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Polysaccharides in *Laminaria japonica* (LP): Extraction, physicochemical properties and their hypolipidemic activities in diet-induced mouse model of atherosclerosis

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ABSTRACT

The crude polysaccharides of *Laminaria japonica* (LP) were extracted with the optimal conditions as follows: raw materials to water 1:50 (w/v), extraction temperature 60 °C and extraction time 60 min. Trypsinase (6 U/ml) in combination with sevag reagent was beneficial to the removal of proteins from crude LP. Three fractions of LP1, LP2 and LP3 were obtained from the crude LP by using a final concentration of 40%, 60% and 80% ethanol to precipitate in turn. The three fractions showed obvious differences in monosaccharide compositions, molecular weight distribution, viscosity, IR spectroscopy and glycosyl linkages. In comparison with the control group, crude LP at a dose of 400 mg/kg/day caused a reduction in total serum cholesterol, triglycerides, high density lipoprotein-cholesterol and low density lipoprotein-cholesterol in serum by 80.6%, 63.4%, 43.8% and 79.8%, respectively. Moreover, crude LP exhibited good potential of enhancing antioxidant enzyme activities in serum of atherosclerosis mice.

1. Introduction

Laminaria japonica, the most important economic seaweed for edible-medicinal use, has high nutritional value and health functions (Wang, Zhang, Zhang, & Li, 2008). It has been reported that *L. japonica* is distributed in most circumlittoral countries to form a seaweed ecosystem and has a high productivity, e.g., 500,000–700,000 tons in dry weight per year from the Chinese coasts (Suzuki, Furuva, & Takeuchi, 2006). Traditionally, it is widely consumed as a marine vegetable for humans in many eastern Asian countries (Li, Zhang, & Song, 2005). Moreover, it has been used in Traditional Chinese Medicine (TCM) as a home remedy for phlegm elimination, detumescence and weight loss for over one thousand years (Li et al., 2005; Luo et al., 2011; Wang et al., 2008). Over the past decades, *L. japonica* has drawn the attention of chemists and pharmacologists on account of the abundance of functional compounds and their biological properties (Kajiwara, Matsui, Akakabe, Murakawa, & Arai, 2006; Li, Wang, & Miao, 2007; Luo et al., 2011; Park, Lee, Sim, & Lee, 2009). Among these compounds, polysaccharides have been recognised as the main active components, which appear to elicit excellent functions in the prevention of diseases, including anti-tumour, anti-apoptosis, anti-virus, anti-coagulant, anti-oxidant, anti-fatigue and anti-radiation properties (Kim, Kim, Kim, Lee, & Lee, 2006; Luo et al., 2011; Tomohiro, Jynji, Takashi, Noriyuki, & Makoto, 2006; Wang et al., 2008; Zhao, Xue, & Li, 2008).

Atherosclerosis, the complex interaction of serum cholesterol with the cellular components of the arterial wall, is the leading cause of cardiovascular disease worldwide, and continues to result in a high incidence of death (Pang et al., 2010). It has become a major health ailment and a serious social problem. Due to the unknown mechanism of the onset and development of atherosclerotic lesions, indirect therapy of prevention of atherosclerosis formation by eliminating or diminishing the risk factors, has become the main approach in the treatment of atherosclerosis in recent years (Chanet et al., 2011; Koh, Han, Oh, Shin, & Quon, 2010). Among various factors leading to atherosclerosis, high blood concentrations of total cholesterol (TC), triglycerides (TG) and low-density lipoprotein cholesterol (LDL-C) have been considered to be the major risk factors in the pathogenesis of atherosclerosis (Chanet et al., 2011; Lusis, 2000). Moreover, atherosclerosis formation is thought to be dependent upon the overproduction of oxygen radicals (Armstrong, Voyle, Armstrong, Fuller, & Rutledge, 2011). It has been indicated that natural polysaccharides extracted from plants and microorganisms have antioxidant and hypolipidemic activities in general and can be developed as novel potential hypolipidemic agents (Chen et al., 2011; Li, Zhang, & Ma, 2010).

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Polysaccharides of *L. japonica* have also been shown to be effective for lowering blood lipids *in vivo* and scavenging superoxide and hydroxyl radicals *in vitro* (Wang, Wang, & Pang, 2007; Zhao et al., 2008). Since atherosclerosis development has been suggested to be related to hyperlipemia and oxidative stress, we guess that the polysaccharides of *L. japonica* might have good potential of preventing atherosclerosis development. However, as far as our literature survey could ascertain, this information has rarely been reported (Godard et al., 2009). The purpose of this study was to develop a better method for extraction and purification of polysaccharides of *L. japonica*, followed by the analysis of physicochemical properties and hypolipidemic activities during atherosclerosis formation in mice.

2. Materials and methods

2.1. Materials and chemicals

L. japonica was obtained from Lianjiang county, Fujian province, China. Male Kunming mice (18 ± 2 g) were purchased from the Experimental Animal Center, Anhui Medical University of China at 8–10 weeks of age. The mice were housed under normal laboratory conditions, i.e., room temperature (RT), 12/12 h light–dark cycles with free access to standard rodent chow and water. D-Galactose (Gal), D-rhamnose (Rha), L-glucose (Glu), L-mannose (Man), L-xylose (Xyl) and L-arabinose (Ara) were purchased from Sigma–Aldrich (St. Louis, MO, USA). All other reagents were of analytical grade.

2.2. Comparison of extraction efficiency of crude polysaccharides and total sugar

All dried materials were ground in a blender to obtain a fine powder, and were then pre-extracted in a soxhlet system with acetone for 24 h and subsequently with methanol for another 24 h. The residue was dried at 40 °C to a constant weight and stored at RT for polysaccharide extraction. The effects of extraction conditions on extraction efficiency of crude polysaccharides and total sugar were investigated. The polysaccharide and total sugar content were measured by the phenol–sulphuric acid method, using D-glucose as a standard (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956).

2.2.1. Effect of the ratio of raw materials to water

Firstly, the dried powder (2 g) after pretreatment in soxhlet system, was mixed with 20 ml, 40 ml, 60 ml, 80 ml, 100 ml and 120 ml of deionized water, and was then heated in a water bath at 60 °C for 2 h, followed a centrifugation for 15 min at 12,000 rpm. After centrifugation, supernatants were removed and the pellet was re-extracted two times to recover the residual water-soluble sugars. The combined supernatants constituted the total water-soluble sugar. The supernatants were further concentrated with a rotary evaporator to a certain volume and added to ethanol to a final concentration of 80% (v/v). Precipitation of polysaccharide proceeded overnight at room temperature and the precipitate was collected by centrifugation at 12,000 rpm for 30 min. The precipitate was then washed with Sevag reagent (Staub, 1965) and freeze-dried to give the crude polysaccharides of *L. japonica* (LP).

2.2.2. Effect of temperature

Based on the above experiments, the extraction temperature was changed from 4 to 100 °C under the fixed ratio of raw material to water of 1:50 (g/ml) and extracted for 2 h to compare the extraction efficiency. The following procedure was the same as mentioned in the section of 2.2.1.

2.2.3. Effect of extraction time

The extraction time was set at 10, 30, 50, 60, 90 and 120 min and the other conditions were fixed as mentioned in Section 2.2.2.

2.3. Removal of proteins

2.3.1. Sevag method

The crude polysaccharide powder (0.3 g) was dissolved in 25 ml distiled water and then transferred into a 250 ml separatory funnel. After addition of sevag reagent (chloroform:*n*-butanol = 4:1, 10 ml), the solution was shaken vigorously for 20 min at room temperature and centrifuged at 12,000 rpm for 20 min. The supernatant were collected and the protein was measured by the Lowry method (Lowry, Rosenbrough, Farr, & Randall, 1951). This process was repeated until the protein content reached a constant value. The polysaccharide in the supernatant was also determined using the phenol–sulphuric acid method, with D-glucose as a standard.

2.3.2. Protease method

Trypsinase and papain were selected as the protease candidates to remove proteins from the crude polysaccharide of *L. japonica*. The crude polysaccharide powder (0.3 g) was dissolved in 25 ml phosphate buffer (0.2 mol/l, pH 8.0) with a 100 ml flask and heated to 60 °C, followed by an addition of proteinase to the solution at the final concentration ranged from 2 U/ml to 6 U/ml, according to the literature with a minor modification (Park, Cheong, & Jung, 1996; Peng & Zhang, 2003). This mixture was stirred and reacted at 60 °C for 2 h using a stable temperature magnetic stirrer, followed a heat fixation at the boiling temperature for 20 min to terminate the reaction, after which centrifugation was performed at 12,000 rpm for 15 min. The supernatant was collected for protein and polysaccharide analysis.

2.3.3. TCA method

The crude polysaccharide (0.3 g) was dissolved in 25 ml distiled water, after which 5 ml of 42%, 60% and 75% TCA aqueous solution were further added separately and stirred at 100 rpm and at 4 °C for 12 h using a stable temperature magnetic stirrer. After centrifugation at 12,000 rpm for 15 min, the supernatant was collected for protein and polysaccharide analysis.

2.3.4. Protease method combined with the Sevag method

Trypsinase was used to remove proteins at 2, 4 and 6 U/ml, respectively, followed by an addition of 10 ml sevag reagent. The other process was the same as describe above.

2.4. Purification of crude polysaccharide

After extraction of crude polysaccharides from the raw materials of *L. japonica*, the purification technology was further performed using ethanol precipitation method at a different final concentration in this study. For preparation of LP1, ethanol was added to LP at a final concentration of 40% (v/v) and centrifuged, and the precipitate was dissolved in appropriate distilled water. The obtained supernatant of 40% ethanol solution (E40) was used to prepare LP2. Ethanol was added to E40 at a final concentration of 60% (v/v) and the precipitate was dissolved in distilled water (LP2) after centrifugation. Similarly, to obtain LP3, ethanol was further added to the ethanol solution obtained in the process of LP2 preparation at a final concentration of 80% (v/v).

2.5. Determination of molecular weight

The molecular weight was measured by a high performance liquid chromatography system (HPLC, 1260 Infinity, Agilent Technologies), equipped with TSKgel column G4000PWXL