International Journal of Biological Macromolecules 93 (2016) 459–467

Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

journal homepage: www.elsevier.com/locate/ijbiomac

Extraction, characterization, and biological activity of polysaccharides from *Sophora flavescens* Ait.

Qihong Zhang [a], Jingbo Yu [a], Leifang Zhang [a], Meiqun Hu [a], Yan Xu [a], Weike Su [a,b,*]

[a] National Engineering Research Center for Process Development of Active Pharmaceutical Ingredients, Collaborative Innovation Center of Yangtze River Delta Region Green Pharmaceuticals, Zhejiang University of Technology, Hangzhou, 310014, PR China
[b] Key Laboratory for Green Pharmaceutical Technologies and Related Equipment of Ministry of Education, College of Pharmaceutical Sciences, Zhejiang University of Technology, Hangzhou, 310014, PR China

ARTICLE INFO

Article history:
Received 2 June 2016
Received in revised form 14 August 2016
Accepted 18 August 2016
Available online 20 August 2016

Keywords:
Sophora flavescens Ait.
Polysaccharide
Mechanochemistry
Antioxidative activity
Immunological activity

ABSTRACT

Four water-soluble polysaccharides, designated as SF1, SF2, SF3 and SF4, were efficiently extracted from the roots of *Sophora flavescens* by mechanochemistry under the conditions of rotational speed of 400 rpm, grinding time of 10 min, powder to ball weight ratio of 1:20, and Na_2CO_3 loading of 7 wt%. The results obtained indicated that all of these four acid heteropolysaccharides are composed of rhamnose, arabinose, xylose, mannose, glucose and galactose, with the average molecular weights of 400.9, 98.6, 99.3, 42.7 kDa, respectively. In vitro, SF4 showed the most significant scavenging activity on superoxide radical, ABTS, and DPPH radical, while SF3 had the most significant scavenging activity on hydroxyl radical. Immunological tests demonstrated that SF1, SF2, SF3 and SF4 significantly stimulated nitric oxide production without cytotoxicity in macrophages and promoted splenocyte proliferation. These data suggest that the four polysaccharides fractions have the potential as novel natural sources of antioxidative and immunopotentiating agents.

1. Introduction

Polysaccharides are biomacromolecules composed of various monosaccharides linked through glycosidic bonds. In recent decades, polysaccharides have been the focus of research in the fields of biochemistry and medicine, due to their antitumor [1], anti-inflammatory [2], antiviral [3], hypoglycaemic activity [4], and immunostimulating [5] activities. Many botanical polysaccharides have been explored as novel antioxidants and other additives in functional foods [6,7]. Macrophages are principal components of innate immune system and play an important role in resistance to pathogens in host defense response. The basic mechanism of the immunostimulatory, antitumor, bactericidal and other therapeutic effects of botanical polysaccharides is thought to occur via macrophage stimulation and modulation of the complement system [8]. In particular, plant polysaccharides have been approved to increase macrophage cytotoxic activity against tumor cells and microorganisms, activate phagocytic activity, increase reactive oxygen species (ROS) and nitric oxide (NO) production [9]. It is well known that splenocyte proliferation is also an important indicator of immunomodulation. Both cellular and humoral immunity, characterized by T cells and B cells respectively, play important roles in the host defense system [10].

Sophora flavescens Ait. (Leguminosae) is a traditional herbal medicine and has been widely used as functional food ingredient because of its potential health benefits, such as antihelminthic, anti-inflammatory, and antimicrobial activities [11–13]. However, most of previous research work focused on small molecular compounds from *S. flavescens*, such as alkaloids and flavonoids. Until now, there have been few reports regarding the polysaccharides from *S. flavescens* and their potential biological activities.

Conventionally, polysaccharides from plants are extracted by using hot water, which are often time consuming, low extraction yield, and high temperature demanding. Mechanochemistry is the study of physico-chemical transformations generated by mechanical force, which has successfully enabled the aqueous extraction of triterpene acids from fir needles [14], and isofraxidin from Eleutherococcus senticosus [15]. Previously, Bai et al. [16] reported that polysaccharide from *S. flavescens* possessed antitumor and immunomodulating activities. However, systemic studies of extraction method, purification process, chemical constituents of extract and their molecular weights have not been reported. Preliminarily investigation indicated that the polysaccharides in *S.*

* Corresponding author at: Zhejiang University of Technology, Chaowang Road No. 18, Xiacheng District, Hangzhou, PR China.
E-mail address: pharmlab@zjut.edu.cn (W. Su).

http://dx.doi.org/10.1016/j.ijbiomac.2016.08.052
0141-8130/

flavescens roots were heterogeneous mixture of neutral and acidic components. Therefore, this study is designed to develop an environmentally benign and easy-handled method for alkali extraction of polysaccharides from *S. flavescens* based on mechanochemistry and evaluate their antioxidative activities and immunological activities via comprehensive chemical and component analysis.

2. Material and methods

2.1. Materials and reagents

S. flavescens roots were purchased from Zhejiang CONBA Pharmaceutical Co., Ltd. and shattered by a HC-500T2 pulverizer (Yongkang, China) to an average particle size of 0.5 mm. They were then stored in a dry place at room temperature. Dulbecco's modified eagle medium (DMEM) and fetal bovine serum (FBS) were purchased from Gibco. Penicillin-streptomycin, lipopolysaccharide(LPS), concanavalin A, 3-(4,5-dimethyl tiazol-2-yl)-2,5 diphenyl tetrazolium bromide (MTT), bovine serum albumin (BSA), D-galacturonic acid were purchased from Sigma–Aldrich (St. Louis, USA). All other chemicals and solvents used were analytical grade.

2.2. Isolation and purification of polysaccharides

S. flavescens roots were pretreated by mechanochemistry. The optimal milling conditions such as rotational speed, powder-to-ball weight ratio, milling time and loading of solid reagent were optimized by single factor experiment through Retsch PM-200 planetary mill. After co-grinding, 500 g of the pretreated powder was extracted with water (5 L) at room temperature twice (15 min for each), and then the extracts were combined and centrifuged at 3077 *g* for 10 min. Meanwhile, as a control group, a mixture of unmilled *S. flavescens* roots powder and solid reagent was extracted with the same process. The pH value of supernatant was adjusted to 7 with citric acid and then condensed, desalted, and precipitated with four volumes of ethanol and kept at 4 °C overnight. The precipitate was collected by centrifugation, washed successively with ethanol. Then the solid was dissolved in distilled water and centrifuged. The supernatant was deproteinated by the Sevage method for 4 times. Finally, the solution was lyophilized in vacuum freeze dryer to obtain crude polysaccharide.

The crude polysaccharide was dissolved in distilled water. The solution was filtered through 0.45 μm filter and then loaded to a DEAE-Sepharose Fast Flow column (XK 26 mm × 100 cm), followed by successive elution with distilled water, 0.1, 0.2 and 0.4 M NaCl solutions at a flow rate of 7 mL/min. The corresponding four fractions were determined by means of the phenol-sulfuric acid assay, concentrated, dialyzed and lyophilized, and further purified with DEAE cellulose chromatography by using eluent of water, 0.075, 0.16 and 0.36 M NaCl respectively. The main polysaccharides fraction (SF1, SF2, SF3 and SF4) were combined, desalted, and concentrated. Further separation (twice) by preparative circulation liquid chromatography (LC-9210NEXT, Japan) used Gel-620 column (2.5 cm × 30 cm), refractive index detector and distilled water as eluent at a flow rate of 3.5 mL/min. Each fraction was collected, lyophilized and stored at −20 °C before use.

2.3. Analysis of physical and chemical characteristics

2.3.1. Preliminary chemical analysis

Carbohydrate content was determined by phenol-sulfuricacid method using D-glucose as standard [17]. Protein was analyzed by Bradford method using bovine serum albumin (BSA) as standard [18]. Uronic acid contents were analyzed by *m*-hydroxybiphenyl method using D-galacturonic acid as standard [19].

2.3.2. Determination of homogeneity and relative molecular weight

Purity and molecular weight were determined by high performance gel permeation chromatography (HPGPC) with a TSK-GEL G 4000W_{XL} column (7.8 mm × 300 mm, Tosoh, Japan), a refractive index detector (Waters 2414), and distilled water as eluent at a flow rate of 0.8 mL/min. Samples (2.0 mg) were dissolved in distilled water, passed through a 0.22 μm filter and injected. The molecular weight was estimated by reference to a calibration curve made by a series of standard dextran (MW: 12,000, 50,000, 80,000, 150,000, 270,000, 670,000 Da).

2.3.3. Analysis of monosaccharide composition

The monosaccharide components of the polysaccharides were analyzed by GC–MS method [20]. The polysaccharide samples were hydrolyzed with trifluoroacetic acid (2 M) in an oven at 110 °C for 2 h. The solution was then concentrated by vacuum evaporation with methanol. The hydrolyzate and standard monosaccharide mixtures were dissolved in distilled water, respectively. After adding $NaBH_4$, the solution was oscillated intermittently for 3 h and then glacial acetic acid was used to remove excess $NaBH_4$ until no bubble forming. The solution was concentrated by vacuum evaporation with methanol below 50 °C. Then the mixture was added 4 mL of acetic anhydride and kept at 110 °C for 1 h, followed by cooling and vacuum evaporation with toluene for several times to remove the excess acetic anhydride. The acetylated mixtures were dissolved in chloroform and analyzed by GC–MS (HP-5 capillary column: 30 mm × 0.32 mm × 0.25 μm, Agilent) with L-rhamnose, L-arabinose, D-xylose, D-mannose, D-glucose, D-galactose as standards. The operation method was as follows: injection temperature was 170 °C; detector temperature was 250 °C; column temperature was set increasing from 120 °C up to 240 °C (standing for 3 min) at 10 °C/min, then increased to 280 °C (standing for 20 min) at 5 °C/min.

2.3.4. IR spectra analysis

The organic functional groups of polysaccharide were identified by Fourier transform infrared (FT-IR) method. Polysaccharide (2 mg) was dried at 35–45 °C under vacuum over P_2O_5 for 48 h and then ground with spectroscopic grade KBr powder and pressed into a 1 mm pellet for FT-IR measurement (Shimadzu 8400S FT-IR infrared spectrometer). The experiment conditions were 400–4000 cm^{-1} frequency, 8 cm^{-1} resolution, and 32 scans.

2.3.5. Scanning electron microscopy

A scanning electron microscope (SEM) (HITACHI S-4700, Japan) was used to get the scanning electron micrographs. The four samples were placed on a specimen holder with the help of double-sided adhesive tape and coated with gold powder.

2.4. In vitro antioxidant activity test

2.4.1. Assay of ABTS radical scavenging activity

The ABTS radical scavenging activity was measured by ABTS radical cation decolorization assay [21]. ABTS solution (7.4 mM) was oxidized with 2.6 mM of potassium persulphate for 12 h in the dark at room temperature. The $ABTS^+$ solution was then diluted with PBS (0.2 M, pH 7.4) to an Abs of 0.70 ± 0.02 at 734 nm, and 2 mL of the resulting $ABTS^+$ solution was mixed with 0.2 mL of sample solution. The mixture was kept for 6 min at room temperature, and the Abs