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Rapid quantification of polysaccharide and the main onosaccharides in *Dendrobium huoshanense* by near-infrared attenuated total reflectance spectroscopy

Jing-Wen Hao[a,b], Nai-Dong Chen[a,c,d,e,*], Cun-Wu Chen[a,c,d], Fu-Cheng Zhu[a], De-Liang Qiao[a,c,d], Yong-Jun Zang[a], Jun Dai[a,c,d], Xiang-Wen Song[a,c], Han Chen[a,c,d]

[a] College of Biotechnology and Pharmaceutical Engineering, West Anhui University, Lu'an City 237012, China
[b] School of Pharmacy, Anhui University of Traditional Chinese Medicine, Hefei 230012, China
[c] The Provincial 2011 Collaborative Innovation Center of Anhui-Dendrobium Huoshanense Industrialization Exploitation Collaborative Innovation Center, Lu'an City 237012, China
[d] West Anhui Biotechnology Research Center of Natural Medicine and Traditional Chinese Medicine, West Anhui University, Lu'an City 237012, China
[e] College of Pharmacy, Anhui Medical University, Hefei 230032, China

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ABSTRACT

A rapid, green, low cost and nondestructive attenuated total reflection near infrared (ATR NIR) method was developed to quantify the total polysaccharide and the main monosaccharides mannose and glucose in *Dendrobium huoshanense*. Total 100 *D. huoshanense* samples from different places were analyzed using ATR NIR method. Potential outlying samples were initially removed from the collected NIR data using the PCA-Mahalanobis distance method. Spectral data preprocessing was studied in the construction of a partial least squares (PLS) model and six different signal pretreatment methods, including multiplicative scattering correction (MSC), standard normal transformation (SNV), first and second derivatives, the combination of MSC with the first derivative, and the combination of SNV with the first derivative, were compared. The results showed that the best signal pretreatment method was the spectral data pretreated by SNV combined with the first derivative due to it showed the lowest root-mean-square error of cross-validation (RMSECV), highest R^2 for both the polysaccharide and its main monosaccharides. In order to improve the performance of the model, the pretreated full spectrum was calculated by different wavelength selection method. The results showed that the optional wavelength selection model was the one simultaneously selecting the NIR wavelength ranges 7500–5750 cm^{-1}, 5250–4700 cm^{-1}, 4450–4300 cm^{-1} and 4200–4100 cm^{-1} because of the lowest RMSECV and the highest R^2 among the ten wavelength selection models. The external validation and the complete external validation confirmed the robustness and reliability of the developed NIR model. The contents of the total polysaccharide and the main monosaccharides are the essential quality assessment criterion for plant medicines while their traditional quantification methods involved sample destruction, tedious sample processing and non-environmentally friendly pretreatment, therefore, our study might provide an efficient technique tool for the rapid, green and nondestructive quantification of the total polysaccharide and the main monosaccharides for *D. huoshanense* and other rich-in-polysaccharide plant medicines.

1. Introduction

Dendrobium huoshanense C.Z. Tang et S.J. Cheng is a rare and endangered medicinal plant and only distributes in the northern mountain regions of Changjiang River of China [1]. The stems of this plant, which are rich in polysaccharide, have long been used as traditional medicines for the treatment of salivary, stomach, and ophthalmic disorders, and also used as functional food materials to make tea drinks, soups and porridges for the protection of eye and liver [2]. It is well known that the chemical constituents of medicine plants, which were the basis for their pharmacological activities, varied depending on their species, cultured places, harvest seasons and even their origins. Therefore, it is vital to develop rapid, nondestructive and accurate methods to quantify the chemical compositions of plant medicines before their clinical application [3]. Polysaccharide is one of the most important active compo-

* Corresponding author at: College of Biotechnology and Pharmaceutical Engineering, West Anhui University, Lu'an City 237012, China.
E-mail address: 2004cnd@163.com (N.-D. Chen).

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Table 1
The list of *D. huoshanense* samples.

Samples	Collection place	Collection time
S1–S24	Jinzhai Count, Anhui Province, China	January, 2015–December, 2015
S25–S60	Huoshan Count, Anhui Province, China	January, 2015–December, 2015
S61–S80	Yingshan Count, Anhui Province, China	January, 2015–December, 2015
S81–S84	Huoshan Count, Anhui Province, China	September, 2015–December, 2015
S85–S88	Huoshan Count, Anhui Province, China	September, 2016–December, 2017
S89–S91	Jinzhai Count, Anhui Province, China	October, 2015–November, 2015
S92–S94	Jinzhai Count, Anhui Province, China	October, 2016–November, 2016
S95–S97	Yingshan Count, Anhui Province, China	October, 2015–December, 2015
S98–S100	Yingshan Count, Anhui Province, China	October, 2016–December, 2016

nents of medicinal plants and its pharmacological activities were influenced greatly by its content and monosaccharide compositions. Thus, the contents of the total polysaccharide and the main monosaccharides are the essential quality assessment criterion for plant medicines, especially for the plants of which the polysaccharide are the main active components such as *D. huoshanense* [4].

Traditional methods for quantification of the polysaccharide and its main monosaccharide in *D. huoshanense* were mainly performed using a colorimetric method involving the anthrone-sulphuric acid method as well as the phenol-sulphuric acid method [5], HPLC, GC–MS and HPCE methods [6–8]. However, these techniques are time-consuming, high cost, labor-intensive, reagent and solvent consuming, chemical waste producing, and are susceptible to human error due to extensive sample preparation. Therefore, a rapid, green, nondestructive and low cost analytical technique to determine the contents of polysaccharide and the main monosaccharides in *D. huoshanense* is in great demand to increase their full application.

ATR NIR spectroscopy, as a rapid, green, nondestructive and low cost analytical technique, has been widely applied to quantitative analysis and quality evaluation in agriculture, pharmaceuticals, polymer production and food industry [9]. The complexity of NIR spectroscopy requires the use of chemometric procedures to extract and visualize the useful analytical information [10]. It usually encounters a collinearity problem because of the strongly overlapped and broad absorption bands [11]. To address this problem, partial least squares (PLS) has been proposed to create a calibration model with NIR data. Typically, the establishment of a calibration model usually covers all of the measured wavelengths [12]. However, such a full spectrum model may obviously contain useless or irrelevant information, which may worsen the predictive ability of the developed model. So it is necessary and quite essential to conduct wavelength selection in a NIR analytical system to gain better prediction performance when creating a calibration model [13,14].

In this study, we firstly established the PLS calibration model between the NIR spectrum data of *D. huoshanense* and the contents of its polysaccharide and main monosaccharides. Then, the prediction results of different wavelength selection methods were compared and the optimal wavelength selection model was obtained based on the prediction performance and model complexity. Thus, a NIR method combined with chemometrics was developed to quickly, accurately and nondestructively quantify the polysaccharide and its main monosachharides in *D. huoshanens*.

2. Materials and methods

2.1. Reagents and sample collection

Deionized water was prepared by double distilled water system from (Millipore Corp, Bedford, MA, USA). Ethanol, acetone, ether, chloroform, *n*-butanol, hydrochloric acid, and pyridine were purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). Hexamethyldisilazane and trimethylchlorosilane were bought from Aladdin Reagents (Shanghai) Co., Ltd (Shanghai, China). Methanol, hexane, myoinositol, xylose, arabinose, rhamnose, glucose, mannose, fructose, galactose, and galacturonic acid were bought from Sigma-Aldrich Co., Ltd (St. Louis, Missouri, USA). The reagents from Sinopharm Chemical Reagent Co., Ltd., and Aladdin Industrial Corporation are of analytical grade. The chemicals and reagents from Sigma-Aldrich are of chromatographic grade.

All *D. huoshanense* samples (Table 1) were provided by Professor Nai-Fu Chen, West Anhui University, China. And that samples were numbered from 1 to 100 for convenience. Samples (S) 1–80 were used for calibration data set and external validation of the NIR model for the quantification of polysaccharide and its main monosaccharides, S81-100 were used for the complete external validation after the NIR quantification model having been established.

2.2. Sample preparation and quantitative analysis

The *D. huoshanense* samples were cut into small pieces and freezing-dried. Before analysis, samples were pulverized and passed through a 60-mesh sieve (particle size-0.2 mm).

2.2.1. Quantitative analysis of the polysaccharide

The polysaccharide content was measured by a TU-1901 UV–vis spectrophotometer (Purkinje General, Beijing, China) with the anthrone-sulphuric acid method [15]. A glucose calibration curve was firstly prepared as the literatures. The polysaccharide was extracted and quantified as our preview study with some modifications. Briefly, as follows: the powder was immersed in methanol by ultrasound condensation reflux. The filter residue was extracted three times with distilled water. Then the filtrate was centrifuged to obtain the crude polysaccharide. The crude polysaccharide solution was operated as the aforementioned calibration curve of glucose. The results were expressed as grams of glucose equivalents per 100 g of dry sample weight (g glucose per 100 g DSW) through the calibration curve with glucose. The content of each sample was determined in triplicate, and the mean of the three measurements was used for further analysis.

2.2.2. Quantitative analysis of the monosaccharides

Ethanol was added into the crude polysaccharide solution and the precipitate was repeatedly washed sequentially with ethanol, acetone, and ether, respectively. The residue was solved in water and then mixed with Sevag reagent to remove the associated proteins. The filtrate was lyophilized to yield diaphanous refined polysaccharide. [7]. The GC–MS analysis of monosaccharides was carried out according to the method proposed by Guadalupe and our previous study [7]. The refined polysaccharide of each sample was hydrolyzed with the methanol analysis reagent (MeOH containing HCl) to their corresponding methyl monosaccharides. The