S. K., Osawa, T., & Kawakishi, S. (1997). Hydroxyl radical scavenging effects of spices and scavengers from brown mustard (*Brassica nira*). *Bioscience Biotechnology and Biochemistry, 61*, 118–123.

Deng, C. H., Yang, X. L., Wang, Y., & Xu, H. B. (2000). Effect of degree of substitution on the antioxidative activities of the sulfated Hunai polysaccharide. *Journal of Huazhong University of Science and Technology, 28*, 104–107.

Diplock, A. T. (1997). Will the good fairies please prove us that vitamin E lessens human degenerative disease? *Free Radical Research, 27*, 511–532.

Du, Y. M., Kong, Z. W., & Li, H. (1994). Studies on separation and structure of lacquer polysaccharide. *Acta Polymerica Sinica, 3*, 301–306.

Du, Y. M., Yang, J. H., Kong, Z. W., & Xiao, L. (1999). Structure and bioactivities of lacquer polysaccharides from Chinese lac trees of wild species and cultispecies. *Chemical Journal of Chinese Universities, 3*, 399–402.

Finkel, T., & Holbrook, N. J. (2000). Oxidants, oxidative stress and the biology of aging. *Nature, 408*, 239–247.

Gamzazade, A., Sklyar, A., Nasibov, S., Sushkov, I., Shashkov, A., & Knirel, Y. (1997). Structural features of sulfated chitosans. *Carbohydrate Polymers, 34*, 113–116.

Grice, H. C. (1988). Safety evaluation of butylated hydroxyanisole from the perspective of effects on forestomach and oesophageal squamous epithelium. *Food and Chemical Toxicology, 26*, 717–723.

Gutteridge, M. C. (1984). Reactivity of hydroxyl and hydroxyl radicals discriminated by release of thiobarbituric acid-reactive material from deoxy sugars, nucleosides and benzoate. *Biochemical Journal, 224*, 761–767.

Gutteridge, M. C. (1987). Ferrous salt promoted damage to deoxyribose and benzoate. *Biochemical Journal, 243*, 709–714.

Halliwell, B., Gutteridge, J. M. C., & Cross, C. E. (1992). Free radicals, antioxidants and human disease: Where are we now. *Journal of Laboratory and Clinical Medicine, 119*, 598–620.

Hu, Y., Xu, J., & Hu, Q. H. (2003). Evaluation of antioxidant potential of aloe vera (*Aloe barbadensis* Miller) extracts. *Journal of Agricultural and Food Chemistry, 51*, 7788–7791.

Jiang, Y. H., Jiang, X. L., Wang, P., & Hu, X. K. (2005). In vitro antioxidant activities of water-soluble polysaccharides extracted from *Isaria farinosa* B05. *Journal of Food Biochemistry, 29*, 323–335.

Kinsella, J. E., Frankel, E., German, B., & Kanner, J. (1993). Possible mechanisms for the protective role of antioxidant in wine and plant foods. *Food Technology, 4*, 85–89.

Liao, M. L., Chiovitti, A., Munro, S. L. A., Craik, D. J., Kraft, G. T., & Bacic, A. (1996). Sulfated galactans from Australian specimens of the red alga *Phacelocarpus peperocarpos* (Gigartinales Rhodophyta). *Carbohydrate Research, 296*, 237–242.

Lu, R., Hattori, K., Xia, Z. Y., Yoshida, T., Yang, J. H., Zhang, L. N., et al. (1999). Structural analysis of polysaccharides in Chinese lacquer by NMR spectroscopy. *Sen'I Gakkaishi, 55*(2), 47–56.

Martinez, A. C., Marcelo, E. L., Marco, A. O., & Moacyr, M. (2001). Differential responses of superoxide dismutase in freezing resistant *Solanum curtibolum* and freezing sensitive *Solanum tuberosum* subjected to oxidative and water stress. *Plant Science, 160*, 505–515.

Mau, J. L., Lin, H. C., & Song, S. F. (2002). Antioxidant properties of several specialty mushroom. *Food Research International, 5*, 519–526.

Mau, Jeng-Leun, Chang, Chieh-No, Huang, Shih-Jeng, & Chen, Chin-Chu. (2004). Antioxidant properties of methanolic extracts from *Grifola frondosa*, *Morchella esculenta* and *Termitomyces albuminosus mycelia*. *Food Chemistry, 87*, 111–118.

Meir, S., Kanner, J., Akiri, B., & Hadas, S. P. (1995). Determination and involvement of aqueous reducing compounds in oxidative defense systems of various senescing leaves. *Journal of Agricultural and Food Chemistry, 43*, 1813–1819.

Nandita, S., & Rajini, P. S. (2004). Free radical scavenging activity of an aqueous extract of potato peel. *Food Chemistry, 85*, 611–616.

Oshima, R., & Kumanotani, J. (1984). Structural studies of plant gum from sap of the lac tree, *Rhus vernicifera*. *Carbohydrate Research, 127*, 43–57.

Oyaizu, M. (1986). Studies on product of browning reaction prepared from flucose amine. *Japanese Journal of Nutrition, 44*, 307–315.

Qi, H. M., Zhang, Q. B., Zhao, T. T., Chen, R., Zhang, H., Niu, X. Z., et al. (2005). Antioxidant activity of different sulfate content derivatives of polysaccharide extracted from *Ulva pertusa* (Chlorophyta) in vitro. *International Journal of Biological Macromolecules, 37*, 195–199.

Qi, H. M., Zhang, Q. B., Zhao, T. T., Hu, R. G., Zhang, K., & Li, Z. E. (2006). In vitro antioxidant activity of acetylated and benzoylated derivatives of polysaccharide extracted from *Ulva pertusa* (Chlorophyta). *Bioorganic and Medicinal Chemistry Letters, 16*, 2441–2445.

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

Servaty, R., Schiller, J., Binder, H., & Arnold, K. (2001). Hydration of polymeric components of cartilage – an infrared spectroscopic study on hyaluronic acid and chondroitin sulfate. *International Journal of Biological Macromolecules, 28*, 121–127.

Sun, C., Wang, J. W., Fang, L., Gao, X. D., & Tan, R. X. (2004). Free radical scavenging and antioxidant activities of EPS2, an exopolysaccharide produced by a marine filamentous fungus *Keissleriella* sp. YS 4108. *Life Science, 75*, 1063–1073.

Uchida, K. (2000). Role of reactive aldehyde in cardiovascular diseases. *Free Radical Biology Medicine., 28*, 1685–1696.

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

Xing, R., Liu, S., Yu, H. H., Guo, Z. Y., Li, Z. E., & Li, P. C. (2005). Preparation of high-molecular weight and high-sulfate content chitosans and their potential antioxidant activity in vitro. *Carbohydrate Polymers, 61*, 148–154.

Yang, J. H., Du, Y. M., Wen, Y., Li, T. Y., & Hu, L. (2003). Sulfation of Chinese lacquer polysaccharides in different solvents. *Carbohydrate Polymers, 52*, 397–403.

Yang, J. H., & Du, Y. M. (2003). Chemical modification, characterization and bioactivity of Chinese lacquer polysaccharides from lac tree *Rhus vernicifera* against leucopenia induced by cyclophosphamide. *Carbohydrate Polymers, 52*, 405–410.

Yang, J. H., Du, Y. M., Huang, R. H., Wan, Y. Y., & Li, T. Y. (2002). Chemical modification, characterization and structure-anticoagulant activity relationships of Chinese lacquer polysaccharides. *International Journal of Biological Macromolecules, 31*, 55–62.

Yang, J. H., Du, Y. M., Huang, R. H., Sun, L. P., Liu, H., Gao, X. H., et al. (2005). Chemical modification and antitumour activity of Chinese lacquer polysaccharide from lac tree *Rhus vernicifera*. *Carbohydrate Polymers, 59*, 101–107.

Yang, J. H., Du, Y. M., Huang, R. H., Wan, Y. Y., & Wen, Y. (2005). The structure–anticoagulant activity relationships of sulfated lacquer polysaccharide. Effect of carboxyl group and position of sulfation. *International Journal of Biological Macromoleculers, 36*, 9–15.

Yang, X. B., Gao, X. D., Han, F., & Tan, R. X. (2005). Sulfation of a polysaccharide produced by a marine filamentous fungus *Phoma herbarum* YS4108 alters its antioxidant properties in vitro. *Biochim. Biophys. Acta, 1725*, 120–127.

Yildirm, A., Mavi, A., Oktay, M., Kara, A. A., Algur, O. F., & Bilaloglu, V. (2000). Comparison of antioxidant and antimicrobial activities of tilia (*Tilia argenta* Desf EX DC), sage (*Salvia triloba* L.) and black tea (*Camellia sinensis*) extracts. *Journal of Agricultural and Food Chemistry, 48*, 5030–5034.

Yuan, H. M., Zhang, W. W., Li, X. G., Lu, X. X., Li, N., Gao, X. L., et al. (2005). Preparation and in vitro antioxidant activity of k-carrageenan oligosaccharides and their oversulfated, acetylated, and phosphorylated derivatives. *Carbohydrate Research, 340*, 685–692.

Zhang, L., Qiu, X., Xu, J., Du, Y., Qian, B., & Kennedy, J. F. (1992). Studies on fractionation and molecular weights of Chinese lacquer polysaccharide. *Carbohydrate Polymers, 19*, 161–166.

Zhu, X., Raina, A. K., Lee, H. G., Casadesus, G., Smith, M. A., & Perry, G. (2004). Oxidative stress signalling in Alzheimer's disease. *Brain Research., 1000*, 32–39.