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Optimization, characterization, sulfation and antitumor activity of neutral polysaccharides from the fruit of *Borojoa sorbilis* cuter

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ABSTRACT

Extraction optimization, purification, characterization, sulfation and antitumor activity of polysaccharides from the fruit body of *Borojoa sorbilis* cuter were investigated in present study. The optimal Ultrahigh Pressure extraction condition was determined as: extraction once with the solid-liquid ratio of 1:10 in 30 °C and 1500 Mpa for crude polysaccharide (BP) and experimental yield was 8.28%. Four water-soluble polysaccharides named as BP1-1, BP1-2, BP1-3 and BP1-4, with molecular weight of 35.8, 32.4, 30.1 and 27.7 kDa, were purified by DEAE Sepharose and Superdex 200 chromatography. On the basis of chemical and spectroscopic analyses, BP1-1–BP1-4 were found to be neutral β-D-galactan containing a (1 → 4)-linked backbone. S-BP1s with the DSS of 1.18, was sulfated by chloro-sulfonic acid-pyridine method. Furthermore, S-BP1s exhibited significant *in vitro* antitumor activity against liver cancer HepG2 and lung cancer A549 cells in a dose-dependent manner. The results indicated that S-BP1s could be potentially developed as functional antitumor drug.

1. Introduction

Natural polysaccharides have drawn much attention in recent years due to their wide variety of pharmacological activities, such as antitumor, immunomodulation and anti-oxidation properties (Li et al., 2012; Cho et al., 2015; Wang, Wang et al., 2015). Borojo is the mature fruit body of *Borojoa sorbilis* cuter, grown in tropical rainforest areas in Latin America, Ecuador (Mosquera & Martinez-Navarrete, 2010). Local residents believe consumption of Borojo benefit human health for its diverse efficacy such as maintaining normal blood pressure, improving cholesterol symptoms, enhancing immunity and anti-inflammatory activity.

Previous investigations have demonstrated that polyphenols (Contreras-Calderon, Calderon-Jaimes, Guerra-Hemandea, & Garcia-Villanova, 2011; Sotelo, Casas, & Camelo, 2010), minerals (Leterme, Buldgen, Estrada, & Londono, 2006), polysaccharide (Lin et al., 2010), amino acids (Liu et al., 2013) and volatile constituents (Xu et al., 2012) are the main chemical components of borojo fruit. Borojo polysaccharide (BP) have been attracted our increasing attentions due to their immune-enhancing activity (Li, Liao et al., 2015; Li, Mao et al., 2015). To the best of our knowledge, studies on polysaccharides from *Borojoa sorbilis* cuter have not been reported.

In order to better explore Borojo polysaccharides (BP), Ultrahigh Pressure extraction method (UHP) that based on biological cell wall broken powder operations, has been developed to improve the extraction rate for its high extracting yield and time saving (Li, Liu, Liu, & Ruan, 2006; Guo, Du, & He, 2001). The optimization of extraction parameters, such as extraction pressure, temperature, solid-liquid ratio and extraction frequence, have been applied to maximum the extraction yield of crude Borojo polysaccharides (Zhang, Zhu, & Wang, 2004).

Sulfated polysaccharides have shown some special bioactivities, such as immunopotentiator, anti-oxidant, anti-coagulant, antitu-

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mor, and anti-HIV (Wang et al., 2010; Wei, Wei, Cheng, & Zhang, 2012; Lu, Mo, Guo, & Zhang, 2012; Zou, Du, Li, Yang, & Zhang, 2010; Urbinati et al., 2004). These activities are closely related to the structural modification, such as the degree of sulfation, the sulfation position, the types of sugar and structures of main chains and branches (Cong, Xiao, Liao, Dong, & Ding, 2014; Cardozo et al., 2013; Zhang, Liu, Park, Xia, & Kim, 2012).

In this paper, we report the extraction, optimization, characterization, sulfation and *in vitro* antitumor activity of polysaccharides from Borojo fruit. Firstly, Ultrahigh Pressure extraction method (UHP) and orthogonal experiment were applied to optimize the extraction conditions, and the resulting extraction conditions were used to prepare crude BP. Secondly, The crude BP was purified by DEAE Sepharose and Superdex 200 column. Fourier transform infrared (FT-IR) spectrometry, high performance gel permeation chromatography (HPGPC), gas chromatography-mass spectrometry (GC–MS) and Nuclear Magnetic Resonance Spectroscopy (NMR) were used to characterize the structural features of the purified polysaccharides. The sulfated derivatives of BP1s (named S-BP1s) was prepared and characterized. Finally, *in vitro* antitumor activities against liver cancer HepG2 and lung cancer A549 cells were evaluated.

2. Materials and methods

2.1. Materials and chemicals

Borojo was obtained from Producto Amazonico, Ecuador, identified by the professor Zhi-hai Huang. The voucher specimen was deposited in The Second Affiliated Hospital of Guangzhou University of Chinese Medicine, Guangzhou, China. Monosaccharide references, Galactose (Gal) was purchased from Yuanye Bio-Technology Co. (Shanghai, China). DEAE Sepharose and superdex 200 were purchased from GE Healthcare (Stockholm, Sweden). The sulfation reagent, sulfur trioxide-pyridine complex (SO_3·Py), was purchased from Aladdin (Shanghai, China). 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium (MTT) was purchased from MP Biomedicals (California, USA). Vinblastine was purchased from Sigma (St. Louis, Mo, USA). Penicillin G and streptomycin, Roswell Park Memorial Institute (RPMI)-1640 medium, phosphate-buffered saline (PBS) and fetal bovine serum were purchased from Thermo Fisher Scientific (Waltham, USA). Liver cancer cell line HepG2 and lung cancer cell line A549 were obtained from Guangdong Provincial Academy of Chinese Medical Sciences. All other chemicals used were of analytical grade.

2.2. General analysis methods

The specific rotation was determined at 25 ± 1 °C using an automatic polarimeter (Autopol I, Rudolph, USA). Ultraviolet and visible absorption spectra were recorded with a UV spectrophotometer (U-2910, Hitachi, Japan). The Fourier transform infrared (FT-IR) spectrum was recorded on a FT-IR spectrophotometer (Spectrum two, Perkin Elmer, USA). NMR spectrum was recorded on a spectrometer (AV-400, Bruker, USA).

2.3. Ultrahigh Pressure extraction of crude Borojo polysaccharide

The crude polysaccharide (named BP) was extracted by Ultrahigh Pressure extraction method using a continuous ultra high pressure homogenizer (JN-10HC, Guangzhou Juneng biological science and Technology Co., China). Borojo was dried and ground to pass a 200-mesh screen. Borojo (10.00 g) was defatted twice with 95% EtOH (100 mL). The residue was then dried at room temperature and treated by Ultrahigh Pressure extraction method, filtered, and the supernatant was collected, concentrated to one tenth of the original volume and then mixed with 4 vols of 95% EtOH at 4 °C. It was stand for overnight and then centrifuged. The precipitate was washed with EtOH and dissolved in water, deproteinized with a combination of Dialysis (MWCO. 10000) and Sevag method (Staub, 1965), and then lyophilized to give the crude polysaccharide BP.

Table 1
The range of independent variables and levels.

variables	A/MPa	B/°C	C/g mL^{-1}	D/times
1	1200	25	10:1	1
2	1500	30	20:1	2
3	1800	35	30:1	3

Table 2
The results of optimization experimental runs.

Run	A	B	C	D	Extraction yield/%
1	1	2	2	2	5.04
2	2	1	3	2	2.87
3	2	3	2	1	5.81
4	3	1	2	3	2.71
5	2	2	1	3	8.25
6	1	3	3	3	2.07
7	1	1	1	1	5.99
8	3	2	3	1	5.63
9	3	3	1	2	6.40
K_1	4.37	3.86	6.88	5.81	
K_2	5.64	6.31	4.52	4.77	
K_3	4.92	4.76	3.52	4.35	
R	0.073	0.277	0.535	0.102	

A, B, C, D, K and R represented for pressure, temperature, solid-liquid ratio, frequence, ranger and variance, respectively.

2.4. Orthogonal experiment

The effects of the major extraction parameters, including extraction pressure, temperature, solid-liquid ratio and extraction frequence were investigated by single-factor tests (data not shown). A three-level, four-variable orthogonal experiment $L_9(3^4)$ was applied to determine the optimal levels of extraction variables including the extraction pressure (A), extraction temperature (B), solid-liquid ratio (C) and extraction frequence (D). The range of independent variables and their levels were shown in Table 1 and 9 experimental runs were conducted and the results were shown in Table 2.

2.5. Purification of BP

The BP was dissolved in distilled water, centrifuged and the supernatant was loaded onto a DEAE Sepharose Fast Flow column (Wang, Li, Liu, Chen, & Wei, 2015) (1.0 cm × 30 cm), eluted with 0, 0.2, 0.4 and 0.6 M sodium chloride (NaCl) solution at a flow rate of 0.5 mL/min.The water fraction (BP1) was further purified on a Superdex 200 prep grade column (1.6 cm × 80 cm) (Zhang, Wang, Nie, Wang, & Cui, 2015) and the column was eluted with water at a flow rate of 1.0 mL/min. The carbohydrate contents were determined by the phenol-sulfuric acid method using D-glucose as standard (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956). As a result, four purified fractions BP1-1, BP1-2, BP1-3 and BP1-4 (BP1s for shorten) were collected and lyophilized for subsequent analysis.

2.6. Characterization of BP

2.6.1. Homogeneity and molecular weight analysis

The homogeneity and molecular weight of BP1s were determined by high performance gel permeation chromatography (HPGPC) using an Agilent 1200 series equipped with refractive