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Preparation, characterization and antioxidant activity of polysaccharide from *Fallopia multiflora* (Thunb.) Harald

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ABSTRACT

This study aimed to investigate antioxidant activities of a polysaccharide from *Fallopia multiflora* (Thunb.) Harald. *F. multiflora* polysaccharide (FMP) was prepared and partially characterized by high performance liquid chromatography, fourier transform infrared spectrum and anion chromatography, and its antioxidant activities both in vitro and in vivo were investigated. FMP, with a molecular weight of 17,758 Da consisted of glucose. In vivo, FMP had high hydroxyl radical scavenging activity and reducing capacity. In vivo, FMP increased serum superoxide dismutase and glutathione peroxidise activities and decreased the level of malondialdehyde. These results indicated that FMP exhibits high antioxidant activity both in vitro and in vivo and might retard human ageing associated with free radicals.

1. Introduction

Fallopia multiflora (Thunb.) Harald. belongs to the family *Polygonum* and is a perennial winding vine plant with root mast and winding stem. *F. multiflora* has been used in traditional Chinese medicine for thousands of years. The root tuber of *F. multiflora* has been used to treat premature greying of the hair, dizziness with tinnitus, weakness and soreness on the knee, limb numbness and lower back [1–3].

The extracts of the root of *F. multiflora* have potent antioxidant, cytoprotective [4], enhanced purgative activities, promoting diuresis and choleretic effects [5], and show a neuroprotective effect against glutamate-induced neurotoxicity [6]. So far, the bioactive components of this herb have been proven to be phospholipids, anthraquinones, and bianthraquinonyl glucosides [7]. However, data regarding the polysaccharide from *F. multiflora* (FMP) are not frequently reported [8].

In this study, FMP was extracted by using hot water extraction methods and the antioxidant activities of FMP were investigated.

2. Materials and methods

2.1. Ethics statement

This study was approved by the ethics committee of Huaihai Institute of Technology, China (ECHHIT20160066). All procedures were performed in compliance with relevant laws and institutional guidelines.

2.2. Materials

Dried *F. multiflora* roots were purchased from Shandong Dingli Rubber Industry Co., Ltd. (Shandong, China). Standard monosaccharides, i.e. glucose, fucose and galactose were obtained from Sigma Chemical Co. (St. Louis, MO, USA). All other reagents were of analytical grade.

2.3. FMP preparation

The dried *F. multiflora* roots was sliced, pulverised and sifted through a 100-mesh sieve to yield fine powder, which was suspended in distilled water under agitation at ~25 °C for 15 min to give a suspension with a concentration of ~2% (w/v). The suspension was incubated in a water bath at 85 °C for 6 h under agitation and then centrifuged at 5000 × *g* for 10 min. The resulting supernatant was protein separated by using Sevag method, precipitated

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with three volumes of absolute ethanol, filtered through a Whatman GF/A filter paper and then freeze dried.

2.4. FMP characterisation

Proximate components, i.e. total sugar, protein and ash, of the products were assayed using phenol–sulphuric acid colorimetric method, Kjeldahl method and method described by the Association of Official Analytical Chemists procedures [9], respectively. The Fourier transform infrared (FTIR) spectra of the representative product samples were obtained from KBr pellets by using a Nicolet Nexus FTIR 470 spectrophotometer over a wavelength range of 400–4000 cm^{-1}. UV spectra were recorded on a UV spectrometer (Spectra Test, Germany). The molecular weights (MWs) of the FMP were determined through high-performance gel filtration chromatography (HPLC) (LC-10A, Shimadzu, Japan) by using an ultrahydrogel size exclusion column (LKB-Prodokter, AB, Bromma, Switzerland) and high-sensitive refractive index detector (Model ERC-7515 A, ERC Inc., Japan). The FMP was eluted with 0.1 N $NaNO_3$ at a flow rate of 0.9 mL/min. Standard pullulan samples (P20–P800, JM Science, Inc., NY, USA) were used as MW standard. The FMP was hydrolysed as per the methods described by Sheng et al. [10]. The monosaccharide compositions of the FMP were assayed by ion chromatography (IC) (ICS-5000, Dionex, USA) using a carbohydrate column (CarboPac PA20, Dionex, USA) and pulse ampere detector (Dionex, USA). The FMP monosaccharides were eluted with mobile phase at a flow rate of 0.5 mL/min. The composition and conditions of the mobile phase are as follows: 0–21.1 min (97.4% of water and 2.6% of 250 mM NaOH); 21.1–30 min (92.4% of water, 2.6% of 250 mM NaOH and 5.0% of NaAc); and 30–50 min (20% of water and 80% of 250 mM NaOH).

2.5. Antioxidant activities in vitro

The reducing capacity of FMP was determined according to the methods of Qiao et al. [11]. FMP (1 g) was mixed with 1.0 mL of 0.2 M phosphate buffer (pH 6.6) and 1.0 mL of 1% (w/v) potassium ferricyanide and then incubated at 50 °C for 20 min before it was cooled at room temperature. Briefly, 1 mL of trichloroacetic acid (10%, w/v) and 0.2 mL of fresh ferric trichloride (0.1%, w/v) were added to the reaction mixture. The resulting mixture was shaken, and its absorbance was assayed at 700 nm against a blank sample (water instead of FMP) after 10 min. The absorbance of the reaction mixture indicates the reducing capacity of the sample.

$$\text{Reducing capacity} = (A_1 - A_2), \quad (1)$$

where A_1 is the absorbance of the sample, and A_2 is the absorbance of the sample under conditions similar to those of A_1 except that water was used instead of ferric trichloride solution.

The hydroxyl radical scavenging activity (HRSA) of the FMP was determined according to previously described methods [12]. The HRSA of FMP was calculated as follows:

$$\text{HRSA}\ (\%) = \frac{A_1 - A_2}{A_1 - A_0} \times 100 \quad (2)$$

where A_0 is the absorbance of the reagent blank, A_1 is the absorbance of the positive control and A_2 is the absorbance of the sample.

2.6. Antioxidant activities in vivo

A total of 60 Kunming mice were purchased from Tumor Hospital Experimental Animal Center of Nanjing Medical University. The Kunming mice (30 males and 30 females) were 8 weeks old and had an average weight of 19–21 g. The mice were maintained under the following environmental conditions: temperature of 25 °C, humidity of 50% and light conditions of 12:12 h light:dark cycle for 1 week before the experimental protocol. During the experimental period, the mice were provided with rodent laboratory chow and tap water ad libitum.

The mice were randomly divided into five groups. In particular, 12 mice were included in the normal control group (NCG), 12 mice in the $_D$-galactose ($_D$-gal) model control group (MCG), and 12 mice each in the 1, 2 and 3 g/kg FMP – treated groups. For the establishment of the ageing model mice, $_D$-gal saline (0.9%) solution with a dose of 100 mg/kg body weight was injected into the back of each mouse once a day for 6 weeks. For the FMP – treated groups, the mice were intragastrically administrated with FMP with doses of 1, 2 or 3 g/kg BW/day. For the NCG, the mice received subcutaneous injection of sterile saline instead of the same volume of $_D$-gal solution. For the MCG group, the mice received normal diet without FMP.

One day after the last drug administration, the mice were sacrificed through cervical dislocation. Blood samples from the retrobulbar venous plexus of the sacrificed mice were centrifuged at 10,000*g* and 4 °C for 15 min to yield blood sera. The sera were stored at −80 °C for further assay. The brains were removed immediately and mixed with 0.9% ice-cold NaCl solution to yield 10% homogenates, which were centrifuged at 4000 × *g* and 4 °C for 15 min. The resulting supernatants were kept at −80 °C for biochemical analysis.

The activities of superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) and level of malondialdehyde (MDA) in the supernatants of the tissue homogenates and sera were determined using commercially available kits. The assays were performed at 4 °C and according to the instructions provided by the manufacturers of the kits.

2.7. Statistical analysis

Data are presented as mean ± SD, and Student–Newman–Kuels multiple range test was performed to compare the means of the two groups. Statistical significance at the 95% probability level was set at $p < 0.05$.

3. Results and discussion

3.1. Product characterisation

The FMP products were water-soluble powders and had a chemical composition of 2.31% moisture, 1.67% protein and 95.46% total sugar. Analysis of the elution curve of the FMP products on the DEAE Sepharose Fast Flow ion exchange chromatography column showed only a fraction in the FMP (Fig. 1). HPLC spectrum analysis of the FMP product indicated that it contained only one kind of polysaccharide, with a molecular weight of 17,758 Da (Fig. 2). Fig. 3 shows that the absorption bands of FMP peak at 3428 cm^{-1} (hydroxyl stretching vibration), 2924 cm^{-1} (C—H stretching vibration), 1636 cm^{-1} (C=O vibration) and 1157–1019 cm^{-1} (pyranose ring). Monosaccharide composition analysis with the IC spectrum showed that the FMP only consisted of glucose (Fig. 4).

3.2. Antioxidant activity of FMP in vitro

Hydroxyl radical ($HO^{\bullet}$) has the highest activity among free radicals and can attack biological molecules, such as lipids, proteins, enzymes, DNA and RNA, leading to cell or tissue injury [11,13]. Furthermore, $HO^{\bullet}$ is widely used to evaluate the scavenging activity of some antioxidants [14]. The HRSA of the FMP are shown in Fig. 5a. The scavenging activities of FMP increased dose-dependently. At 200 mg/mL concentration, the HRSA of the FMP