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Characterization and antioxidant activities of polysaccharides from the leaves of *Lilium lancifolium* Thunb.

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ABSTRACT

In this study, LLP-1, LLP-2, and LLP-3 three novel polysaccharide fractions were purified from the leaves of *Lilium lancifolium*, and their physicochemical characterizations and antioxidant properties were investigated by chemical methods, high performance gel permeation chromatography (HPGPC), high performance liquid chromatography (HPLC), fourier transform infrared spectrometry (FT-IR), scanning electron microscopy (SEM), and DPPH radical assay, hydroxyl radical assay, superoxide radical assay and ferrous ion chelating assay, respectively. Results showed that LLP-1, LLP-2, and LLP-3 had low protein and uronic acid contents, meanwhile, their weight-average molecular weight were estimated to be 2.25×10^6, 2.02×10^6, and 2.08×10^6 Da, respectively. Mannose, rhamnose, glucuronic acid, galacturonic acid, glucose, galactose, arabinose glucose and galactose were the major monosaccaherides components presenting in the polysaccharide fractions. Three polysaccharide fractions were not observed triple-helical conformation, while possessed variant surface structure. In addition, three polysaccharide fractions all exhibited significantly scavenging activities against free radical and chelate Fe^{2+} *in vitro*.

1. Introduction

Lilium lancifolium Thunb., known as juandan and tiger lilje, a perennial bulbous herb belongs to the genus *Lilium* of the family Liliaceae, originates from East Asia and be widely cultivated as an ornamental in Europe and North America. In China, *L. lancifolium* is extensively used as spice, food as well as traditional Chinese medicinal material to treat bronchitis, tuberculosis, pneumonia, chromic gastritis and ulcers [1,2]. Phytochemical researches have shown that a variety of chemical active substances such as steroidal saponins, flavonoids, alkaloids, and colchicine isolated from *L. lancifolium* exhibited excellent antioxidant, antibacterial, and anti-inflammatory effects [2–4]. In addition, polysaccharides from its edible bulbs have received significant attention due to its hypotensive, antineoplasmic, antioxidant and immunomodulating activities [5,6]. In 2015, Chinese *L. lancifolium* planting areas approximate to 40,000 ha. To improve the edible bulbs yield, a large amount of aerial portion be pruned resulting in serious resource waste and environment pollution, which in not conducive to the comprehensive utilization of this valuable species.

Reactive oxygen species (ROS), such as superoxide (O2-), peroxyl (ROO•), alkoxyl (RO•), hydroxyl (HO•), and nitric oxide (NO•), are generated during normal mitochondrial oxidative metabolism as well as in cellular response to xenobiotics, cytokines, and bacterial invasion [7,8]. ROS play an important signaling role in organism controlling processes such as growth, development, and homeostasis [9]. Unfortunately, all ROS are extremely harmful to organisms at high concentrations, which may cause various diseases, such as diabetes, atherosclerosis, cancer, neurodegeneration, and aging [10]. Generally, synthetic antioxidants including butylated hydroxyl anisole (BHA), butylated hydroxyltoluene (BHT) and tertiary butyl hydroquinone (TBHQ) have been used as strategic clinical medicine, but several studies suggest that they could promote liver damage and carcinogenesis [11,12]. Meanwhile, polysaccharides from natural sources have gained extensive attention due to its good bioactive and non-toxic properties. However, few study has embarked on polysaccharides from leaves of *L. lancifolium* (LLPs). Therefore, in this paper, a water-soluble LLPs was isolated from the leaves of *L. lancifolium* and fractionated by DEAE-52 cellulose anion-exchange chromatography column to obtained LLP-1, LLP-2, and LLP-3. Then, chemical compositions, molecular weights, monosaccharide com-

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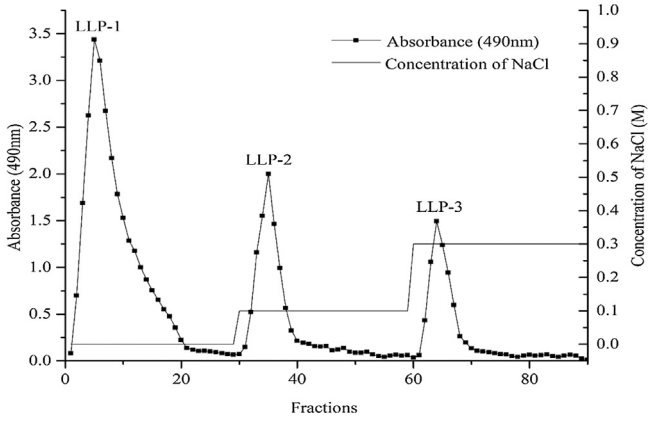

Fig. 1. Stepwise elution curve of crude LLPs on DEAE-52 Cellulose anion-exchange column.

positions, and structures of the three fractions were investigated. Moreover, the antioxidant scavenging effect of LLPs were evaluated by *in vitro* antioxidant assay, including DPPH radical, hydroxyl radical, and superoxide radical scavenging activity and ferrous iron chelating activity.

2. Materials and methods

2.1. Materials and reagents

L. lancifolium leaves were collected from Dazhou (Sichuan Province, China) in June 2015. The fresh sample was rinsed carefully with tap water and hot-air dried at 70 °C. The dried leaves were ground in a powerful mill (FW177, Taisite Instrument Co., Ltd.) to ultrafine powder and stored in a desiccator at room temperature.

DEAE Cellulose-52, 1-phenyl-3-methyl-5-pyrazolone (PMP), DPPH (1,1-diphenyl-2-picrylhydrazyl), nitroblue tetrazolium (NBT), dihydronicotinamide adenine dinucleotide (NADH), phenazine methosulfate (PMS), and ferrozine were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Ethanol, ferrous sulfate and all other chemicals were obtained from Chengdu Kelong Chemical Factory (Chengdu, China).

2.2. Extraction and purification of polysaccharides

The dried leaves power of *L. lancifolium* was treated with petroleum ether, acetone, and ethanol to remove pigments, monosaccharides, oligosaccharides, and other small moleculars, respectively. The resulting residue was dried and extracted with deionized water for 1 h at 70 °C for three times. The extraction solution was combined, condensed and mixed with a quadruple volumes of anhydrous ethanol at 4 °C overnight. Precipitate was dissolved in deionized water. Then, the solution was treated with α-amylase to digest starch and Sevage solution (chloroform: butyl alcohol, 4:1) was used to remove protein. Finally, the solution was vacuum dried to yield crude polysaccharides (LLPs).

Crude LLPs was dissolved in deionized water and loaded on DEAE-52 cellulose column (4.5 cm × 30 cm). Then, the column was stepwise eluted with 0, 0.1, 0.3 M sodium chloride (NaCl) solution at a flow rate of 60 mL/h. Eluate (40 mL/tube) was collected and the carbohydrates were monitored by the phenol–sulfuric acid method [13]. Finally, three purified polysaccharides fractions, LLP-1, LLP-2, and LLP-3 were obtained, further purified by Sephadex G-100 columns (2.5 cm × 60 cm), and vacuum dried for further study.

2.3. Physicochemical characterisation analysis

Total carbohydrate contents of LLP-1, LLP-2, and LLP-3 were determined by the phenol-sulfuric acid method, using d-glucose as standard [13]. Protein contents of LLP-1, LLP-2, and LLP-3 were measured by bicinchoninic acid/CuSO4 (BCA) method, using bovine serum albumin as a standard [14]. Uronic acid contents of LLP-1, LLP-2, and LLP-3 were measured by m-hydroxydiphenyl method, using d-galacturonic acid as standard [15]. The homogeneity and molecular weights of LLP-1, LLP-2, and LLP-3 were identified by high performance gel permeation chromatography (HPGPC) with a Refractive Index Detector (RID) on a PL aquagel-OH (7.5 × 300 μm) column (Agilent Technologies, Palo, Alto, CA, USA). The test operation conditions were as follows: flow rate: 10 μL; mobile phase: deionized water; flow rate: 1 mL/min; column temperature: 30 °C; detecting temperature: 40 °C.

2.4. Fourier transform infrared spectrometry (FT-IR) analysis

FT-IR of the three purified polysaccharide fractions were measured by the potassium bromide (KBr) pellet method on a Shimadzu 8400S spectrophotometer (Japan) in the range of 400–4000 cm^{-1} [16].

2.5. Monosaccharide analysis

Monosaccharide compositions of LLP-1, LLP-2, and LLP-3 were analyzed by High Performance Liquid Chromatography (HPLC) method [17]. Briefly, 10 mg polysaccharide samples were hydrolyzed with 3 mL of 3 M trifluoroacetic acid (TFA) at 100 °C for 6 h in a sealed ampoule bottle. Then, the hydrolysates were evaporated under a reduced pressure to remove the excess acid with methanol. Finally, the dried hydrolyzed samples were derivatized with 250 μL of 0.5 mol/L PMP under alkaline condition. The PMP-labeled monosaccharides were analyzed by a Agilent 1260 HPLC system equipped with a Zorbax SB-C18 column (150 × 4.6 mm, 5.0 μm), and detected by a UV–vis DAD detector at 250 nm. The mobile phase consisted of 0.05 M KH_2PO_4 (pH = 6.9) with 15% (solvent A) and 40% (solvent B) acetonitrile, at a flow rate of 1 mL/min with the following gradient: 0–8–20% solvent B by a linear increase from 0 to 10 to 28 min.

2.6. Tertiary structure determination

The conformational structure of LLP-1, LLP-2, and LLP-3 was determined by the Congo red method described by Lee et al. [18]. Briefly, 100 μL polysaccharide sample solution (1 mg/mL) was thoroughly mixed with 100 μL Congo red solution (80 μM) in a concentration gradient of NaOH solution (0–0.5 M). The absorbance of each mixture was scanned in the range of 200–700 nm by a spectrophotometric microplate reader (Molecular Devices, Sunnyvale, CA, USA).

2.7. Scanning electron microscopy (SEM) analysis

The morphological characteristics of LLP-1, LLP-2, and LLP-3 were observed by scanning electron microscopy (JSM-7500, JEOL, Japan). The vacuum dried sample was placed on a specimen holder with aluminum, and sputtered with gold powder by vacuum coating apparatus at an accelerating voltage of 5 kV, as well as image magnifications of 400×.

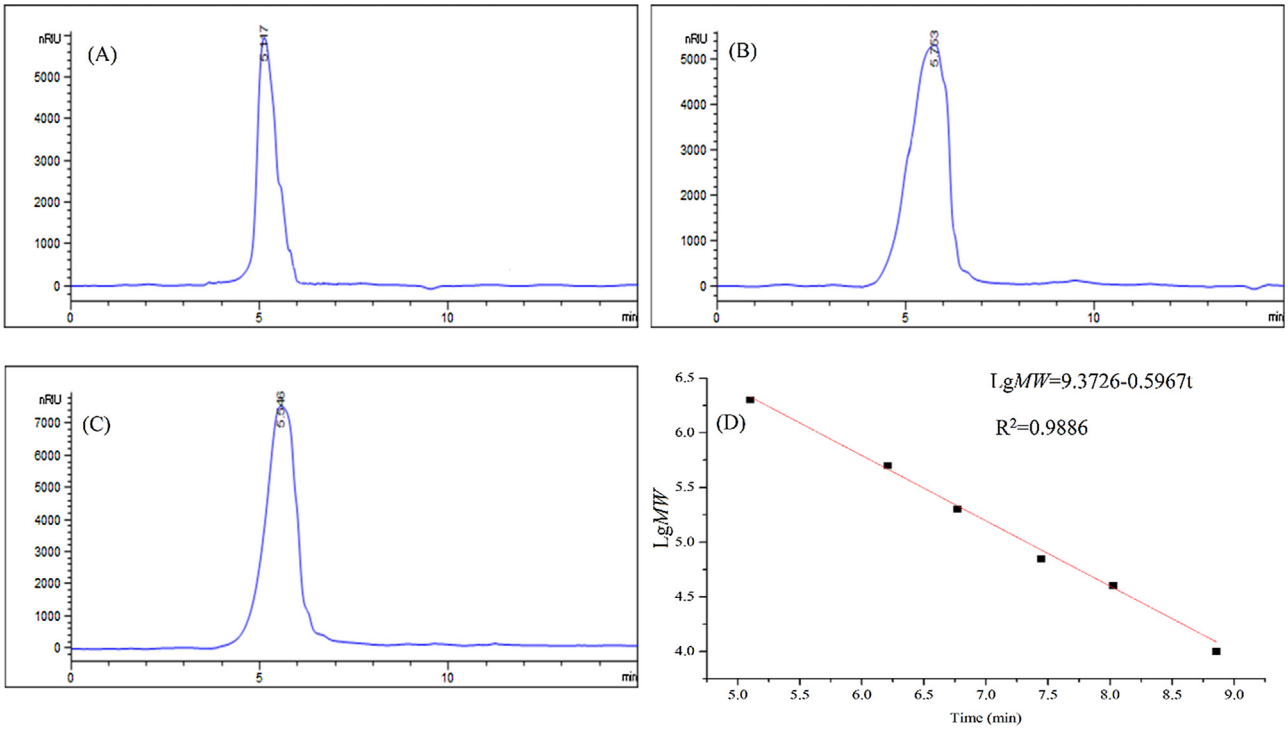

Fig. 2. HPGPC profiles of (A) LLP-1; (B) LLP-2; (C) LLP-3; and (D) calibration curve of Dextran T-series standards.

2.8. In vitro *antioxidant activity test*

2.8.1. *DPPH radical scavenging activity*

The DPPH radical scavenging activity of LLPs was measured according to the reported method [19] with slight modifications. Briefly, different concentrations (0.125, 0.25, 0.5, 1.0, 2.0, 3.0, 4.0 and 5.0 mg/mL) of LLP sample was prepared in deionized water. Then, 70 μL sample solution was mixed with 140 μL DPPH-ethanol solution (0.4 mM) in a 96-well plate. The reaction solution was incubated at 37 °C in oven for 30 min, and the absorbance at 517 nm was measured in a spectrophotometric microplate reader. In this study, deionized water and ascorbic acid (Vc) were served as blank and positive control, respectively. The scavenging activity of DPPH radicals was calculated by the following equation:

$$\text{DPPH radical scavenging activity}\,(\%) = \left[1 - \frac{(\text{Abs}_1 - \text{Abs}_2)}{\text{Abs}_0}\right] \times 100$$

where Abs_0 was the absorbance of the control (deionized water instead of sample), Abs_1 was the absorbance of a mixture of DPPH solution with the sample and Abs_2 was the absorbance of the sample only (ethanol instead of DPPH-ethanol solution).

2.8.2. *Hydroxyl radical scavenging activity*

The hydroxyl radical scavenging activity was assayed based on the Fenton reaction [20] with slight modifications. Briefly, 50 μL sample solution was incubated with 50 μL sodium salicylate (9 mM), 50 μL $FeSO_4$ (9 mM) and 50 μL H_2O_2 (0.025%, w/v) at 37 °C for 30 min and the absorbance at 562 nm was determined. The deionized water was used as blank control and ascorbic acid (Vc) was served as blank and positive control. The scavenging activity of hydroxyl radical was calculated by the following equation:

$$\text{Hydroxyl radical scavenging activity}\,(\%) = \left[1 - \frac{(\text{Abs}_1 - \text{Abs}_2)}{\text{Abs}_0}\right] \times 100$$

where Abs_0 was the absorbance of the control (deionized water instead of sample), Abs_1 was the absorbance of the test sample mixed with reaction solution and Abs_2 was the absorbance of the sample only (deionized water instead of hydroxyl radical generating system solution).

2.8.3. *Superoxide radical scavenging activity*

The superoxide radical scavenging activity assay of LLP was determined by the method described by Xu et al. [16]. 50 μL sample solution was mixed with 50 μL NBT (156 μM), 50 μL NADH (156 μM) and 50 μL PMS (60 μM). Then, the mixture was incubated at room temperature for 5 min, and the absorbance at 560 nm was measured. The deionized water was used as the blank control and ascorbic acid was used as positive control. The scavenging activity of superoxide radical was calculated by the following equation:

$$\text{Superoxide radical scavenging activity}\,(\%) = \left[1 - \frac{(\text{Abs}_1 - \text{Abs}_2)}{\text{Abs}_0}\right] \times 100$$

where Abs_0 was the Abs of the control (deionized water instead of sample), Abs_1 was the absorbance of the test sample mixed with reaction solution and Abs_2 was the absorbance of the sample only (deionized water instead of superoxide radical generating system solution).

2.8.4. *Fe^{2+} chelating activity*

The Fe^{2+} chelating activity of sample was measured as reported method [21] with a minor modification. Briefly, 50 μL sample solution was mixed with 100 μL FeSO4 (0.125 mM) and 50 μL ferrozine (1.0 mM). Then, the mixture reacted at room temperature for 10 min, and the absorbance of the solution was determined at 562 nm. A 200 μL deionized water was used as blank control and ethylene diamine tetraacetic acid disodium salt (EDTA-2Na) was served as blank and positive control. The Fe^{2+} chelating activity was calculated by the following equation:

$$Fe^{2+}\text{chelating activity}\,(\%) = \left[1 - \frac{(\text{Abs}_1 - \text{Abs}_2)}{\text{Abs}_0}\right] \times 100$$

where Abs_0 was the Abs of the control (deionized water instead of sample), Abs_1 was the absorbance of the test sample mixed with

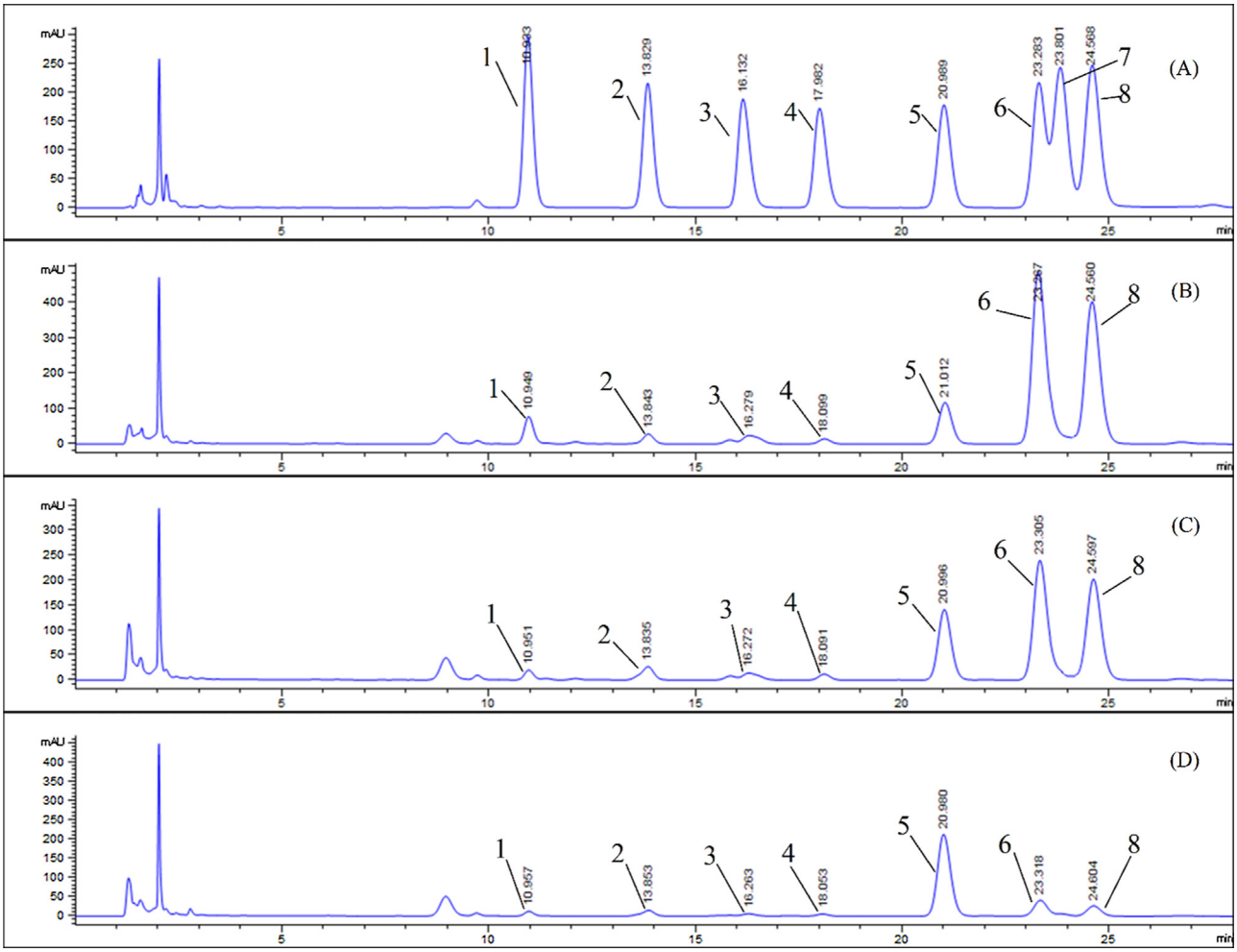

Fig. 3. HPLC chromatograms of PMP derivatives of (A)standard monosaccharide mixture solution; (B) LLP-1; (C) LLP-2; and (D) LLP-3. Peak identity: 1. mannose; 2. rhamnose; 3. glucuronic acid; 4. galacturonic acid; 5. glucose; 6. galactose; 7. xylose; 8. arabinose.

reaction solution and Abs_2 was the absorbance of the sample only (deionized water instead of $FeSO_4$ and ferrozine solution).

2.9. Statistical analysis

The experiments used completely randomized block designs and the analyses were carried out in triplicate. Data were statistically analyzed by One-Way ANOVA procedure with SPSS software Version 22.0, followed by the Duncan test. The significance of difference among mean values was determined at ($p < 0.05$).

3. Results and discussion

3.1. Isolation and purification of LLPs

A water-soluble crude polysaccharide LLPs was isolated from the leaves of *L. lancifolium* with a yield of 11.27% (w/w). Then, the crude LLPs was separated by a DEAE-52 Cellulose anion-exchange column chromatography with NaCl solutions gradient elution. As shown in Fig. 1, three independent fractions LLP-1, LLP-2, and LLP-3 were collected, concentrated, further purified by Sephadex G-100 columns and vacuum dried. The recovery rates of LLP-1, LLP-2, and LLP-3 based on the amount of crude LLPs used were 20.13, 13.07, and 9.85%, respectively.

Table 1
Molecular weight distribution of LLP-1, LLP-2, and LLP-3.

Fraction	Mw[a]	Mn[b]	Mp[c]	Mw/Mn
LLP-1	2.25×10^6	1.91×10^6	2.09×10^6	1.18
LLP-2	2.02×10^6	1.30×10^6	0.87×10^6	1.55
LLP-3	2.08×10^6	1.36×10^6	1.16×10^6	1.53

[a] Mw: Weight-average molecular weight.
[b] Mn: Number-average molecular weight.
[c] Mp: Peak-position molecular weight.

3.2. Homogeneity and average molecular weights of LLP-1, LLP-2, and LLP-3

The homogeneity and average molecular weights of LLP-1, LLP-2, and LLP-3 were determined by high performance gel permeation chromatography (HPGPC) with RID on a PL aquagel-OH (7.5×300 μm) column. As shown in Fig. 2A–C, the HPGPC profiles of the three polysaccharide fractions all showed a single peak, which indicated each purified fraction was a homogeneous polysaccharide. The weight-average molecular weight (Mw) of LLP-1, LLP-2, and LLP-3 were around 2.25×10^6, 2.02×10^6, and 2.08×10^6 Da, respectively (Table 1). In addition, the Mw/Mn value of LLP-1 was closer to 1 and less than that of LLP-2 and LLP-3, indicating that LLP-1 had higher of homogenicity. Meanwhile, these results were different from those of Gao et al. who found that the molecular weight of the polysaccharide fraction was 8.52×10^6 Da, which was

Table 2
Chemical characteristics and monosaccharide compositions of LLP-1, LLP-2, and LLP-3.

Item	LLP-1	LLP-2	LLP-3
Total carbohydrate (%)	83.03 ± 1.51	75.79 ± 2.72	63.07 ± 1.38
Protein (%)	1.69 ± 0.35	7.41 ± 1.03	9.46 ± 0.41
Uronic acid (%)	4.61 ± 0.10	4.67 ± 0.67	1.90 ± 0.56
Monosaccharide composition (molar ratio, %)			
Mannose	4.57	2.02	3.04
Rhamnose	1.96	3.88	4.77
Glucuronic acid	2.65	2.63	1.76
Galacturonic acid	1.09	1.57	1.53
Glucose	9.56	20.23	68.55
Galactose	43.05	37.20	11.60
Arabinose	37.11	32.47	8.75

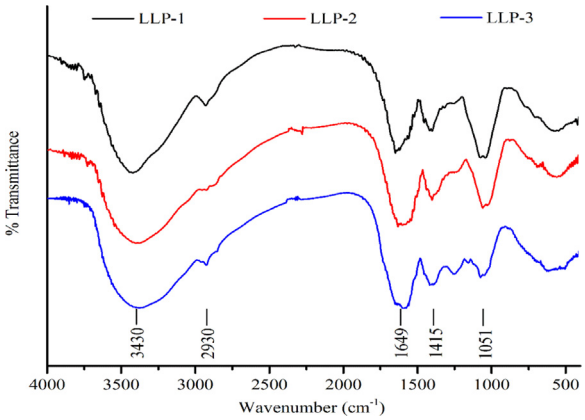

Fig. 4. Infrared spectra of LLP-1, LLP-2, and LLP-3.

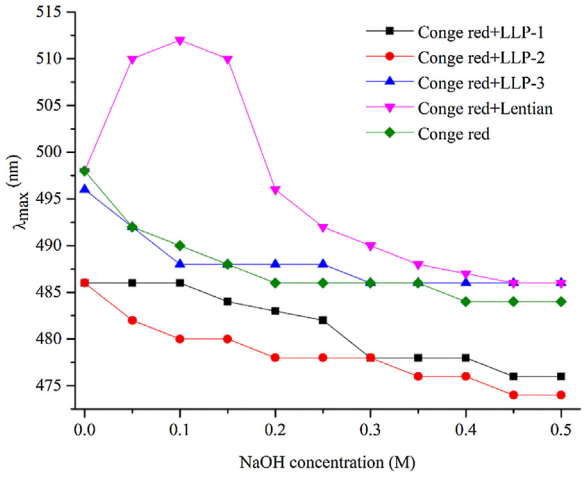

Fig. 5. The absorbance maximum (λ_{max}) of Congo red, LLP-1, LLP-2, LLP-3, and Lentinan at various NaOH concentrations.

isolated from the bulbs of *L. lancifolium* using a DEAE cellulose column [5].

3.3. Chemical characteristics and monosaccharide compositions of LLP-1, LLP-2, and LLP-3

The chemical constitutions and monosaccharide compositions of LLP-1, LLP-2, and LLP-3 were summarized in Table 2. Briefly, three purified polysaccharide fractions all contained high levels of total carbohydrate, but low protein and uronic acid. The total carbohydrate in LLP-1, LLP-2, and LLP-3 was 83.03, 75.79, and 63.07%, respectively, whereas the protein content in respective order was 1.69, 7.41, and 9.46%. Moreover, their uronic acid contents were all below 5%. HPLC chromatograms depicted that three polysaccharide fractions had the same monosaccharide composition but different mole percentage (Fig. 3). As shown in Table 2, LLP-1, LLP-2, and LLP-3 primarily were consisted of mannose, rhamnose, glucuronic acid, galacturonic acid, glucose, galactose, and arabinose with a molar ratio percentage of 4.57:1.96:2.56:1.09:9.56:43.05:37.11, 2.02:3.88:2.63:1.57:20.23:30.20:32.47, and 3.04:4.77:1.76:1.53:68.55:11.60:8.75, respectively. Obviously, galactose and arabinose were the main monosaccharides in both LLP-1 and LLP-2 while glucose was the significant predominant component in LLP-3. Meanwhile, glucuronic acid and galacturonic acid showed low peaks in three polysaccharide fractions HPLC chromatograms, which was in line with the results of m-hydroxydiphenyl assay.

3.4. FT-IR spectra of LLP-1, LLP-2, and LLP-3

The FT-IR spectra of LLP-1, LLP-2, and LLP-3 were shown in Fig. 4. The strong band at 3430 cm^{-1} was attributed to the presence of O—H stretching [22]. The band at 2930 cm^{-1} was due to C—H stretching and bending vibration. The absorption bands at 1649 cm^{-1} was corresponded with characteristic of ester carbonyl and carboxyl groups [23]. Moreover, the strong absorption bands at 1051 cm^{-1} was assigned to the stretching vibration of a-pyranose ring in the glucosyl residue [24]. Overall, these results showed that three polysaccharide fractions obtained in this study possessed the typical absorption peaks of polysaccharide, and there were no significant differences among the three polysaccharide fractions.

3.5. Tertiary structure of LLP-1, LLP-2, and LLP-3

Polysaccharide could form special complexes with Congo-red and induce a shift in the visible absorbance maximum (λ_{max}) of the dye, which is a rapid method for detecting tertiary structure of polysaccharide. Generally, the λ_{max} of Congo-red shifts towards a longer wavelength when Congo-red combined with a triple-helix polysaccharide, while the λ_{max} decreases as Congo-red mixed with a random coil polysaccharide [25]. As shown in Fig. 5, the λ_{max} of lentian increased to a maximum with the C_{NaOH} increased initially and decreased dramatically when the C_{NaOH} reached 0.15 M, which indicated that lentian displayed a triple-helical structure and this special structure was disrupted with C_{NaOH} increased [26]. However, at any concentration of NaOH, all LLP fractions showed no specific shift of the λ_{max}, indicating that LLP-1, LLP-2, and LLP-3 three polysaccharide fractions formed random coils or large aggregates in aqueous solution.

3.6. SEM of LLP-1, LLP-2, and LLP-3

Scanning electron microscopy (SEM) was used to explore the surface ultrastructures of LLP-1, LLP-2, and LLP-3. Fig. 6A showed

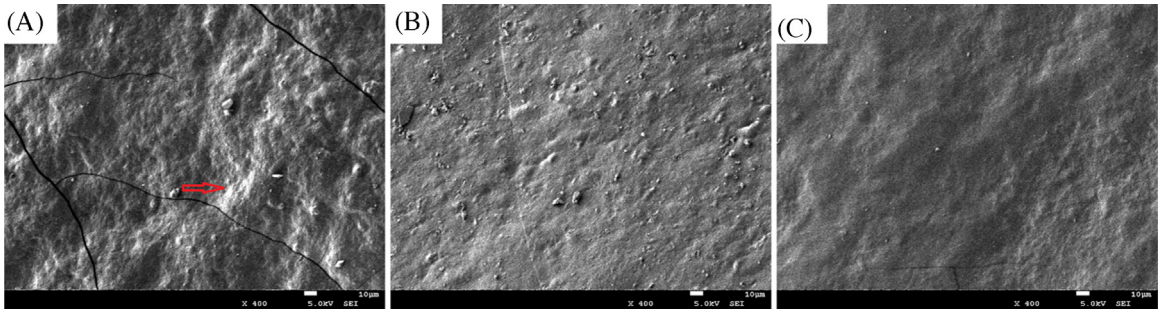

Fig. 6. Scanning electron micrographs of (A) LLP-1; (B) LLP-2; and (C) LLP-3.

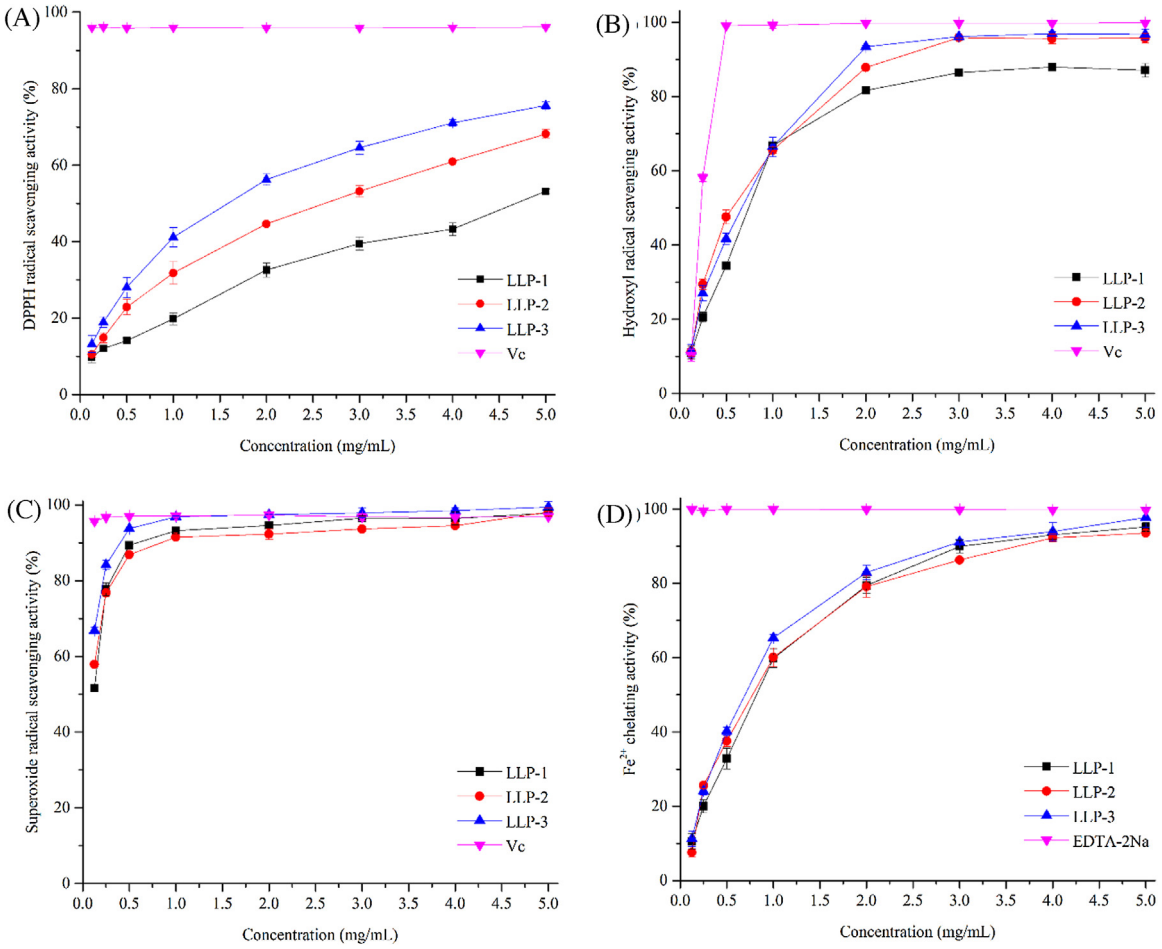

Fig. 7. Antioxidant activities of three polysaccharide fractions. (A) DPPH radical scavenging activity; (B) Hydroxyl radical scavenging activity; (C) Superoxide radical scavenging activity; and (D) Fe^{2+} chelating activity.

that LLP-1 had a rough surface with characteristic large dents. Fig. 6B showed that LLP-2 had a rough surface with many irregular bulges. Fig. 6C showed that LLP-3 very smooth, was very distinct from those of the others. The results showed that different material induced different morphological changes.

3.7. Antioxidant properties

3.7.1. DPPH radical scavenging activity

DPPH, a stable free radical, has been widely used as a reagent to evaluate the free radical scavenging ability of antioxidants [27]. As shown in Fig. 7A, three polysaccharide fractions exhibited obvious scavenging effect on DPPH radical in a dose-dependent manner within the range of concentrations from 0.125 to 5.0 mg/mL, whereas the all overall DPPH radical scavenging activity was not as strong as Vc. The half-effective concentration (EC_{50}) of LLP-1, LLP-2, and LLP-3 were 5.54, 2.34, and 1.43 mg/mL, respectively, which implied that DPPH radical scavenging potential of LLP-3 was the best of all.

3.7.2. Hydroxyl radical scavenging activity

Hydroxyl radical, one of the most reactive and harmful reactive oxygen species, can easily attack lipids, proteins, and DNA, thus resulting in cell death and tissue damage [28]. The results of scavenging hydroxyl radical ability of Vc, LLP-1, LLP-2, and LLP-3 were showed in Fig. 7B. The scavenging effects of all samples displayed a dose dependent manner. At the concentration of 3.0 mg/mL, the scavenging activity of hydroxyl radical was 86.46, 95.85, 96.16 and

99.76% for LLP-1, LLP-2, LLP-3 and Vc, respectively. The scavenging effect of LLP-1, LLP-2 and LLP-3 on hydroxyl radical was close to Vc. In addition, the EC_{50} of LLP-1, LLP-2, LLP-3 and Vc were 0.71, 0.52, 0.53, and 0.22 mg/mL, respectively. These results demonstrated that the polysaccharide fractions from *L. lancifolium* leaves had potential antioxidant ability for scavenging hydroxyl radicals.

3.7.3. Superoxide radical scavenging activity

Although superoxide was a relatively weak oxidant in most organisms, it could easily react with other molecules and further form secondary radicals, including hydroxyl radicals, peroxynitrate, hydrogen peroxide and singlet oxygen, and then induce pathological incidents such as inflammation, cancer and neurodegeneration [29,30]. In this study, superoxide radical scavenging activity of different fractions and different concentrations of polysaccharide are shown in Fig. 7C. As shown in Fig. 7C, three polysaccharide fractions aslo showed a dose-response relationship. At the concentration of 1.0 mg/mL, the scavenging activities of LLP-1, LLP-2, LLP-3 and Vc were 93.20, 91.49, 96.83 and 97.10%, respectively. LLP-1, LLP-2, and LLP-3 superoxide radical scavenging activity was weaker than Vc at the concentrations ranging from 0.125 to 1.0 mg/mL, however, the scavenging activity was rapidly close to Vc with continuously increasing concentration. Meanwhile, the EC_{50} of LLP-1, LLP-2, and LLP-3 were 0.09, 0.07, and 0.06 mg/mL, respectively. These results reveal that the three polysaccharide fractions from *L. lancifolium* leaves had significant superoxide radical scavenging activity.

3.7.4. Fe^{2+} chelating activity

Iron (Fe) is an essential metallic element for respiration, oxygen transport and activity of many enzymes. However, iron is an extremely active metal and will promote oxidative reactions in nucleic acid, proteins, lipids and other cellular components via the Fenton reaction [31,32]. Thus, ferrous ion chelating activity is recognized as a correlative activity to antioxidant. As shown in Fig. 7D, chelating abilities of three polysaccharide fractions climbed up as their concentrations increased. At the concentration of 5.0 mg/mL, the chelating activities of LLP-1, LLP-2 and LLP-3 on Fe^{2+} were 95.21, 93.57, and 97.79%, respectively, were close to the chelating activity of EDTA-2Na (99.84%). Furthermore, the EC_{50} of LLP-1, LLP-2, and LLP-3 were 0.71, 0.70, and 0.60 mg/mL, respectively. These results reveal that the polysaccharide fractions demonstrate an effective capacity for Fe^{2+} chelating.

4. Conclusion

In this study, three novel polysaccharide fractions (LLP-1, LLP-2, and LLP-3) were successfully prepared from the polysaccharides extracted from the leaves of *L. lancifolium*. by DEAE-52 cellulose anion-exchange chromatography column. The physicochemical and structural study revealed that LLP-1, LLP-2, and LLP-3 possessed different chemical compositions and average molecular weights. Meanwhile, three polysaccharide fractions were not observed triple-helical conformation, while possessing variant surface structure. In addition, three polysaccharide fractions all exhibited significantly scavenging activities against free radical and chelating Fe^{2+} *in vitro*. The overall findings indicated that the extracted LLPs could be explored as novel natural antioxidants for application in medicine or functional foods.

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