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Characterization and comparison of bioactive polysaccharides from the tubers of *Gymnadenia conopsea*

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ABSTRACT

Water soluble polysaccharides from seven batches of *Gymnadenia conopsea* were firstly investigated and compared using high performance size exclusion chromatography coupled with multi-angle laser light scattering/refractive index detector (HPSEC-MALLS/RID) and saccharide mapping based on polysaccharide analysis by carbohydrate gel electrophoresis (PACE), respectively. The results showed that the weight-average molecular weight (M_w) and the radius of gyration ($<S^2>_z^{1/2}$) of polysaccharides were ranging from 4.46×10^5 to 7.41×10^5 Da and 73.3–94.2 nm, respectively. By applying the polymer solution theory, the exponent (ν) values of $<S^2>_z^{1/2} = kM_w^{\nu}$ were ranging from 0.36 to 0.42, which indicated that polysaccharides from *G. conopsea* existed as globular in the aqueous solution. Furthermore, the results showed that α-1,4- and β-1,3(4)-glucosidic, α-1,5-arabinosidic, β-1,4-mannosidic and α-1,4-D-galactosiduronic linkages existed in polysaccharides from *G. conopsea*. The similarity of the hydrolysates of polysaccharides in *G. conopsea* collected from different regions was high. Moreover, the nitric oxide released from RAW 264.7 cells induced by polysaccharides were significantly affected by their α-1,5-arabinosidic and β-1,3(4)-glucosidic, especially α-1,4-D-galactosiduronic and β-1,4-mannosidic linkages. These results are beneficial for better understanding of the structures–bioactivity relationship of polysaccharides from *G. conopsea*, and helpful to improve their pharmacological activity-based quality control.

1. Introduction

Polysaccharides exist as one of the mainly bioactive compounds in the medicinal plants, which have attracted a great deal of attention in the biomedical and functional foods area (Li, Wu, Lv, & Zhao, 2013). Polysaccharides, especially water-soluble, from medicinal plants are most important for their significant pharmaceutical activities, such as anti-cancer (Yang & Zhang, 2009; Zong, Cao, & Wang, 2012), anti-oxidant (Jin, Zhao, Huang, & Shang, 2014; Sun, 2011), immunomodulatory (Chlubnova et al., 2011; Sun, 2011), and anti-diabetic activities (Jin et al., 2014; Zhang & Jiang, 2012). Furthermore, the bioactivities of polysaccharides are closely correlated with their physico-chemical properties such as molecular size, features of glycosidic linkages and chain conformation (Li et al., 2013). Therefore, the bioactive polysaccharides analysis is very important for pharmacological activity-based quality control of medicinal plants. Additionally, the discovery and evaluation of novel polysaccharides from the various plants as safe compounds for medicine and functional foods has become a hot research spot.

Gymnadenia conopsea R. Br. is a plant of the Orchidaceae family (Fig. 1), which is widely distributed in China (Zi et al., 2008). The tubers of this plant have long been used as traditional medicines for the treatment of asthma, neurasthenia and chronic hepatitis (Lang, Chen, & Zhu, 1999), and also used as tonic foods especially in Mongolia and Tibet for invigorating vital energy, promoting the production of body fluids and enhancing intelligence (Zi et al., 2008). Small molecules, such as phenanthrenes and stilbenes with anti-allergic activity (Matsuda, Morikawa, Xie, & Yoshikawa, 2004), and 2-isobutylmalate derivatives with neuroprotective effects have been investigated (Zi et al., 2008). Moreover, the

Abbreviations: ANTS, 8-Aminonaphthalene-1,3,6 trisulphonic acid; HPSEC, High performance size exclusion chromatography; PACE, Polysaccharide analysis by carbohydrate gel electrophoresis; RID, Refractive index detector; MALLS, Multi-angle laser light scattering; MTT, 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide; SMC, Simulative mean chromatogram.

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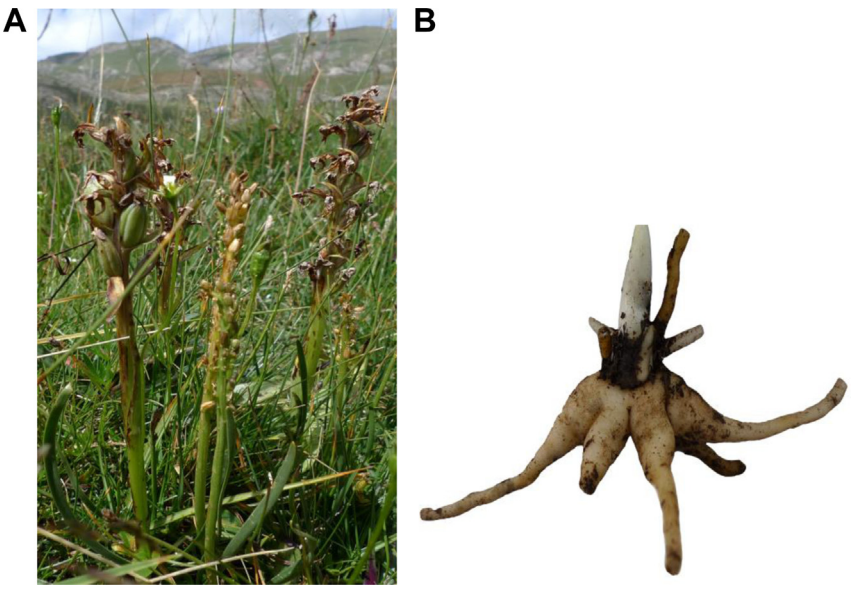

Fig. 1. The original plant (A) and tuber (B) of *G. conopsea*.

chemical characteristics of the methanol (Cai, Zhou, Gesang, Bianba, & Ding, 2006) and ethanol extracts (Li, Guo, Wang, & Xiao, 2009) of *G. conopsea* collected from different regions have also been compared. However, the molecular structures and bioactivities of polysaccharides from *G. conopsea* have seldom been investigated. Therefore, comparison of the molecular structures and bioactivities of polysaccharides from *G. conopsea* is beneficial for better understanding the chemistry and bioactivity of *G. conopsea* and improving their pharmacological activity-based quality control.

High performance size exclusion chromatography coupled with multi-angle laser light scattering (HPSEC-MALLS) has been used for the quality evaluation of lentinan (β-glucan isolated from *Lentinus edodes* (Berk.) sing) injection in our previous studies (Chen et al., 2013), which has been proven as one of the most powerful techniques for the investigation of the molecular parameters such as absolute molecular mass, radius of gyration and chain conformation of macromolecules. Furthermore, saccharide mapping based on partial acid/enzymatic hydrolysis followed by polysaccharide analysis by carbohydrate gel electrophoresis (PACE) has been employed for the analysis of polysaccharides from *Ganoderma* spp. (Wu, Xie, Hu, Zhao, & Li, 2013) and *Cordyceps* spp. (Wu et al., 2014). Such strategy has been proven to have high repeatability, stability, sensitivity and throughput for the analysis of the chemical characters of the hydrolysates of polysaccharides. Therefore, the combination of HESEC-MALLS and saccharide mapping based PACE is a good choice for understanding the comprehensive molecular characters of polysaccharides in *G. conopsea* collected from different regions of China. Furthermore, the effects of the glycosidic linkages of polysaccharides on their macrophages functions were investigated. The results are helpful to improve the pharmacological activity-based quality control of polysaccharides from *G. conopsea*, and beneficial to develop a unique health and functional product in future.

2. Materials and methods

2.1. Materials and chemicals

Seven batches of the dried tubers (GC1 to GC7) of *G. conopsea* (GC) were collected from different places of China. Identity of the *G. conopsea* was confirmed by Doctor Chun-Feng Qiao, University of Macau, Macau SAR, China. The voucher specimens were deposited at the Institute of Chinese Medical Sciences, University of Macau, Macao, China.

D-glucose, starch (ST), α-amylase (EC 3.2.1.1), pectinase (EC 3.2.1.15), β-D-glucanase (EC 3.2.1.6), Griess reagent, lipopolysaccharide (LPS) and 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) were purchased from Sigma (St. Louis, MO, USA). Laminaribiose (Lam, 95%), β-1,3(4)-D-glucan, linear arabinan, galactomannan, pectic galactan, endo-arabinanase (EC 3.2.1.99), β-mannanase (EC 3.2.1.78) and 1,3-β-D-glucanase (EC 3.2.1.39) were purchased from Megazyme (Wicklow, Ireland). 8-Aminonaphthalene-1,3,6-trisulphonic acid (ANTS) was purchased from Tokyo Chemical Industry (Tokyo, Japan). Polyacrylamide containing a ratio of acrylamide/N,N-methylenebisacrylamide (19:1, w/w) was obtained from Bio-Rad (Hercules, CA, USA). Deionized water was prepared by a Millipore Milli-Q Plus system (Millipore, Bedford, MA, USA). All the other reagents were of analytical grade.

2.2. Preparation of polysaccharides from the tubers of G. conopsea

The samples were dried at 45 °C for 24 h, and pulverized. Sample materials (1 g) were immersed in water (30 mL) and refluxed in a Syncore parallel reactor (Büchi, Flawil, Switzerland) for 1.5 h at 100 °C with stirring at 150 rpm, respectively. Then the extract solution was centrifuged at 4000 × g for 10 min (Allegre X-15 centrifuge; Beckman Coulter, Fullerton, CA, USA). Subsequently, ethanol (95%, w/v) was added to the final concentration of 80% (v/v) for precipitation of crude polysaccharides. After standing for 8 h at 4 °C, centrifugation (4000 × g for 10 min) was performed. The precipitate was redissolved in 20 mL of hot water (60 °C). After centrifugation (4500 × g for 15 min), the supernatant was collected and the powder of the supernatant was obtained by freeze-drying. Finally, the crude polysaccharides were prepared in duplicates for further analysis. The yield of the crude polysaccharides from *G. conopsea* collected from different regions was ranging from 8.7% to 11.3%.

2.3. High performance size exclusion chromatography coupled with multi-angle laser light scattering (HPSEC-MALLS) analysis

The values of weight-average molecular weight (M_w), the polydispersity index (PDI) and the radius of gyration ($<S^2>_z^{1/2}$) of

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