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The isolation and characterization of polysaccharides from longan pulp

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ABSTRACT

Crude water-soluble longan polysaccharides (LPSs) were extracted from longan pulp using hot water assisted by microwave pretreatment and ethanol precipitation. Neutral and acidic polysaccharides were separated by DEAE-cellulose anion-exchange chromatography. High performance gel permeation chromatography (HPGPC) analysis showed that the average molecular weight (M_w) of neutral polysaccharide (LPS-N) was approximately 13.8 kDa, and those of two acidic polysaccharides (LPS-A1 and LPS-A2) were approximately 1382 and 571 kDa, respectively. Structural properties and compositions of these three LPS were examined by FTIR and HPLC. It was hypothesized that LPS belong to β-type acidic heteropolysaccharides with pyran group, among which LPS-N was composed of xylose and glucose at the molar ratio of 1:1.9; LPS-A1 consisted of rhamnose, xylose, arabinose and galactose at the molar ratio of 1:1.64:4.33:2.28; and LPS-A2 comprised only rhamnose. The total uronic acid content of LPS-A1 and LPS-A2 were 6% and 19%, respectively.

1. Introduction

Polysaccharide is an important kind of carbohydrates in nature. Depending on the structure and properties of constituent monosaccharide, polysaccharides show different chemical and biological activities. Polysaccharides from different sources, such as fungi and algae, have been demonstrated to possess a variety of medical activities [1–6]. Due to the rich variety and availability of polysaccharides from the nature, exploitation of polysaccharides for pharmaceutical application is very promising. However, identifying polysaccharides with desired biological activity is not easy because of the separation and purification difficulties.

Longan (Latin name: *Dimocarpus longan* Lour., Chinese name: *Longyan*; family name: *Sapindaceae*) is a valuable subtropical plant, which is widely distributed in southeastern Asian countries, such as China, Vietnam and Thailand. Longan is historically planted as an edible fruit, but it can also be used for medical purposes. In Chinese medicine, longan has been used to promote blood metabolism, soothe nerves, relieve insomnia, etc. Several studies have been conducted on the bioactivity of longan polysaccharides (LPSs). For example, Wu and Li have found that longan polysaccharides were capable of removing oxygen free radicals [7,8]. However, to date, the chemical compositions and the structural properties of longan polysaccharides are still unknown. Recently, microwave, electrofiltration and sonication technology have been widely used in polysaccharide extraction and achieved a lot of success [9–11]. Our group has developed an efficient LPS extraction method that combined the hot water extraction and microwave-assisted extraction [12].

Numerous studies were conducted to further isolate and characterize polysaccharides of plant and microbial sources. For example, the chemical composition and structure of water-soluble polysaccharides from *Asparagus* has been identified [13]. Tallon et al. characterized the exopolysaccharides produced by microbe *Lactobacillus* and determined their monosaccharides content [14]. Although polysaccharides isolation and characterization were successful in many plants and microorganisms, difficulties still exist when the impurities strongly bind to the target polysaccharides molecule. Given the fact that polysaccharides themselves are a mixture composed of different monosaccharide, the difficulties could be very hard to overcome. The emergence of more powerful separation technologies, for example, high performance liquid chromatography (HPLC) [15], provide a potent solution to this issue. In the present paper, we studied the chemical characterization of LPSs based on the successful isolation. LPS were first extracted using hot water assisted by microwave pretreatment. The extract was then fractionated through anion-exchange chromatography (DEAE52-cellulose) and the molecular weight of each sub-fraction was determined by high performance gel permeation

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chromatography (HPGPC). The structure and carbohydrate compositions of LPS were investigated by FTIR and HPLC.

2. Experimental

2.1. Materials

The species of longan used in this study was "Fenglisui", which was grown in Fujian Province of China and supplied by Xiang'an Science and Technology Bureau of Xiamen, Fujian, China.

Dextran standards (Dextran T standards) were purchased from Pharmacia (Sweden). Carbohydrate standards were purchased from Sigma (USA).

2.2. Longan polysaccharides extraction

Hot water extraction assisted by microwave pretreatment was performed using the method described in our previous study [12].

2.3. Quantitative determination of water-soluble polysaccharides and protein content

The polysaccharides concentration was determined by phenol-sulfuric acid assay [16]. The protein content was determined by the method described by Bradford [17].

2.4. Fractionation

Anion-exchange DEAE52-cellulose chromatography (35 cm × 2 cm) was used to fractionate LPS. 0.5 g crude LPS was dissolved in distilled water, filtered by 0.5 μm membrane and then injected into the column. The LPS was first eluted with distilled water for 7.5 h followed by the elution with NaCl linear gradient (0–1.0 M) for another 10 h. The flow rate was maintained at 0.4 mL/min. 6 mL of each fraction was analyzed for polysaccharide concentration. Sub-fractions obtained by separation of DEAE52-cellulose chromatography were dialyzed and concentrated by rotary evaporation. The obtained precipitate was then purified by adding ethanol and then centrifuged under 3600 rpm for 10 min. The precipitate was dissolved in distilled water for the following HPGPC identification.

2.5. Molecular weight determination

Molecular weight of the LPS sub-fractions was determined by HPGPC coupled to refractive index (RI) detection. Each fraction sample was diluted to a concentration of approximately 2 mg/mL by adding 0.001% NaN_3 solution. 50 μL of sample injected into HPLC system with TSK G4000PWxl (300 mm × 7.8 mm i.d., 13 μm, 500 A) column (TOSOH Corp. of Japan). The 0.001% NaN_3 solution was pumped to HPLC system at the flow rate of 0.5 mL/min. The column temperature was constantly kept at 30 °C. Calibration curve for molecular weight determination was made using a series of Dextran T (Pharmacia) standards, following the method described by Alosp and Vlachogiannis [18]. The developed calibration curve correlated the molecular weight with the HPGPC retention time of the standards, which was subsequently used for molecular weight evaluation of unknown fractions. The molecular weight-retention time equation developed by calibration curve was $\log \bar{M}_w = 6.264 - 2.902K_{av} = 6.264 - 2.902[(T_e - T_0)/(T_t - T_0)]$ with $R^2 = 0.9937$ ($\bar{M}_w$: the average molecular weight of dextran standards; T_e: sample's retention time; T_t: glucose standard retention time; T_0: retention time of dextran standard with $M_w = 2 \times 10^6$; K_{av}: partition coefficient of sample/standard in the chromatography column). The mass ratio of LPS-N, LPS-A1 and LPS-A2 in the crude polysaccharides was calculated after weighing the corresponding dried fractions.

2.6. Structure and property analysis of LPS

The hydrolysis of LPS was conducted using the method described by Carnachan and Harris [19]. 20 mg of each three sub-fractions of LPS was dissolved in 2 mL of 2 mol/L trifluoroacetic acid solution (TFA) and hydrolyzed at 100 °C for 4 h, respectively. The hydrolysates of LPS were then analyzed by HPLC-RI. The column used was Zorbax NH_2 (250 mm × 4.6 mm, 5 μm). An eluent of acetonitrile–water (75:25, v/v) was pumped into HPLC at the flow rate of 1 mL/min. A carbohydrate standard mixture (10 mg/mL), including rhamnose, xylose, glucose and galactose, was used for LPS monosaccharide composition identification. The quantification of uronic acid of LPS was performed using vitriol-carbazole method [20]. The organic groups of LPS purities were detected with Avatar 360 FTIR spectrophotometer (Nicolet, USA).

3. Results and discussion

3.1. Isolation of polysaccharide fractions

The microwave-assisted hot water extraction has been shown to be an efficient method for longan polysaccharides extraction [12]. The water-soluble LPS obtained using this method was separated into three main sub-fractions (LPS-N, LPS-A1 and LPS-A2) after elution from DEAE52-cellulose column, which was as shown in Fig. 1.

Sub-fraction LPS-N was a neutral polysaccharide as it was eluted with water. Polysaccharides eluted with NaCl solution from anion-exchange chromatography should be acidic polysaccharides [22]. Two sub-fractions, LPS-A1 and LPS-A2, were eluted by salt gradient elution in the range of 0.05–0.25 M NaCl. LPS-A1 and LPS-A2 are the major component of LPS crude extract, which was shown by their peak areas in anion-exchange chromatogram (Fig. 1). Since salt solution can elute acidic polysaccharides from cellulose column by anion exchange, the use of NaCl gradient indicated the acidity of LPS-A1 and LPS-A2.

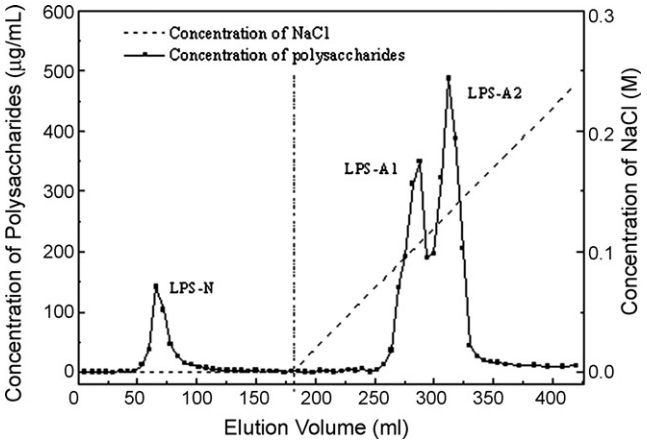

Fig. 1. Anion-exchange chromatogram of LPS. Column: DEAE52-cellulose (35 cm × 2 cm); flow rate: 0.4 mL/min; fraction volume: 6 mL. LPS-N: neutral polysaccharide (eluted with water); LPS-A1 and LPS-A2: acidic polysaccharides (eluted by salt gradient elution).