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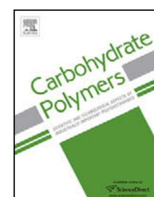

Characterization, antioxidant and antitumor activities of polysaccharides from purple sweet potato

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ABSTRACT

Three polysaccharides, PSPP1-1, PSPP2-1 and PSPP3-1, were isolated from purple sweet potato. The three polysaccharides belonged to β-type polysaccharides and contained low proportions of proteins and uronic acids. PSPP1-1 and PSPP3-1 with molecular weights of 33.3 and 75.3 kDa, respectively, were composed of rhamnose, xylose, glucose and galactose, whereas PSPP2-1 with molecular weight of 17.8 kDa was composed of rhamnose and galactose. The three polysaccharides possessed *in vitro* antioxidant (scavenging DPPH radicals, chelating ferrous ions and reducing power) and antitumor activities (against SGC7901 and SW620 cells) in a dose-dependent manner. Among the three polysaccharides, PSPP2-1 exhibited the strongest reducing power, scavenging activity on DPPH radicals and chelating capability on ferrous ions. PSPP1-1 showed the strongest inhibitory activities on the growth of SGC7901 and SW620 cells. In addition, flow cytometry results showed that PSPP1-1 could induce apoptosis in SGC7901 and SW620 cells. These results suggest that polysaccharides from purple sweet potato are potential natural antioxidant and antitumor agents that can be used as drugs or functional food ingredients.

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1. Introduction

Sweet potato (*Ipomoea batatas* L.) belongs to the family *Convolvulaceae* (Shekhar, Mishra, Buragohain, Chakraborty, & Chakraborty, 2015). This plant is used for both food and animal feed or industrial raw material purposes. It is widely cultivated in the tropical, subtropical and temperate regions around the world (Shekhar et al., 2015). Sweet potato is considered to be one of the most promising economic crops because of its high yielding and strong resistance capabilities for different environmental, soil, and temperature conditions (Ahn et al., 2010; Sun, Mu, Xi, Zhang, & Chen, 2014a). Nowadays, sweet potato is considered as the seventh most important food crop in the world (Lee et al., 2012). In China, sweet potato accounts for 90% of worldwide sweet potato production with an annual production of 117 million tons (Guo, Liu, Lian, Li, & Wu, 2014). Purple sweet potato belongs to a member of the sweet potato family. It is a highly nutritious vegetable, containing a variety of vitamins, amino acids, minerals, dietary fiber, phenolic acids, anthocyanins, tocopherol and β-carotene etc (He et al., 2012; Teow et al., 2007; Wu et al., 2008; Zhang, Mu, & Sun, 2014b). Thus, there is a growing interest in purple sweet potato because of its nutritive values and biological activities.

Polysaccharides have garnered attention mainly attributed to their diverse biological activities, including antioxidant, antimicrobial, anticancer and anti-inflammatory activities (Abou Zeid, Aboutabl, Sleem, & El-Rafie, 2014; Facchini et al., 2014; Pakrokh Ghavi, 2015; Popov et al., 2014). In previous study, we revealed that purple sweet potato had 5.42% of water-soluble polysaccharides (Qu, Wu, & Jia, 2014). The content for polysaccharide from purple sweet potato was similar to this content (5.56%) for polysaccharide from chickpea flours (Mokni Ghribi et al., 2015). However, and to our knowledge, limited data is currently available on the characterization and bioactivities of polysaccharides from purple sweet potato. The aim of the present study is, first of all, to isolate polysaccharides from purple sweet potato and to analyze their physicochemical properties. Additionally, the *in vitro* antioxidant activities of isolated polysaccharides were evaluated by 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay, ferrous ions chelating

Abbreviations: PSPP, purple sweet potato polysaccharide; DPPH, 1,1-diphenyl-2-picrylhydrazyl; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; DMSO, dimethyl sulfoxide; DMEM, dulbecco's modified eagle medium; FBS, fetal bovine serum; GC, gas chromatography; TFA, trifluoroacetic acid; SEM, scanning electron microscopy; FT-IR, Fourier transformation infrared.

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activity assay and reducing power activity assay. Finally, the *in vitro* antitumor activities of isolated polysaccharides were evaluated by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assays using SGC7901 and SW620 cells.

2. Materials and methods

2.1. Materials and chemicals

Purple sweet potato (*I. batatas* L.) (variety NING No. 1) was grown at planting base of Jiangsu Academy of Agricultural Sciences (China) and sold by local supermarket (Jiangsu, China). The purple sweet potato was washed in running tap water to remove its surface impurities. The washed purple sweet potato was cut and then dried in a hot air oven (DNG-9143BS, Xinmiao Medical Device Manufacturing Co., Shanghai, China) at 60 °C. Then the dried purple sweet potato was pulverized and sifted through an 80-mesh sieve for getting purple sweet potato powder.

The α-amylase from *Bacillus subtilis* was purchased from Yuanye Biotechnology Co. (Shanghai, China). DEAE-52 cellulose and Sephadex G-100 were purchased from Auyoo Biotechnology Co. (Shanghai, China). 1,1-diphenyl-2-picrylhydrazyl (DPPH), T-series dextrans, monosaccharide standards, 3-(4,5-dimethylthiazol-2-yl) -2,5-diphenyltetrazolium bromide (MTT) and dimethyl sulfoxide (DMSO), were purchased from Sigma-Aldrich Trading Co. (Shanghai, China). Dulbecco's modified eagle medium (DMEM) and fetal bovine serum (FBS) were purchased from Gibico (Grand Island, NY, USA). Annexin V-EGFP/PI apoptosis detection kit was purchased from Keygen Biotechnology Co. (Nanjing, China). All other chemicals and solvents were of analytical grade and purchased from Limai Biotechnology Co. (Shanghai, China).

2.2. Tumor cells

Human gastric carcinoma SGC7901 and human colon cancer SW620 cells were obtained from Shanghai Institute of Cell Biology (Shanghai, China) and were cultured in DMEM medium containing 10% FBS, penicillin (100 unit/ml) and streptomycin (100 μg/ml) at 37 °C in an atmosphere containing 5% CO_2.

2.3. Separation and purification of PSPP

Purple sweet potato powder was first defatted with petroleum ether and de-pigmented with 80% ethanol (v/v). The de-pigmented dried powder (8 g) was dispersed by stirring for 10 min in 200 ml of deionized water. The suspension was treated using an ultrasonic cleaner (SY-360, Shanghai Ningshang Ultrasonic Instruments Co., Ltd., China) at a power of 210 W for 30 min. After sonication, the treated suspension was hydrolyzed for 1.5 h using α-amylase (enzyme-substrate ratio, 60 U/g) at natural pH and 66 °C with stirring, and then the suspension was centrifuged at 3500 × *g* for 10 min. The supernatant was concentrated to a tenth of its original volume using a rotatory evaporator. The concentrated liquid was precipitated with 3 volumes of 95% ethanol (v/v), and centrifuged again at 3500 × *g* for 15 min. The precipitated polysaccharide was washed twice with 95% ethanol (v/v) and deproteinated by Sevag method (Zhang et al., 2014a). The deproteinated polysaccharide was lyophilized. The lyophilized purple sweet potato polysaccharide (PSPP) was re-dissolved in distilled water, and then purified using a DEAE-52 cellulose column (2.0 cm × 30 cm) which was eluted with distilled water, 0.1, 0.2, 0.3 and 0.4 mol/l NaCl, sequentially, at a flow rate of 1.0 ml/min. The fractions were collected at 3 min intervals with a fraction collector, and measured by the phenol-sulfuric acid method. The major fractions were pooled, desalted, concentrated and further separated using Sephadex G-100 column (1.5 cm × 30 cm) which was eluted with distilled water at a flow rate of 0.5 ml/min. The fractions were collected and monitored under the same conditions as that used for the DEAE-52 cellulose column. Each fraction was collected, lyophilized and stored at −20 °C until use.

2.4. Determination of contents of protein and uronic acid

The protein content of purified polysaccharide was determined according to Bradford method (Bradford, 1976). The uronic acid content of purified polysaccharide was determined according to the carbazole–sulfuric acid method (Bitter & Muir, 1962).

2.5. Determination of polysaccharide composition

Polysaccharide composition of the polysaccharide was analyzed by gas chromatography (GC) as described previously (Zhang et al., 2014a). Fructose, rhamnose, arabinose, xylose, mannose, glucose and galactose were used as the monosaccharide standards. The monosaccharides were acetylated by adding 1-methylimidazole and acetic anhydride after the polysaccharide was degraded with trifluoroacetic acid (TFA). The acetylated monosaccharides were detected on an Agilent 6820 GC, using an Agilent DB-1701 capillary column (30 m × 0.25 mm × 0.25 μm) and nitrogen as a carrier gas. The temperature program was: 130 °C for 20 min, then to 190 °C at 5 °C/min holding for 20 min and then to 230 °C at 5 °C/min holding for 10 min.

2.6. Molecular weight determination

The molecular weight of the polysaccharide was determined by gel filtration on a TSK-GEL G3000PWxl column (7.5 × 300 mm, Tosoh Co., Japan) coupled to a high performance liquid chromatography system. The column was eluted with 0.01 mol/l NaCl solution at a flow rate of 0.5 ml/min. The fractions were collected at 4 min intervals with a fraction collector. The phenol-sulphuric acid method was used to determine the elution profiles of the polysaccharide fractions. Dextrans T-3, T-10, T-40, T-70 and T-500 were used as the standards for molecular weight measurement.

2.7. Fourier transformation infrared (FT-IR) spectroscopy analysis

The FT-IR spectrum of polysaccharide was recorded with a FT-IR spectrometer (Tensor 27, Bruker Co., Germany) using KBr pellet method. The dried polysaccharide was grounded with KBr powder and then pressed into 1 mm pellets for FT-IR measurement from 4000 to 400 cm^{-1}.

2.8. Scanning electron microscopy (SEM)

The sample was lyophilized and then the dried polysaccharide granules were mounted on the copper sample-holder, using a double sided carbon tape and coated with gold of 5–10 nm thicknesses to make the polysaccharide granules conductive. SEM studies were carried out using a scanning electron microscope (S4800-II, Hitachi High-Technologies Co., Japan) at acceleration voltage of 15 kV.

2.9. In vitro *antioxidant activity test*

2.9.1. DPPH radical-scavenging assay

DPPH radical scavenging activity was determined as the procedure described by Cumby, Zhong, Naczk, and Shahidi (2008) with some modifications. Two milliliter of polysaccharide samples at different concentrations (0–600 μg/ml) was added to 2 ml of DPPH (0.1 mmol/l in 95% ethanol). The mixture was shaken vigorously and then incubated at room temperature for 30 min in the dark. The absorbance was recorded at 517 nm using ascorbic acid as a positive

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