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Effects of extraction methods on the yield, chemical structure and anti-tumor activity of polysaccharides from *Cordyceps gunnii* mycelia

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Chloroform (PubChem CID: 6212)
Cellulase (PubChem CID: 3084041)
Dimethylsulfoxide (PubChem CID: 21912085)
Trifluoroacetic acid (PubChem CID: 6422)
Pyridine (PubChem CID: 1049)
Methanol (PubChem CID: 887)
Acetic acid (PubChem CID: 176)
Ethanol (PubChem CID: 702)
Acetic anhydride (PubChem CID: 7918)

ABSTRACT

This study was to investigate the effects of different extraction methods on the yield, chemical structure and antitumor activity of polysaccharides from *Cordyceps gunnii* (*C. gunnii*) mycelia. Five extraction methods were used to extract crude polysaccharides (CPS), which include room-temperature water extraction (RWE), hot-water extraction (HWE), microwave-assisted extraction (MAE), ultrasound-assisted extraction (UAE) and cellulase-assisted extraction (CAE). Then Sephadex G-100 was used for purification of CPS. As a result, the antitumor activities of CPS and PPS on S180 cells were evaluated. Five CPS and purified polysaccharides (PPS) were obtained. The yield of CPS by microwave-assisted extraction (CPS_{MAE}) was the highest and its anti-tumor activity was the best and its macromolecular polysaccharide (3000–1000 kDa) ratio was the largest. The PPS had the same monosaccharide composition, but their obvious difference was in the antitumor activity and the physicochemical characteristics, such as intrinsic viscosity, specific rotation, scanning electron microscopy and circular dichroism spectra.

1. Introduction

Cordyceps, one of the famous traditional Chinese medicines, has been used as health food for a long time in China. Recently, due to its anti-inflammatory activity, anti-tumor activity, anti-aging effect and immunomodulatory effects, *Cordyceps* has attracted much attention (Wang et al., 2011). *Cordyceps gunnii (berk.) Berk (C. Gunnii)*, one particular *Cordyceps* species, is also well known as the Chinese rare caterpillar fungus and has similar pharmacological activity with *C. sinensis*. The anamorph of *Paecilomyces gunnii* has been isolated, verified and identified (Liang, 1985). Many important secondary metabolic products were found in *C. gunnii* mycelia including cordycepin, cordycepic acid, polysaccharides and anti-ultraviolet radiation constituents. Many reports indicated that *C. Gunnii* polysaccharides have strong anti-tumor activities (Xiao et al., 2004; Zhu et al., 2012).

Various novel techniques for extraction of polysaccharides have been developed, including microwave-assisted extraction (MAE) (Wang et al., 2010), enzyme-assisted extraction (EAE) (Yin, You, & Jiang, 2011) and ultrasonic-assisted extraction (UAE) (Yan et al., 2014). Previous studies on the polysaccharide extraction have mainly focused on the yield or the effect of single variable, and have not investigated to obtain a comprehensive evaluation of the yield and activity of polysaccharides, furthermore, the

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relationship between chemical structure and anti-tumor activity of polysaccharides. Wang et al. (2009) had confirmed that degradation occurred in the molecular weight of polysaccharides during the microwave-assisted extraction process, but polysaccharides extracted by microwave-assisted extraction showed higher antioxidant activity compared to the hot water extraction method. Wei et al. compared the effects of different extraction methods on the composition and bioactivity of tea flower polysaccharides, and traditional water extraction was found to be the main and most conventional extraction method for polysaccharides (Guan, Zhang, Yang, Xin, & Liu, 2011). However, it is usually associated with a longer extraction time, more than 2 h, but has lower extraction efficiency with a yield of less than 70.00 mg/g (Xu, Wu, & Chen, 2011). Ultrasonic assisted extraction has been employed widely in the extraction of target compounds from different materials because of its shorter times, simplified manipulation, and lowered energy input and solvent consumption (Khan, Maryline, Fabiano-Tixier, Dangles, & Chemat, 2010), but it has a lower extraction efficiency of polysaccharide. Moreover, some studies have revealed that different extraction methods have effects on the structure and antioxidant activity of polysaccharide (Wang, Sun, Zhang, Chen, & Liu, 2012). Zhang, Lv, He, Shi, Pan, and Fan (2013) reported that the polysaccharides extracted by four different methods showed significantly different SEM images and antioxidant activities. Therefore, each extraction method has its own advantages and disadvantages, and a comprehensive evaluation method of the yield and activity of polysaccharides is a promising new technology.

The objective of this study was to investigate the influences of different extraction methods on the yield, the relationship between chemical structure and anti-tumor activity of polysaccharides from *C. gunnii* mycelia, and to find the potential extraction method, which is the comprehensive evaluation of yield and activity of polysaccharides. First, The dried *C. gunnii* mycelia powder was extracted to gain crude polysaccharides by using RWE, HWE, MAE, UAE and CAE, and CPS was purified by AB-8 resin, Sephadex G-100. Then the different characterizations of five CPS were analyzed by high-performance liquid chromatography (HPLC), and five PPS were analyzed by Fourier transform-infrared spectroscopy (FT-IR), HPLC, Scanning electron microscopy (SEM), intrinsic viscosity circular dichroism. Finally, anti-tumor activities of CPS and PPS *in vitro* were compared respectively.

2. Materials and methods

2.1. Materials

C. gunnii mycelia and the S180 cells used in this study were obtained from the Key Laboratory of Food Nutrition and Safety, Ministry of Education, College of Food Science and Biotechnology, Tianjin University of Science and Technology, Tianjin, China. The macroporous resin AB-8 and Sephadex G-100 and the standard monosaccharides (D-glucose, D-xylose, D-galactose, L-rhamnose, D-mannose, and D-arabinose) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Cellulase (R10) and dextran standards of different molecular weights were purchased from Pharmacia Biotech (Uppsala, Sweden). 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was purchased from Sigma-Aldrich, Inc. (St. Louis, MO, USA). Dimethylsulfoxide (DMSO), trifluoroacetic acid (TFA), and Congo red were purchased from Tianjin Kemiou Chemical Reagent Company (Tianjin, China). *N*-butyl alcohol, chloroform, pyridine, methanol, and acetic acid, ethanol, acetic anhydride and all other chemicals and reagents were of grade (AR) and obtained from Beijing Qison Biotechnology Co., Ltd. (Beijing, China).

2.2. Extraction and fractionation of CPS by various methods

2.2.1. Extraction and fractionation with room -temperature water extraction

The dried *C. gunnii* powder (10 g) was mixed with distilled water (1:20 ratio of raw material to water, w/v) in a round-bottom flask (500 mL). The flask was placed into a water-bath and connected with cooling water at 25 °C for 3 h. Then, the suspension was centrifuged with a centrifugal machine (Sorvall ST16R, Thermo, USA) and the insoluble residue was handled again twice as mentioned above. The above supernatant was combined and concentrated to 100 mL using a rotary evaporator (Sy-2000, Shanghai Ya Rong biochemical instrument factory, China) at 60 °C. The protein was removed by following the Sevag method (Staub, 1965). When materials balanced, removing the protein compounds and the upper polysaccharide mixture was precipitated by using 4 volumes of ethanol at 4 °C for 12 h. The CPS was obtained by centrifugation at 5500 r/min for 15 min, and the precipitate fraction was collected, washed with dehydrated alcohol 3 times and lyophilized to yield the CPS (named CPS_{RWE}). The polysaccharide content of CPS_{RWE} was measured by the phenol–sulfuric acid method using glucose as a standard substance to obtain the polysaccharide yield (Zhang, Li, Xiong, Jiang, & Lai, 2013) in a table. Then the samples were freeze-dried (SA-15, Shenzhen Yujia Electrical Instrument Co., China) and stored at −20 °C until needed.

2.2.2. Extraction and fractionation with hot-water extraction

The dried *C. gunnii* powder (10 g) was mixed with distilled water (1:20, w/v) in a round-bottom flask (500 mL). The flask was placed into a water-bath and connected with cooling water, and then extracted at 78 °C for 3 h. Then, the suspension was centrifuged and the insoluble residue was handled again twice as mentioned above. The extracted solution was cooled to room temperature and the following procedures were consulted for RWE, and the crude polysaccharide was named CPS_{HWE}.

2.2.3. Extraction and fractionation with microwave-assisted water extraction

The dried *C. gunnii* powder (10 g) was mixed with distilled water (1:20, w/v) in a round-bottom flask (500 mL). The flask was placed into the microwave-assisted extraction equipment (Galanz WG800CSL26-K6, made by Guangdong Galanz Group Co., China) for 5 min with microwave power setting at 280 W, while the temperature was kept steady at 70 °C during the process (Chen, Shao, Tao, & Wen, 2015). Then, the pretreated sample solution was cooled to room temperature and the following extraction procedures were consulted for RWE, and the crude polysaccharide was named CPS_{MAE}.

2.2.4. Extraction and fractionation with ultrasound-assisted water extraction

The dried *C. gunnii* powder (10 g) was extracted three times with distilled water (1:20, w/v) in an Erlenmeyer flask (500 mL). The flask was placed into an ultrasound-assisted extraction machine (DL-70, made in Shanghai Dalong machine factory, China) for 13 min, with ultrasound power setting at 250 W, then, the flask was partially immersed into the ultrasonic bath, which contained 2 L water, the water in the ultrasonic bath was circulated and regulated at 45 °C (Xu, Wang, Liang, Zhang, & Li, 2015). The pretreated sample solution was cooled to room temperature and the following procedures were consulted for RWE, and the crude polysaccharide was named CPS_{UAE}.