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# Antioxidant activities of different fractions of polysaccharide purified from *Gynostemma pentaphyllum Makino*

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**Abstract**

The water-soluble crude polysaccharide GM, obtained from the *Gynostemma pentaphyllum Makino* by boiling-water extraction and ethanol precipitation, was fractionated by DEAE–Sepharose CL-6B column chromatography, and purified by Sephadex G-100 column chromatography, giving three polysaccharide fractions termed GMA, GMB and GMC. The monosaccharide components of them were studied by PC and GC. On the basis of superoxide radical assay, hydroxyl radical assay and self-oxidation of 1,2,3-phentriol assay, the antioxidant activities of GM, GMA, GMB and GMC were investigated. Among these contents, GMC had the higher scavenging effects on superoxide radicals and inhibitory effects on self-oxidation of 1,2,3-phentriol, and so should be explored as a novel potential antioxidant.


*Keywords:* *Gynostemma pentaphyllum Makino*; Polysaccharide; Purification; Antioxidant activity

## 1. Introduction

Oxidation is essential to many organisms for the production of energy to fuel biological processes. However, the uncontrolled production of oxygen derived free radicals is involved in onset of many diseases such as cancer, rheumatoid arthritis, and atherosclerosis as well as in degenerative processes associated with aging (Mau, Lin, & Song, 2002). In order to reduce damage to the human body, synthetic antioxidants are used for industrial processing at the present time. However, the most commonly have been suspected of being responsible for liver damage and carcinogenesis (Grice, 1988; Qi et al., 2005). Thus, it is essential to develop and utilize effective and natural antioxidants so that they can protect the human body from free radicals and retard the progress of many chronic diseases (Kinsella, Frankel, German, & Kanner, 1993; Nandita & Rajini, 2004). Published data indicates that plant polysaccharides in general have strong antioxidant activities and can be explored as novel potential antioxidants (Hu, Xu, & Hu, 2003; Jiang, Jiang, Wang, & Hu, 2005; Ramarahnam, Osawa, Ochi, & Kawaishi, 1995). The extraction and characterization of active compounds from medicinal plants and the search for these new pharmacologically active agents has led to the discovery of many clinically useful drugs that play a major role in the treatment of human disease (Colegate & Molyneux, 1993; Donehower & Rowinsky, 1993).

*Gynostemma pentaphyllum Makino* is a well known edible and medicinal plant in oriental countries (Hu, Chen, & Xie, 1996). Recently, *Gynostemma pentaphyllum Makino* has attracted great attention owing to its anti-tumor activities (Zhou, Liang, & Hu, 2001), anti-gastric ulcer effect (Rujjanawate, Kanjanapothi, & Amornlerdpison, 2004), immunomodulatory effect (Qian, Wang, & Tang, 1998), and treating hyperlipidaemia (Birgitte, Per, & Zhao, 1995). The cultures of *Gynostemma pentaphyllum Makino* or their extracts processed in health care have been put into production on a large scale. To date, no investigation has been

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carried out on polysaccharides that may account for the textural properties and antioxidant activities of *Gynostemma pentaphyllum Makino*. Identification of the polysaccharides is necessary to better effectively exploit the structure and functional properties of these substances.

In this study, we report on the extraction and purification of the major polysaccharides of *Gynostemma pentaphyllum Makino* using a DEAE–Sepharose CL-6B column chromatography and a Sephadex G-100 column chromatography. In addition, the properties and antioxidant activities of these major polysaccharides are also identified.

## 2. Materials and methods

### 2.1. Materials and chemicals

Dried *Gynostemma pentaphyllum Makino* was purchased from a local store (Quanzhou, Fujian Province, China).

Nitro blue tetrazolium (NBT), phenazine methosulfate (PMS), dihydronicotineamidadenine dinucleotide (NADH), thiobarbituaric acid (TBA), deoxyribose, L-rhamnose, D-glucuronic, D-arabinose, D-xylose, D-fructose, D-galactose and D-mannose were purchased from Sigma Chemical Co. (St. Louis, MO, USA), while DEAE–Sepharose CL-6B and Sephadex G-100 were from the Pharmacia Co. (Sweden). All other reagents used were of analytical grade.

### 2.2. Isolation and purification of polysaccharides

The *Gynostemma pentaphyllum Makino* (250 g) was extracted with 95% ethanol at 50 °C for 6 h, dried, and then extracted with distilled water at 95 °C for 1.5 h twice. After each extraction, the soluble polymers were separated from residues by filtration, and extracts were combined, concentrated and dialyzed against running water for 48 h. The above extract was submitted to graded precipitation with four volumes of ethanol and the mixture was kept overnight at 4 °C to precipitate the polysaccharides. The precipitate was collected by centrifugation, washed successively with ethanol and ether, and dried at reduced pressure, giving GM as a crude polysaccharide.

Size-exclusion and anion-exchange chromatography were used for the fractionation of this preparation. The sample GM (800 mg) was dissolved in 10 mL distilled water, centrifuged, and then the supernatant was injected to a column (4.6 × 30 cm) of DEAE–Sepharose CL-6B equilibrated with distilled water. After loading with sample, the column was eluted with distilled water for 500 mL at 4 mL/6 min/tube, followed stepwise by NaCl aqueous solution (0 and 2 M) for 400 mL, respectively, at 8 mL/12 min/tube. The major polysaccharide fractions were collected with a fraction collector, dialyzed against tap water and distilled water for 48 h, respectively, and then purified by gel-filtration chromatography on a column of Sephadex G-100 (2.6 × 70 cm).

### 2.3. Monosaccharide composition and properties

Total carbohydrate and protein of these polysaccharides were determined by the phenol–sulfuric acid (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956) and Bradford (Bradford, 1976), respectively. Paper chromatography (PC, Wang, Luo, & Liang, 2004) and gas chromatography (GC) were used for identification and quantification. GC was performed on a HP5890 instrument (Hewlett-Packard Component, USA) with a column HP-5 (30 m × 0.32 mm × 0.25 μm). First, the polysaccharide (5 mg) was hydrolyzed with 1 mL of HCl–methanol at 80 °C for 20 h, then hydrolyzed products were neutralized to pH 6.0 by KOH–methanol and dried at reduced pressure. Derivation was then carried out using the trimethylsilylation reagent according to the method of Guentas et al. (2001) with some modifications. Above dried product was dissolved with 0.2 mL pyridine of at 75 °C for 30 min, added 0.2 mL hexamethyl disilazane and 0.1 mL trimethychlorosilane, and mixed rapidly. The derivatives were loaded onto a HP 5 capillary gas chromatography (GC) column equipped with flame-ionization detector (FID), using mannitol as the internal standard. The operation was performed using the following conditions: $H_2$: 20 mL/min; air: 200 mL/min; $N_2$: 20 mL/min; injection temperature: 250 °C; detector temperature: 250 °C; column temperature programmed from 160 to 180 °C at 20 °C/min, then increasing to 220 °C at 8 °C/min and holding for 1 min at 220 °C (Yang et al., 2006).

The IR spectrum of the polysaccharide was determined using a Fourier transform infrared spectrophotometer (FTIR, Bruker, Germany) equipped. The purified polysaccharide was ground with KBr powder and then pressed into pellets for FTIR measurement in the frequency range of 4000–500 $cm^{-1}$ (Kumar, Joo, Choi, Koo, & Chang, 2004). The molecular weight was calculated by the calibration curve obtained by using various standard dextrans (Wang, Liang, & Zhang, 2001).

### 2.4. Determination of the polysaccharides purification

The sample were dissolved in 0.9% sodium chloride, centrifuged, and then the filtrate was applied to a Sephadex G-100 column (1.6 × 80 cm), which was eluted with 0.9% sodium chloride at 2.2 mL/12 min/tube. Polysaccharides were detected by the phenol–sulfuric acid method (Dubois et al., 1956). Elution curve was drawn by tube number as abscissa and absorbance as vertical coordinate. In addition, the polysaccharides purification was also identified by cellulose acetate pellicle electrophoresis (borax–sodium hydroxide buffer, pH 10) at 40 V for 50 min with detection using Toluidine Blue.

### 2.5. Assay for antioxidant activities

#### 2.5.1. Superoxide radical assay

The superoxide radical assay was measured by the method of Robak and Gryglewski (1988) with a minor