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# Comparison of different extraction methods for polysaccharides from Dendrobium officinale stem



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#### ABSTRACT

The purpose of this study was to screen the optimum extraction of polysaccharides (DOP) from *Dendrobium officinale* stem. Firstly, different methods, including hot water extraction (HWE), cold-pressing (CP), freeze-thawing cold-pressing (FTCP), ultrasonic-assisted hot water extraction (UHWE), microwave-assisted hot water extraction (MHWE) and enzyme-assisted hot water extraction (EHWE), were employed to extract DOP under their respective best parameters. Then, the extraction yield, structure and antioxidant activity of the polysaccharides from different extraction methods were compared under the same condition. The data implied that UHWE and FTCP possessed higher extraction yield than the other extraction methods. Besides,  $DOP_{CP}$  and  $DOP_{FTCP}$  had higher molecular weight than the other polysaccharide samples. More importantly,  $DOP_{FTCP}$  had the highest antioxidant activity. Overall,  $DOP_{FTCP}$  exhibit high extraction yield, well-preserved molecular chains and best antioxidant activity, all these indicated FTCP was the most suitable method to extract DOP.

#### 1. Introduction

The extraction of polysaccharides from a plant source needs to follow a series of procedures including solvent infiltration, polysaccharide dissolution and diffusion (Fig. 1A). The improvement of solvent penetration into cells and solute diffusion are usually the key factors to facilitate the extraction progress (Chen, Meng, Zhang, & Liu, 2009). Hot water extraction method (HWE) could easily accelerate the diffusion rate of polysaccharides and improve the extraction efficiency with the increased temperature of water. The extraction process can also be further facilitated by physical methods. Ultrasonic-assisted hot water extraction (UHWE) (Zhang, Li, Xiong, Jiang, & Lai, 2013), microwave-assisted hot water extraction (MHWE) (Tao & Xu, 2008) and enzyme-assisted hot water extraction (EHWE) (Pan, Wang, Ye, Zha, & Luo, 2015) were commonly used and proved to be more efficient, as they can largely promote the dissolution of polysaccharides through biodegradation or mechanical destruction of plant cell walls. Recently,

freeze-thawing cold-pressing (FTCP) method has been favored, because the plant cell wall can be effectively destructed, thus the polysaccharide dissolution rate are high (Guo, Liu, Bai, & Yang, 2013). In addition, this method can avoid the degradation of polysaccharides caused by heat or ultrasound (Zhang, Chen et al., 2013), and retain good bioactivity.

Dendrobium officinale (Orchidaceae) is a valuable Chinese herbal medicine for its polysaccharide (DOP). It has showed a variety of pharmacological properties, such as antioxidative (Huang et al., 2016; Luo, Ge, Fan, Chun, & He, 2011), immune-stimulating (Cai et al., 2015), hypoglycemic (Zhao, Son, Kim, Jang, & Lee, 2007) and anti-tumor activities. There is a growing number of research and increasing demand of the DOP-based supplements and Chinese patent medicines. HWE (Huang et al., 2016), UHWE (Fan, Luo, Luo, & Chun, 2009) and MHWE (Liu & Xiong, 2009) have been used in previous studies for the extraction of polysaccharides from *Dendrobium officinale*, but these research did not specify the best extract method for DOP. Thus, study of the efficiency of different extraction methods can potentially benefit the

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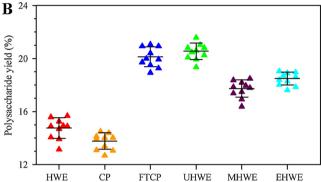


Fig. 1. The mechanism (A) and yield (B) of different extraction methods.

relevant pharmaceutical industry by providing evidence-based information for improvement of industrial equipment and materials.

Detailed structural studies of *Dendrobium officinale* revealed that the neutral polysaccharide was dominantly composed of  $\beta$ -D-glucopyranose and  $\beta$ -D-mannopyranose, both of which possess hydroxyl radical scavenging activity and thus anti-oxidative capacity (Xing, Cui, Nie, Phillips, Goff & Wang, 2013). Oxidative stress has been proved to be implicated in aging (Remmen & Richardson, 2001), neurodegeneration (Deng, Thompson, Gao, & Hall, 2007), diabetes mellitus (Wolff, Jiang, & Hunt, 1991), cardiovascular disease (Siti, Kamisah, & Kamsiah, 2015), and cancer (Reuter, Gupta, Chaturvedi, & Aggarwal, 2010). Antioxidants might be useful in combating with these processes and diseases. In current study, the antioxidant capacity after different extraction method can be used as an indicator to determine the remained amounts of active polysaccharides.

The present study was aimed to evaluate and screen the most suitable extraction method for DOP by comparing the outcomes of DOP's yields of different extraction methods, including hot water extraction (HWE), cold-pressing (CP), freeze-thawing cold-pressing (FTCP), ultrasonic-assisted hot water extraction (UHWE), microwave-assisted hot water extraction (EHWE). Then, DOP samples were characterized by chemical analysis, gas chromatography (GC), high-performance gel permeation chromatography (HPGPC), Fourier transform infrared spectoscopy (FT-IR) and scanning electron microscopy (SEM). Finally, the antioxidant activities *in vitro* of DOP samples were evaluated and compared to find the most bioactive sample.

#### 2. Materials and methods

#### 2.1. Materials and reagents

Dendrobium officinale stems was provided by Guangdong Yongshengyuan Biological Technology Co., Ltd. (Guangdong, China). Dendrobium officinale stems used in present study was 3-year-old and collected in May of the third year after planting. Their quality was identified by Prof. Danyan Zhang from Guangzhou University of

Chinese medicine. They were cultivated on the GAP base of Raoping County, Guangdong Province.

Dextrans with different molecular weight, 2,2'-azino-bis (3- ethylbenzothiazoline- 6-sulfonic acid) (ABTS) and Cellulase (comes from Aspergillus niger, 10000U/g), were purchased from Shanghai Aladdin Biochemical Technology Co., Ltd (Shanghai, China). Monosaccharide standards (xylose, arabinose, fucose, mannose, rhamnose, glucose and galactose) were obtained from Sigma Chemical Co., Ltd. (Sigma-Aldrich, St. Louis, MO, USA). All other reagents used in this study were of analytical grade.

#### 2.2. Extraction methods of DOP

#### 2.2.1. Cold-pressing method

Fresh Dendrobium officinale stem was extracted under the optimal parameter shown in Supplementary Section 1.2.1. Briefly, Fresh Dendrobium officinale stem sample  $(50.0\,g)$  was cut into small pieces  $(2-3\,cm)$ , and placed in a juicer, which was then added with 1000 ml water and squeezed for 5 min. The juice was filtrated and the residue was squeezed for another 2 times. The filtrates were combined, concentrated to 50 ml, and added with 200 ml of anhydrous ethanol for precipitation for 3 h. The precipitate was separated by centrifugation  $(3000\,\mathrm{rpm},\ 20\,\mathrm{min})$ , and then refluxed with 150 ml of 75% ethanol solution for 2 h. The residue was treated with ethanol solution in the same way until being colorless. Then the precipitate was evaporated to dryness. The dry DOP was dissolved in water and deproteinized by Sevage method (Xiong et al., 2015) and freeze-dried to obtain DOP sample (DOP<sub>CP</sub>).

#### 2.2.2. Freeze thawing cold-pressing method

Fresh Dendrobium officinale stem was extracted under the optimal parameter shown in Supplementary Section 1.2.2. Fresh Dendrobium officinale stems (50.0 g) were cut into small pieces (2–3 cm), and placed in a homogenizer. The sample was homogenized for 2 min after being added with some water. The homogenate was placed in -80°C for 24 h, then quickly thawed at 60°C. The sample was treated by the same method for 3 times; then the thawing solution was placed in a juicer. The following steps were the same with those in Section 2.2.1, and then DOP samples (DOP $_{\rm FTCP}$ ) were obtained.

#### 2.2.3. Hot water extraction method

Fresh Dendrobium officinale stem was extracted under the optimal parameter shown in Supplementary Section 1.2.3. The pieces of the Dendrobium officinale stems  $(50.0\,g)$  were placed in a homogenizer. 1000 ml water was added and homogenized for 2 min. Homogenate was transferred to a reflux device, and extracted in a boiling water bath to reflux for 3 h. The extract solution was centrifuged and the residue was extracted twice. The extract solutions were combined. The next step was with the same shown in Section 2.2.1, which lead to the DOP samples (DOP<sub>HWE</sub>).

#### 2.2.4. Ultrasonic- assisted hot water extraction

Fresh Dendrobium officinale stem was extracted under the optimal parameter shown in Supplementary Section 1.2.4. The pretreatment process was the same as in Section 2.2.3. Then homogenate was transferred to the ultrasonic extraction device (KQ-400KDE, Kunshan Ultrasound Instrument Co., Jiangsu, China), and ultrasonic extracted for 1 h at 65°C. The extract solution was centrifuged and the residue was extracted twice. The next step was according to Section 2.2.1, and DOP samples (DOP\_ $_{\rm UHWE}$ ) were obtained.

#### 2.2.5. Microwave- assisted hot water extraction

Fresh Dendrobium officinale stem was extracted under the optimal parameter shown in Supplementary Section 1.2.5. The pretreatment process was the same as in Section 2.2.3. The homogenate was treated for 90 s in a microwave extraction device with the power set up at

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