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Characterization and antioxidative activities of polysaccharide in Chinese angelica and its processed products

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ABSTRACT

Five polysaccharides from unprocessed Chinese angelica (UCAP), parched one with alcohol (ACAP), soil (SCAP), sesame oil (OCAP) and parched into charred (CCAP) were extracted and purified. Their structures were identified by Fourier transform-infrared spectroscopy (FT-IR), compositions were analyzed by gas chromatography–mass spectrometry (GC–MS) and antioxidative activities were compared by determining MDA contents and SOD activities of liver tissue in mice damaged with CCl_4 after gavage. The results showed that the FT-IR spectra of CCAP and OCAP displayed lower transmittance at around 1050 cm^{-1} in comparison with that of UCAP. Five polysaccharides were all composed of rhamnose, arabinose, mannose, glucose and galactose. In CCAP, ACAP, OCAP and SCAP, the proportions of arabinose were significantly increased in comparison with that of UCAP. The SOD activities in CCAP and SCAP groups were significantly enhanced, and MDA contents in CCAP, OCAP and SCAP groups were significantly decreased as compared with UCAP group. This indicated that processing could change the structure, composition and enhance antioxidative activity of polysaccharide in Chinese angelica, and CCAP possessed the strongest antioxidative activity.

1. Introduction

Chinese angelica (CA), a kind of Chinese traditional herbal medicine (CTHM), is mainly planted in the northwest of China [1,2]. The root is the main medicinal part [3]. It is used to treat female irregular menstruation [4], anemia, gastrointestinal disease, cardiovascular disease, chronic bronchitis, asthma, rheumatism, and so on [5].

In clinical use, CTHM is always processed before being applied because processing can increase its therapeutic effect and reduce its side effects [6]. Moreover, some kinds of CTHM can be most effective if processed by a certain method, such as frying with some specific supplementary material. At present, processed products of CA that are widely prescribed in CTHM mainly include Chinese angelica parched with alcohol (ACA), Chinese angelica parched with soil (SCA), Chinese angelica parched with sesame oil (OCA), and charred Chinese angelica (CCA). Moreover, the functions of CA and its processed products can be different [7]. For example, its function on scavenging free radicals and resisting lipid peroxidation can be enhanced after processed [8]. The reason might be that chemical components in CA were altered after being processed. It was reported that over 70 kinds of compounds had been identified in CA, such as polysaccharides, essential oils, organic acids and esters [9,10]. Among them, polysaccharide, a very important biological macromolecule, is proven to have antioxidative activities [11–13] and hepatoprotective effects [14]. After CA was processed, its polysaccharide content became different [15]. However, studies about whether processing could alter the structure, monosaccharide composition, and antioxidative activities of the polysaccharides remain elusive [16].

In the present study, UCAP, ACAP, OCAP, SCAP and CCAP were extracted from UCA, ACA, OCA, SCA and CCA, respectively, and then purified. In addition, their carbohydrate contents were measured using the phenol-sulfuric acid method. Some carbohydrate

Abbreviations: CA, Chinese angelica; UCA, unprocessed Chinese angelica; ACA, Chinese angelica parched with alcohol; SCA, Chinese angelica parched with soil; OCA, Chinese angelica parched with sesame oil; CCA, charred Chinese angelica; CAP, Chinese angelica polysaccharides; UCAP, polysaccharides in UCA; ACAP, polysaccharides in ACA; OCAP, polysaccharides in OCA; SCAP, polysaccharides in SCA; CCAP, polysaccharides in CCA; FT-IR, Fourier transform-infrared spectroscopy; GC–MS, gas chromatography–mass spectrometry; CCl4, carbon tetrachloride; MDA, malonaldehyde; SOD, superoxide dismutase; CTHM, Chinese traditional herbal medicine; SM, standard mixture; ROS, reactive oxygen species.

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characteristic absorption peaks were detected in FT-IR. Monosaccharide composition was analyzed using GC–MS. The antioxidative activities of all kinds of CAP were investigated by comparing MDA contents and SOD activities in liver tissue of mice for all the groups. The pathological changes in liver tissue of mice were used to verify their antioxidative activities. The objectives of this study were to characterize and compare the polysaccharides in CA and its four processed products, to investigate the effects of processing on antioxidative activities of CAP and to determine which has the strongest antioxidative activities, and to offer theoretical evidence for the study of a new-type of antioxidant.

2. Materials and methods

2.1. Materials and chemicals

CA was purchased from Min County, Gansu Province, China and authenticated by Dr. Yanming Wei (School of Veterinary Medicine, Gansu Agricultural University, Lanzhou, China). Glucose, arabinose, galactose, xylose, mannose, rhamnose and CCl_4 were purchased from Sigma Chemical Co. (St. Louis, USA). Assay kits for MDA and SOD were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). All reagents used were analytical grade or chromatographic grade.

2.2. Acquisition of Chinese angelica and its four processed products

UCA was produced after the original CA was decontaminated, cleaned, humidified, cut into slice and dried. ACA was produced after the original CA was cut into slice, mixed thoroughly in alcohol, braised, fried until yellow and then cooled. The amount of alcohol used was 0.1 kg (200 mL/L) for every 1 kg CA. OCA was produced after the original CA was sliced, mixed thoroughly with 3% sesame oil, braised, fried into dark yellow using soft fire, and then cooled. SCA was produced after the original CA was cooked with sizzling soil by soft fire and then cooled. The amount of soil used was 0.3 kg for every 1 kg CA. CCA was produced after the original CA was sliced, cooked in medium heat until it was coke black and its internal portion was brown, and then cooled when it had charred taste and was crisp.

2.3. Extraction and purification of UCAP, ACAP, OCAP, SCAP and CCAP

Five kinds of crude polysaccharides were extracted from UCA, ACA, OCA, SCA and CCA according to the following optimized procedure: 100 g of every CA sample was prepared, and 837.6 mL water was put in; 229.12 mL solution was obtained after concentrated in a rotary evaporator under reduced pressure; according to the formula:

Alcohol density (%) = $V_{\text{anhydrous alcohol}}/(V_{\text{anhydrous alcohol}} + V_{\text{solution}})$, the alcohol density was 65.80%, and the reflux extraction time was set at 120 min. Protein was then removed using papain and Sevage reagent [chloroform: n-butanol, 4:1 (v/v)], while pigments were removed using hydrogen peroxide. In order to remove the impurities with MW <7000 Da in crude polysaccharide, it was dissolved in distilled water and poured into a dialysis bag (molecular weight cut-off 7000 Da) for dialysis to proceed for 24 h. The resulting dialyzed solution was concentrated and then lyophilized to obtain crude polysaccharides. All crude polysaccharides were dissolved in distilled water and purified using a Sephadex G-100 (Pharmacia, USA) chromatographic column (65 cm × 3 cm). In the end, UCAP, ACAP, OCAP, SCAP and CCAP were obtained.

2.4. Structural characterization of UCAP, ACAP, OCAP, SCAP and CCAP

2.4.1. Carbohydrate content determination

Carbohydrate contents of UCAP, ACAP, OCAP, SCAP and CCAP were measured by the phenol-sulfuric acid method.

2.4.2. FT-IR spectra

The FT-IR spectra of five kinds of homogeneous polysaccharides in a wavenumber range of 4000–500 cm^{-1} were obtained using a NEXUS 670 Fourier-transform infrared spectrometer (Nicolet, USA) through KBr pellet method.

2.4.3. GC–MS analysis

2.4.3.1. Chromatography. Standard mixture (SM) and the extracted UCAP, ACAP, OCAP, SCAP, and CCAP were analyzed using GC–MS. Chromatographic analysis was performed in an Agilent 6890N/5973N series GC–MS (Agilent Corporation, USA) equipped with an OV-1701 capillary column (30 m × 0.15 μm × 15 mm).

2.4.3.2. Analytical procedure. The initial temperature (85 °C) was held constant for 3 min and then raised to 280 °C at a rate of 10 °C/min. All samples were injected in split mode. The injection temperature was at 270 °C. The mass spectrometer was operated in EI mode (positive ion, 70 eV), and the quadrupole was set at 150 °C. Mass spectra were acquired in full scan mode with repetitive scanning from 60 *m*/*z* to 600 *m*/*z* in 1 s. Ion source temperature was at 230 °C.

2.4.3.3. Sample preparation. Twenty (20) mg of UCAP, ACAP, OCAP, SCAP, and CCAP were weighed and placed into ampoule bottles. They were then hydrolyzed using 2 mL TFA (2 mol/L). Methyl alcohol was added, and the resulting mixture dried in drying oven. And 2 mL water was added before application [17]. Standard Mixture (SM) sample was prepared using equal amounts of 6 monosaccharides, namely, glucose, arabinose, galactose, rhamnose, xylose, and mannose. 20 mg of SM was weighed and placed into an ampoule bottle. All the samples were derivatized as follows: 10 mg of hydroxylamine hydrochloride and 0.5 ml of pyridine were added to the samples; the resulting mixture was then reacted at 85 °C for 40 min; after cooling, 0.5 mL acetic anhydride derivatization reagent was added. The sample solution was then filtered and transferred into a microvial for GC–MS analysis.

2.5. Antioxidative activities research

2.5.1. Animal grouping and experimental design

Forty nine (49) Kunming male mice (SCXKZ(Gan)2009-0004) (6 weeks old and weighing 18.0–22.0 g) were purchased from Experimental Animal core, School of Medical Science, Lanzhou University (China), and maintained under controlled conditions of 22 ± 0.5 °C, 50 ± 2.0% RH, and under a normal day/night cycle. The mice were also allowed free access to basal pellet diet and tap water. 49 mice were randomly divided into seven groups (n = 7 per group): normal control group, model group, SCAP treatment group, OCAP treatment group, ACAP treatment group, CCAP treatment group and UCAP treatment group. During the first two days of the experiment, all mice of the CAP treatment group were given different CAP solutions by gavage (120 mg/kg, one time/day, dosage of 0.3 mL for each mouse). The mice of other groups were given equal amount deionized water. On the third day, the mice of other groups, except for normal control group, were given 0.2 mL/10 g soybean oil with 0.1% CCl_4 through intraperitoneal injection. The mice of normal control group were given the equal amount soybean oil through intraperitoneal injection.