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# Characterization and mesenteric lymph node cells-mediated immunomodulatory activity of litchi pulp polysaccharide fractions

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ABSTRACT

Three water-soluble hetero-polysaccharides, designated LP1–3, were isolated from litchi pulp. Their structures, solution properties and immunomodulatory activities were evaluated. LP1 contained (1 → 4,6)-β-D-Glc and (1 → 4)-α-L-Gal, while LP2 contained (1 → 3)-α-L-Ara and (l → 2)-β-D-Gal, and LP3 contained α-L-Ara and (l → 4)-β-Rha. Their molecular weights ranged from 105,880 to 986,470 g/mol. LP1 had a spherical conformation with hyper-branched structure and LP2 was semi-flexible chain, while the polysaccharide chains of LP3 were cross linked to form network-like conformation in solution. In addition, all fractions strongly stimulated mesenteric lymph node cell proliferation, IFN-γ and IL-6 secretion in the dose range of 25–100 μg/mL compared with untreated control group ($p < 0.05$). LP1 exhibited the strongest stimulation of mesenteric lymph node cell proliferation and cytokine secretion, which may be attributed to its unique chemical structure and chain conformation. This is the first report on the solution properties and intestinal immunity activities of polysaccharides from litchi pulp.

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## 1. Introduction

Polysaccharides isolated from natural sources are recognised as effective biological response modifiers for their antioxidant, anti-tumour and immunomodulatory activities. The biological activities of polysaccharides are affected by their structures, such as molecular size, type and ratio of the constituent monosaccharides, glycosidic linkages features, chain conformations and solution properties. For example, the anti-tumour and immunomodulatory activities of polysaccharides from *Rhizoma Panacis Japonici* (Huang et al., 2012) and *Poria cocos* (Huang, Jin, Zhang, Cheung, & Kennedy, 2007) are related to the sphere-like conformations while the random coil polysaccharides from *Cordyceps sinensis* (Yan, Wang, Li, & Wu, 2011) and *Longan* (Yi et al., 2013) exhibited more significant anti-tumour and immuno-stimulating effects than the highly branched polysaccharides. By analysing the relationship between the structures and bioactivities of polysaccharides, it can be found that, although the biological activities are directly related to the structures of the polysaccharides, no definite rule can be inferred between the structures and biological activities. Therefore, it is necessary to carry out individual investigation of the chemical structure, chain conformations and solution properties of various polysaccharides from different sources, in order to reveal their structure-bioactivity relationship.

Litchi (*Litchi chinensis* Sonn.) is a delicious fruit and widely distributed in subtropical areas. Its pulp has been used as a traditional Chinese medicine to promote blood metabolism, relieve insomnia and prevent amnesia in folk remedies. Early studies suggested that the numerous health benefits of litchi pulp might be related to the polysaccharides, which serve as the main bioactive ingredients (Huang, Zhang et al., 2014; Huang et al., 2015; Jing et al., 2013). Fruit polysaccharides, including litchi polysaccharides, are typically hetero-polysaccharides. They are composed of various fractions with different chemical and advanced structures, which cause differences in bioactivities. Although researchers have purified litchi polysaccharides and analysed their structures (Jing et al., 2013; Yang, Prasad, & Jiang, 2016), the advanced structure and solution properties of purified polysaccharide fractions are still unclear.

Because the human genome does not encode adequate carbohydrate active enzymes, it is difficult for the orally bioactive polysaccharides to be digested and degraded in the gastrointestinal

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system (Xu, Xu, Ma, Tang, & Zhang, 2013). Active polysaccharides will directly contact with the intestinal mucosa after oral administration. The intestinal mucosal immune system is considered the largest immune organ of the body; and it can help the intestines maintain immuno-homeostasis. Studies have found that intestinal immunity was an important way for bioactive polysaccharides to exert their immunomodulatory activity (Suh et al., 2013; Zuo et al., 2014). Mesenteric lymph node (MLN) cells are an important component of the intestinal mucosal immune system. They mainly regulate cell proliferation, cytokines secretion and assist immunoglobulin synthesis to exert intestinal immunity. *Lentinan* polysaccharide had been found to be able to activate the proportion of T cells and promote IL-4 secretion in MLN cells in Zhang' study (Zhang, 2012). Although litchi polysaccharide have showed their stimulation on splenocyte proliferation (Huang, Zhang et al., 2014; Jing et al., 2013), it is unclear whether litchi polysaccharides could regulate the function of MLN cells to exert intestinal immunity activity.

Therefore, three fractions were purified from a polysaccharide extract of litchi pulp using DEAE-52 cellulose and Sephadex G-100 column. The chemical structures, solution properties and stimulation of MLN cells proliferation and cytokine secretion of three polysaccharide fractions were analysed in the present study. The objectives were (1) to explore the detailed structural features of the different litchi polysaccharide fractions, (2) to reveal the relationship between the structure and the immunomodulatory activity of litchi polysaccharides.

## 2. Material and methods

### 2.1. Materials and chemicals

#### 2.1.1. Chemicals and reagents

Standard dextrans (a series of specific molecular weight dextrans, including $5.2 \times 10^3$ g/mol, $11.6 \times 10^3$ g/mol, $23.8 \times 10^3$ g/mol, $48.6 \times 10^3$ g/mol, $148 \times 10^3$ g/mol, $273 \times 10^3$ g/mol, $410 \times 10^3$ g/mol, $668 \times 10^3$ g/mol, and $14 \times 10^5$ g/mol), arabinose, mannose, rhamnose, galactose, xylose, glucose, anion–exchange DEAE52-cellulose, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), Sephadex G-100 and penicillin–streptomycin solution were purchased from Sigma Chemical Co. (St. Louis, MO, USA). RPMI-1640 medium and foetal calf serum was purchased from Gibco Life Technologies (Grand Island, NY, USA). Enzyme-linked immunosorbent assay (ELISA) kits were purchased from Neobioscience (Shenzhen, China). All other reagents were analytical grade.

#### 2.1.2. Animals and cells

BALB/c mice (male, $20.0 \pm 2.0$ g) aged 6–8 weeks were provided by the Experimental Animal Laboratory of Sun Yat-Sen University. The mice were acclimatised for 1 week before being used for study. All animal treatments were performed in accordance with the Guide for the Care and Use of Laboratory Animals.

### 2.2. Isolation and purification of polysaccharides

Fresh fruits of litchi (cv. Gui-wei) were dried by hot air drying and dried pulps were soaked in 80% ethanol to remove the pigments, monosaccharides and oligosaccharides. After filtration, the residues were homogenized and then extracted twice with distilled water (1:20, g/mL) at 85 °C for 4 h. The extracts were filtered, concentrated, and precipitated with 4 volumes of absolute ethanol to obtain precipitates. The precipitates were lyophilized to obtain dried litchi pulp polysaccharides (LP) (Huang, Zhang et al., 2014). The yield of LP was 3.58%. LP consisted of 65.73% polysaccharides, 4.72% uronic acid and 6.48% protein. The polysaccharides were mainly composed of galactose, glucose, mannose and arabinose at the molar ratio of 3.90:1.97:1.67:1.00 (Huang, Zhang et al., 2014).

The litchi polysaccharide fractions were prepared as follows. Firstly, 100 mg of LP was dissolved in 10 mL of distilled water and centrifuged at 4000*g* for 15 min. The supernatant was added in a DEAE52-cellulose anion-exchange column (60 cm × 2.6 cm) which was equilibrated with distilled water at 25 °C. Then, the column was successively eluted by distilled 0.1, 0.2 and 0.3 M NaCl for 10 h each. The eluent was collected in test tubes with 6 mL per tube at a flow rate of 1.0 mL/min. The polysaccharide concentration of the eluent in each tube was determined using the phenol-sulfuric acid method with some modification and the mixture of arabinose, glucose, mannose and galactose was used as calibration curves (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956; Saha & Brewer, 1994). The protein absorbance was measured on a UV–vis spectrophotometer (UV 1800, Shimadzu, Japan) at 280 nm. The eluents containing polysaccharides were combined individually. The eluents were injected into regenerated cellulose filter bags (MWCO3500, Spectrum, USA) and then the bags were put in distilled water at 4 °C for 3 days. The distilled water was replaced every 12 h. The components with molecular weight less than 3500 g/mol would penetrate out of the bags. The dialysed polysaccharide fractions were concentrated using a vacuum rotary evaporator (BC–R203, Shanghai Biochemical Equipment Co., Shanghai, China) at 55 °C and lyophilised. Then, 50 mg fractions were dissolved in 5 mL water and centrifuged at 4000*g* for 15 min. Five millilitres of supernatant was added into a column (40 cm × 2.0 cm) of Sephadex G-100 and eluted with distilled water at a flow rate of 0.1 mL/min at 25 °C. The purified fractions were collected as described above and lyophilised to obtain LP1, LP2 and LP3. The lyophilised samples were weighed to calculate the yields. The polysaccharide contents of LP1-3 were determined using the phenol–sulphuric acid method (Dubois et al., 1956; Saha & Brewer, 1994). The samples were stored in a desiccator at room temperature until further use.

### 2.3. Characterisation analysis

#### 2.3.1. GC–MS

According to our previous study (Huang et al., 2015), the monosaccharide composition of the litchi polysaccharide fractions were determined by GC–MS. Briefly, polysaccharide samples (40 mg) were dissolved in 2 mol/L $H_2SO_4$ (10 mL) and hydrolyzed at 100 °C for 6 h. After neutralizing the residual acid with 2 mol/L $BaCO_3$, the hydrolysate was filtered through 0.2 μm syringe filters (Whatman, Sanford, ME, UK) and dried under a stream of $N_2$. Dried hydrolysate was then dissolved in 5 mL pyridine containing 14 mg/mL hydroxylamine at 90 °C for 30 min. The samples were cooled to room temperature and 1 mL acetic anhydride was added and the mixture was incubated at 90 °C for 30 min. The acetylated hydrolysate was extracted by trichloromethane, following by evaporation under a stream of $N_2$. The final product was analysed by GC–MS, using an Agilent 6890 GC instrument (Agilent Technologies Co., Ltd., Colorado Springs, CO, USA) equipped with a DB-1 column and an Agilent 5973 MS detector. The temperature program was set as follows: the initial temperature of column was 190 °C, increased to 230 °C at 2 °C/min, holding for 2 min, then to 240 °C at 5 °C/min, holding for 2 min. The detector temperature was 290 °C and vaporizing chamber temperature was set at 260 °C. The GC/MSD ChemStation software was used. Six monosaccharides (arabinose, mannose, rhamnose, galactose, xylose, and glucose) were used as the external standards to identify the composition of the polysaccharides. Each sample was analysed three times.

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