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# Comparison of polysaccharides from different *Dendrobium* using saccharide mapping

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ABSTRACT

Multiple species of *Dendrobium* are widely used as *Shihu*, a well known Chinese herb, for medicinal purpose in China. Small molecules such as phenols, alkaloids and coumarins are obviously varied in different species of *Dendrobium*. But there are few reports on polysaccharides, one of major active components, from *Dendrobium*. In this study, polysaccharides from different species or locations of *Dendrobium* were compared using saccharide mapping. The results showed that polysaccharides of *Dendrobium* from different species or locations were obviously varied in spite of they had some similar characters, which is helpful to control the quality of *Dendrobium*.



## 1. Introduction

The plants of *Dendrobium* genus, with more than 1100 species, are widely distributed throughout Asia, Europe and Australia. There are 78 species of *Dendrobium* plants found in China [1], and about 30 species of them, well known as *Shihu* in China, are employed in traditional or folk medicine [2]. According to the record of China Pharmacopeia (2010 version), *Dendrobium nobile* Lindl., *D. chrysotoxum* Lindl., *D. fimbriatum* Hook., and the related species of *Dendrobium* genus are all officially used as *Shihu*. Due to multiple origins, their active compounds including coumarins [3,4], phenols [2,5,6] and alkaloids [7] were greatly varied [8]. Actually, polysaccharides in *Dendrobium* have also been demonstrated their various beneficial effects, such as antioxidant, anti-hyperglycemic [9,10], immuno-stimulating [11,12] and antitumor [13] activities. However, to the best of our knowledge, there is no report on the specific characters of polysaccharides, due to the complexity, in different species or locations of *Dendrobium*, though compositional monosaccharides in several species of *Dendrobium* were investigated [14].

In this study, polysaccharides from eight *Dendrobium* samples with different species or locations were first compared using saccharides mapping, a method developed base on their carbohydrase enzymatic digestion properties and chromatographic characteristics of the enzymatic hydrolysates in our lab [15].

## 2. Experimental

### 2.1. Chemicals, reagents and materials

Eight samples of *Shihu*, including *D. huoshanense* Tang et Cheng and *D. officinale* Kimura et Migo from Anhui, *D. fimbriatum* Hook., *D. Chrysanthum* Lindl., *D. nobile* Lindl., and *D. officinale* Kimura et Migo from Yunnan, *D. nobile* Lindl. from Guizhou and *D. officinale* Kimura et Migo from Zhejiang, were collected in 2008 and 2009 by ourselves. The botanical origin of material was identified by Professor Dongxia Shen from China Pharmaceutical University and Yunnan Jinling Botanical Medicine Co., Ltd., Simao, China, and the voucher specimens were deposited at the Institute of Chinese Medical Sciences, University of Macau, Macao, China.

Acetonitrile and ammonium acetate for HPLC analysis were purchased from Merck (Darmstadt, Germany) and Riedel-de Haën, (Seelze, Germany), respectively. Deionized water was prepared by Millipore Milli Q-Plus system (Millipore, Bedford, MA). Sodium acetate, sodium phosphate monobasic and sodium phosphate dibasic from Riedel-de Haën were used in preparation of buffer solution for enzymatic digestion of polysaccharides. 1-phenyl-3-methyl-5-pyrazolone (PMP) was purchased from Sigma (St. Louis, MO, USA).

*Abbreviations:* HPSEC, high-performance size-exclusion chromatography; PMP, 1-phenyl-3-methyl-5-pyrazolone.

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**Table 1**
Conditions for enzymatic hydrolysis modified from the operation manual.

| Enzyme | EC number | Buffer solution | pH | Temperature (°C) |
|---|---|---|---|---|
| Arabinanase | 3.2.1.99 | 50 mM sodium acetate | 4.0 | 40 |
| Xylanase | 3.2.1.8 | 25 mM sodium acetate | 4.7 | 40 |
| 1,4-β-D-Galactanase | 3.2.1.89 | 25 mM sodium acetate | 4.0 | 40 |
| Cellulase | 3.2.1.4 | 25 mM sodium acetate | 4.5 | 40 |
| Pectinase | 3.2.1.15 | 50 mM sodium acetate | 5.5 | 40 |
| β-Mannanase | 3.2.1.78 | 50 mM sodium acetate | 4.5 | 40 |
| 1,3-β-Glucanase | 3.2.1.39 | 50 mM sodium acetate | 6.0 | 40 |
| Lichenase | 3.2.1.73 | 25 mM sodium phosphate | 6.5 | 40 |
| α-Amylase | 3.2.1.1 | 100 mM sodium acetate | 7.0 | 40 |
| Isoamylase | 3.2.1.68 | 100 mM sodium acetate | 4.0 | 40 |

D-galacturonic acid monohydrate (GalA), D-glucuronic acid (GlcA), D-arabinose (Ara), D-mannose (Man), D-galactose (Gal), D-glucose (Glc) were purchased from Fluka (Buchs, France). L-Rhamnose monohydrate (Rha), D-xylose (Xyl), maltose (Malt), pectinase (endopolygalacturonase, EC 3.2.1.15), cellulase (endo-1,4-β-D-glucanase, EC 3.2.1.4) and α-amylase (EC 3.2.1.1) were purchased from Sigma (St. Louis, MO, USA). Endo-arabinanase (EC 3.2.1.99), isoamylase (glycogen 6-glucanohydrolase, EC 3.2.1.68),

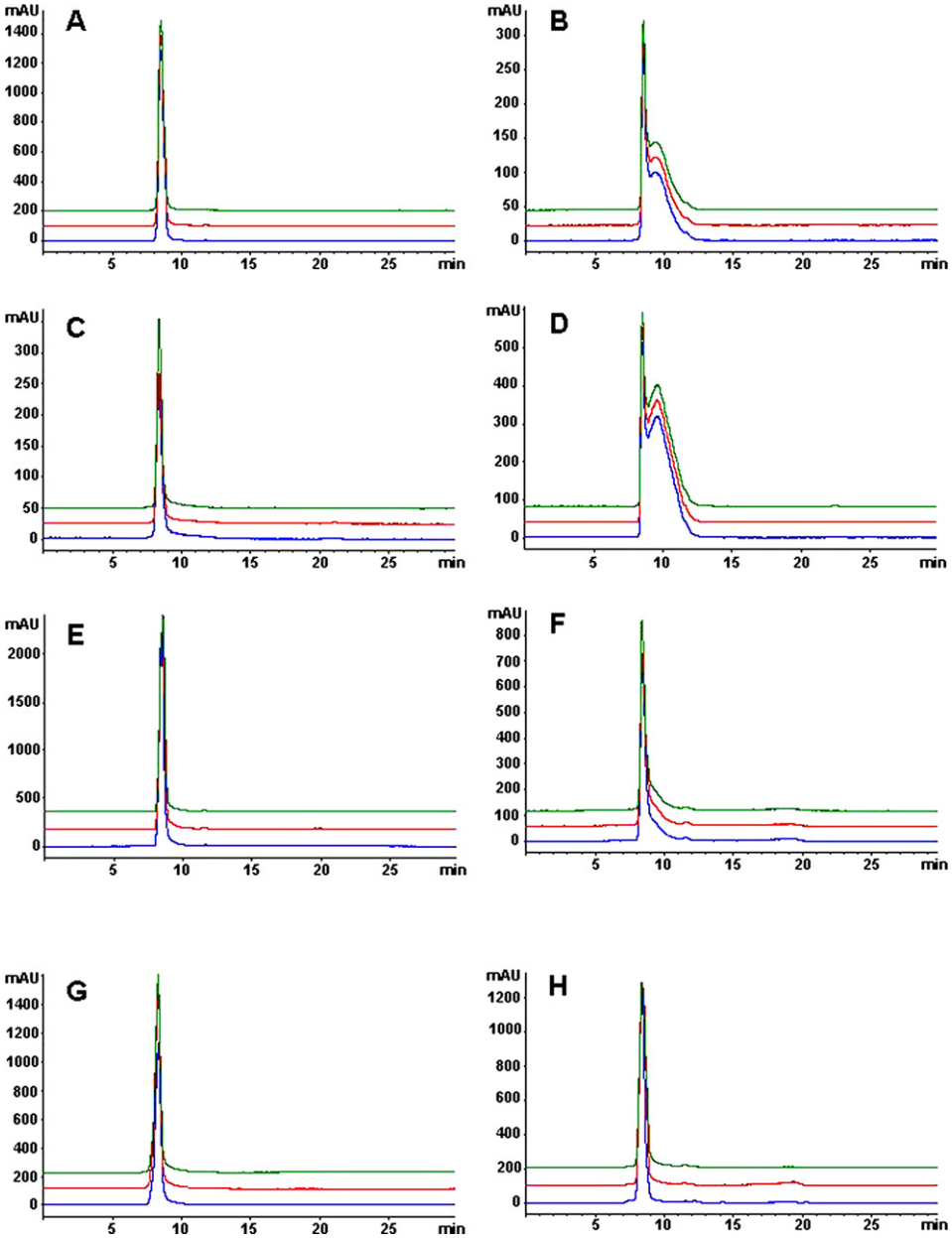


**Fig. 1.** HPSEC-ELSD profiles of three individual extracted polysaccharides from investigated *Dendrobium* spp. The samples were from different locations of China. (A) *D. huoshanense* from Anhui; (B) *D. fimbriatum* from Yunnan; (C–E) *D. officinale* from Anhui, Yunnan and Zhejiang, respectively; (F) *D. chrysanthum* from Yunnan; (G and H) *D. nobile* from Guizhou and Yunnan, respectively.