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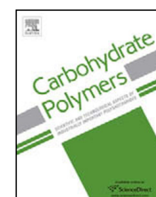

Purification, structural characterization and anticancer activity of the novel polysaccharides from *Rhynchosia minima* root

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ABSTRACT

Three novel acidic polysaccharides termed PRM1, PRM3 and PRM5 were purified from *Rhynchosia minima* root using DEAE-52 cellulose and sephadex G-150 column chromatography. Their structures were characterized by ultraviolet (UV) and Fourier transform infrared (FTIR) spectrometry, gel permeation chromatography (GPC), gas chromatography–mass spectrometry (GC–MS), and differential scanning colorimeter (DSC) analysis. The uronic acid contents of PRM1, PRM3 and PRM5 were 30.7%, 12.7% and 47.7%, respectively. PRM1 (143.2 kDa), PRM3 (105.3 kDa) and PRM5 (162.1 kDa) were heteropolysaccharides because they were composed of arabinose, mannose, glucose and galactose. Their enthalpy values were 201.0, 111.0 and 206.8 J/g, respectively. PRM3 and PRM1 exhibited strong *in vitro* anticancer activity against lung cancer A549 and liver cancer HepG2 cells in a dose-dependent manner. These findings suggested that PRM1 and PRM3 could be potentially developed as natural anticancer agents.

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1. Introduction

Polysaccharides, as a type of secondary metabolites, are one of the most abundant biopolymers that naturally exist in plants (Ding, Hou, & Hou, 2012). Polysaccharides are relatively non-toxic and so have no significant side effects (Schepetkin & Quinn, 2006), therefore, they play pivotal roles in food, pharmacy and material industries (Jiao, Yu, Zhang, & Ewart, 2011). Plant polysaccharides are linked by glycosidic bonds and displayed wide biological activities, such as immunomodulation (Teng et al., 2015), anticancer (Suresh et al., 2013), antioxidation (Yang, Wang, Li, & Yu, 2015), antimicrobial (Krichen et al., 2015), and wound-healing (Lazareva et al., 2001). Anticancer activities of polysaccharides are particularly interesting to be intensively investigated. A considerable amount of evidence indicated that polysaccharides possess anticancer activities or could enhance the activity of conventional chemotherapy drugs (Zong, Cao, & Wang, 2012). Treatment with *lycium barbarum* polysaccharide could inhibit the growth of human hepatoma QGY7703 cell growth, arrest the cell cycle in S phase, and induce apoptotic cell death (Zhang et al., 2005). A novel high molecular weight polysaccharide extracted from *Phellinus linteus* mycelia could inhibit the growth of HepG2 cells in a dose-dependent manner (Pei, Wang, Ma, & Yan, 2015). Additionally, polysaccharides of porcine cartilage reveal strong anticancer activity against human K562 cells *via* inducing apoptosis (Song et al., 2014). Thus, polysaccharides could be beneficial to our health not only as functional foods but also as potential therapeutic agents.

Rhynchosia minima (family: Papilionaceae/Fabaceae), commonly known as least snout-bean, burn-mouth-vine, and jumby bean, can be found on every continent and used as a medicinal herb in China. It was traditionally used to alleviate boils, colds, upper respiratory infections, and joint pains. Chemical studies had shown that *R. minima* contained essential oils, tannins, flavonoids, triterpene steroids, *etc.* (Gweru et al., 2009; Ikhiri, Boureima, Dicko, & Koulodo, 1992). It also exhibited other bioactivities, such as antimicrobial and anthelmintic activities (Mali & Mahale, 2008). However, to our knowledge, there was little information on polysaccharides and their anticancer activity.

The bioactivities of polysaccharides were mostly related to their physicochemical properties, including the content of polysaccharide and uronic acid, molecular weight, and types of sugar

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residues (Xie et al., 2013). Therefore, the objective of this study was to investigate the physicochemical property and anticancer activity of polysaccharides purified from *R. minima* root. Crude polysaccharides were extracted using hot water extraction and purified by DEAE-52 cellulose and sephadex G-150 column chromatography. Ultraviolet (UV) and Fourier transform infrared (FTIR) spectrometry, gel permeation chromatography (GPC), gas chromatography–mass spectrometry (GC–MS) and differential scanning colorimeter (DSC) were used to characterize the structural features of the purified polysaccharides. Moreover, the *in vitro* anticancer activities against lung cancer A549 and liver cancer HepG2 cells were also evaluated.

2. Materials and methods

2.1. Materials and reagents

R. minima roots were purchased from apothecary shop (Pingtan County, Fujian Province, China), and identified using DNA barcoding method (Han et al., 2013). The herb was ground into fine powder using a powerful mill (FW177, Tianjin Taisite Instrument Co. Ltd., China), and stored at room temperature in a desiccator until use.

DEAE Cellulose-52 and sephadex G-150 were purchased from Sigma–Aldrich Co. Ltd. (St. Louis, MO, USA). All other reagents of analytical grade were obtained from Aladdin Industrial Corporation (Shanghai, China).

2.2. Isolation and purification of polysaccharides

The powder of *R. minima* root was refluxed with petroleum ether for 2 h to remove pigments and lipids, and then dried in a fume hood at room temperature. The residues were extracted using hot water extraction at 70 °C for three times (each for 2 h). After vacuum filtration, the supernatants were collected and condensed to about 100 mL, and then mixed with 5 volumes of anhydrous ethanol at 4 °C overnight. Precipitates were dissolved in deionized water and deproteinized by Sevag solution (chloroform: butyl alcohol, 4:1). The deproteinized solution was re-precipitated in anhydrous ethanol, then centrifuged and lyophilized. The obtained powder was crude polysaccharide.

Crude polysaccharide was dissolved in deionized water and centrifuged at 3000 rpm for 10 min. The supernatants were passed through DEAE cellulose-52 column (5 cm × 30 cm) and stepwise eluted with 0, 0.1, 0.3, 0.5 and 0.7 mol/L NaCl at a rate of 3 mL/min (6 mL/tube). Total carbohydrates in each tube were measured using phenol–sulphuric acid method. Three fractions named PRM1, PRM3, and PRM5 were obtained. Each fraction was collected, concentrated and lyophilized for further use.

2.3. Physicochemical property analysis

Total sugar content of PRM1, PRM3 and PRM5 was measured by phenol–sulfuric acid colorimetric method (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956). Protein content of PRM1, PRM3 and PRM5 was measured by bicinchoninic acid/$CuSO_4$ (BCA) method (Tyllianakis, Kakabakos, Evangelatos, & Ithakissios, 1994). Uronic acid content of PRM1, PRM3 and PRM5 was measured by m-hydroxydiphenyl method using D-galacturonic acid as the standard (Liang et al., 2012). Molecular weight of PRM1, PRM3 and PRM5 was determined by gel permeation chromatography (GPC) method at a flow rate of 1 mL/min, using a sephadex G150 column (1.5 cm × 55 cm). A standard curve was constructed using the dextran standards (23,800, 80,900, 147,600, 2,000,000 Da).

2.4. UV and IR spectroscopy analysis

UV spectra were tested for the identification of protein in PRM1, PRM3 and PRM5. The UV spectra of each polysaccharides solution were scanned with a microplate reader (SpectraMax M5, Molecular Devices, USA) from 200 to 400 nm.

Fourier-transform infrared spectra (FTIR) of the three purified polysaccharides were measured on spectrum 100 FTIR spectrometer (PerkinElmer). PRM1, PRM3 and PRM5 were evenly mixed with dried KBr powder, and then pressed into a 1 mm disk for FTIR measurements in mid-infrared wavelengths of 4000–450 cm^{-1}.

2.5. Monosaccharide analysis

Monosaccharide composition of PRM1, PRM3 and PRM5 was performed by GC–MS (TRACE™ 1300, Thermo Scientific, USA). Each sample (10 mg) was hydrolyzed with 2 mol/L trifluoroacetic acid at 100 °C for 3 h. The excessive acid was removed by methyl alcohol for 4 times. The hydrolysates were reduced by $NaBH_4$ (10 mg), and acetylated by pyridine (0.5 mL) and acetic anhydride (0.5 mL) at 40 °C for 2 h. The alditol acetates were dissolved in 2 mL methyl alcohol and filtrated *via* a 0.45 μm filter membrane before injecting into the GC–MS for analyzing. The operation condition for GC analysis was as follows: injection temperature was 170 °C; detector temperature was 280 °C; column temperature was set changing from 120 °C up to 170 °C (standing for 3 min) at 10 °C/min, then increased to 280 °C (standing for 20 min) at 5 °C/min. Helium was used as the carrier gas and maintained at a flow rate of 1 mL/min.

2.6. Thermal properties analysis

Thermal properties of PRM1, PRM3 and PRM5 were performed using differential scanning colorimeter (DSC) thermogram (DSC-60A, Shimadzu, Japan). Two milligrams of sample were kept in an aluminum pans and sealed. Empty closed aluminum pan was used as the reference. Samples were scanned from 50 °C up to 350 °Cat a ramping rate of 10 °C/min. Nitrogen was used as a purging gas (35 mL/min). The onset temperature (T_o), peak temperature (T_P), and enthalpy change (ΔH) were measured.

2.7. In vitro *anticancer activity analysis*

The *in vitro* anticancer activities of PRM1, PRM3 and PRM5 were measured by MTT colorimetric method (Pei et al., 2015). A549 and HepG2 cancer cells were used and cultured in RPMI 1640 culture solution (10% fetal bovine serum, 100 U/mL penicillin, and 100 μg/mL streptomycin) with 5% CO_2 at 37 °C. Each cell suspension was seeded in 96-well plates and incubated for 24 h at a concentration of 1×10^5 cell/mL. PRM1, PRM3 and PRM5 solutions (0.125, 0.25, 0.5, 1, 2, and 4 mg/mL) were added into the wells. After incubation for 24 h, 100 μL MTT (5 mg/mL) was added and incubated for 4 h at 37 °C. Then, culture media were removed and 100 μL DMSO was added to each well. Absorbance was measured at 570 nm using a microplate reader (SpectraMax M5, Molecular Devices, USA). The inhibition rate of cell growth was calculated according to the formula:

$$\text{Inhibitory rate}\,(\%) = \left[\frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}}\right] \times 100$$

2.8. Statistical analysis

Results were expressed as means ± standard deviation (SD) and each experiment was repeated three times.

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