International Journal of Biological Macromolecules 51 (2012) 815–818

Contents lists available at SciVerse ScienceDirect

International Journal of Biological Macromolecules

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

Extraction, partial characterization and bioactivity of polysaccharides from boat-fruited sterculia seeds

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ARTICLE INFO

Article history:
Received 29 June 2012
Received in revised form 23 July 2012
Accepted 5 August 2012
Available online 10 August 2012

Keywords:
Boat-fruited sterculia seeds
Polysaccharides
Extraction
Physicochemical characterization
Bioactivity

ABSTRACT

Three polysaccharides (water-soluble (WSP), alkali-soluble (ASP) and insoluble (IMP)) from boat-fruited sterculia seeds were obtained using different extraction methods. Moisture, ash, protein and total carbohydrate content of WSP, ASP and IMP were analyzed. WSP was rich in glucose, rhamnose, arabinose and galactose while small amount of xylose was also detected. The monosaccharide composition as well its relative content for WSP and ASP were similar. The intrinsic viscosity results demonstrated that ASP had much lower intrinsic viscosity than WSP, indicating partial polysaccharides were degraded into low molecular weight polymers during alkaline extraction. The acute anti-inflammatory bioactive results of polysaccharides indicated that WSP demonstrated an inhibitive effect toward acute inflammation.

1. Introduction

Boat-fruited sterculia seed (*Semen Sterculiae Lychnophorae*) is traditionally both an edible and medicinal resource, specified as the ripe seed of *Sterculia lychnophora Hance* in the Chinese pharmacopoeia. The original plant is a tropical herb of the *Sterculiaceae* family, mainly distributed in Vietnam, Thailand, Malaysia, Indonesia as well as Southern China due to the limitation of region and climate [1]. The seed contains a large amount of mucilaginous substance which is used as a traditional medicine in Southeast Asia as well as in China. The mucilage product can be sweetened and consumed as a dessert or canned juice [2]. The seeds are commonly used to treat hoarseness of voice and sore throat by clearing heat from the lungs to resolve phlegm, and treat constipation by relaxing the bowels to clear away toxic substances [3]. The boat-fruited sterculia seeds are just primarily used for making tea, desserts and healthy buccal tablets; however, to our best knowledge, little systematic study for their bioactive components has been done so far.

In the past several years, medicinal polysaccharides from plant cell walls have been widely studied for their physicochemical properties and biological activities such as anti-tumor, free radical scavenging, anti-inflammatory, antioxidant, immunomodulating and antimicrobial properties [4–10]. However, attention paid to the polysaccharides from boat-fruited sterculia seeds was rather limited, perhaps due to the limitation of its habitat mainly in Southeast Asia. Only a few studies on the content and chemical compositions of the polysaccharides from boat-fruited sterculia seeds have been reported [2,11,12]. Animal experiments of aqueous extracts from seeds revealed that the active component with the anti-inflammatory activity was polysaccharides, which exhibited the function of promoting the peristalsis of small intestines [13]. In order to explore the traditional pharmacological effects of boat-fruited sterculia seeds, it is significant to investigate the structural features and functional properties of these polysaccharides. The objectives of the current project were to sequentially extract the polysaccharides from boat-fruited sterculia seeds by different solvents, and investigate the physicochemical properties and characterize the anti-inflammatory bioactivity after oral administration to mice.

2. Materials and methods

2.1. Plant material and chemicals

The boat-fruited sterculia seeds harvested in Vietnam were provided by Shanhe Pharmaceutical Co. Ltd. (Wuxi, China). Meta-hydroxydiphenyl, galacturonic acid, glucose, rhamnose, arabinose, galactose, xylose and mannose were purchased from

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http://dx.doi.org/10.1016/j.ijbiomac.2012.08.006

Sigma–Aldrich Trading Co., Ltd. (Shanghai, China). All other chemicals and solvents used were of analytical grade unless otherwise specified.

2.2. Analytical methods

Moisture and ash contents of the seeds and polysaccharides were determined according to AOCS (1997) [14] and AOAC (2005) [15] methods until no further decrease in weight (AOCS, Ba 2a-38; AOAC 942.05), while crude protein (CP) content in the seeds and solid polysaccharides was determined using the Kjeldahl method with a conversion factor of 6.25 (AOCS, Ba 4a-38). Crude fat content was determined by Soxhlet apparatus extraction using petroleum ether according to AOAC 945.16. Crude fiber (CF) content was determined according to AOAC 962.09. The total carbohydrate content (%) in the seeds was estimated as: {100 − (%Moisture + %Ash + %CF + %CP + % Crude fat)} [16]. Total reducing sugars in the seeds were determined by the Munson–Walker method (AOAC 945.66). Total carbohydrate in the polysaccharides was determined by the phenol–sulphuric acid colorimetric method using glucose as a standard at 490 nm [17]. Proteins in the solution were estimated by the method of binding of Coomassie Brilliant Blue G-250 to protein using bovine serum albumin as a standard [18]. The total uronic acid was colorimetrically determined according to Blumenkrantz and Asboe-Hansen's method by measuring the absorbance at 525 nm with galacturonic acid as a standard [19].

The composition of neutral monosaccharide was analyzed by gas chromatography (GC-14A, Shimadzu, Japan). The polysaccharides were dissolved in 2 M trifluoroacetic acid (TFA) and hydrolyzed at 121 °C for 1 h in a sealed glass tube into monosaccharides. The mixture of monosaccharides was reduced with hydroxylamine hydrochloride dissolved in pyridine, and then acetylated using acetic anhydride at 90 °C for 30 min into alditol acetates. The alditol acetate derivatives were separated by a capillary column at a temperature program and then detected with a flame ionization detector (FID). The percentage of monosaccharides in the sample was calculated by the peak areas using normalization with the correction factor.

2.3. Extraction procedure of polysaccharides from boat-fruited sterculia seeds

2.3.1. Water extraction of polysaccharides

Boat-fruited sterculia seeds were pretreated into defatted powder, and removed some colored materials, oligosaccharides and other low molecular weight compounds, as described previously [20]. Subsequently, the dried powder was extracted twice with deionized water at 70 °C under constant stirring for 3 h. The supernatant aqueous extract was separated from insoluble mucilage with nylon cloth, and then concentrated in a rotary evaporator under reduced pressure at 45 °C and finally precipitated with four volumes of 95% ethanol at 4 °C for 12 h. After collection by centrifugation, washing with acetone, and then drying in the vacuum state, water-soluble polysaccharide (WSP) was obtained.

2.3.2. Mild alkaline extraction of polysaccharides from insoluble mucilage

To avoid the partial degradation of polysaccharides, alkali-soluble polysaccharide (ASP) was extracted twice from insoluble substance mentioned above with 0.05 mol/L NaOH solution (solution to powder ratio at 40:1, at 40 °C, for 2 h). The supernatant alkali-soluble extract was separated, concentrated, precipitated by ethanol, collected and dried, just as WSP was done, and then ASP was obtained. The filtered insoluble mucilage obtained was dried in the vacuum, and then insoluble polysaccharide (IMP) was obtained.

The three polysaccharides (WSP, ASP and IMP) were purified further to remove the free proteins by Sevag method, and then redissolved in distilled water, dialyzed against distilled water (Molecular weight cutoff 10,000 Da), and concentrated and lyophilized, consecutively.

2.4. Calculating the yield and purity of polysaccharides

The yield and purity of polysaccharides were calculated by the following formulas:

$$Y(\%) = \frac{M_2}{M_1} \times 100, \qquad P(\%) = \frac{M_3}{M_2} \times 100$$

where Y and P are the yield and purity of polysaccharides, respectively. M_1, M_2 and M_3 are respectively the weight of seed powder, polysaccharides and purified polysaccharides.

2.5. Intrinsic viscosity measurement of polysaccharides

The aqueous solutions of polysaccharides were prepared by dispersing in deionized water for 1 h at 60 °C using a magnetic stirrer, and then filtering through 0.45 μm nylon syringe filter (Chromatographic Specialties Inc., USA) to remove any insoluble particulate matter. The intrinsic viscosities were determined by dilute solution viscometry using a Cannon-Ubbelohde semi-micro dilution glass viscometer (size 75, viscometer constant 8.433×10^{-3} mm^2/s^2, Kinematic viscosity range 1.6–8 mm^2/s; Cannon Instrument Co., USA) in a constant temperature water bath at 25 °C. The polysaccharide viscosity was measured in duplicates in a concentration range. While the relative viscosity (η_r) was kept from 1.2 to 2.0, the polysaccharide solution was essentially Newtonian fluid without end effect correction. Intrinsic viscosity [η] was calculated using the following relationship [21].

$$[\eta] = \lim_{c \to 0} \left(\frac{\eta_{sp}}{c} \right) = \lim_{c \to 0} \left(\frac{\ln \eta_r}{c} \right)$$

where c is concentration of polysaccharide, η_r relative viscosity, and η_{sp} specific viscosity defined as $\eta_r - 1$. Huggins–Kramer plots of η_{sp}/c and $(\ln \eta_r)/c$ versus c were then used to estimate the intrinsic viscosity [η] by extrapolation to zero concentration.

2.6. Acute anti-inflammatory bioactivity assessment of polysaccharides

The acute anti-inflammatory activity was evaluated by dimethylbenzene-induced mice ear edema, as described previously [20,22,23]. Male Kunming (KM) mice weighing (20 ± 2) g (provided and identified by Nanjing Qinglongshan Animal Center) were used for the assessment of the acute anti-inflammatory activity. The experiment was not done until the animals were settled to adapt themselves to the new environment. The mice were divided randomly into five groups (eight mice per group). WSP (200 mg/kg day), ASP (200 mg/kg day) and IMP (200 mg/kg day) solutions were prepared with normal saline, and the dose of polysaccharides was determined by the pre-experiments and each administered to a test group of mice. Positive control group mice were treated orally with aspirin at a dose of 100 mg/kg day dissolved in normal saline. Normal group received the same amount of normal saline.

3. Results and discussion

3.1. Chemical composition analysis of boat-fruited sterculia seeds

The chemical composition of boat-fruited sterculia seeds from Vietnam as analyzed is presented in Table 1. 54.54% (w/w) of