



Monosaccharide Analysis of *Dendrobium moniliforme* Polysaccharides by High Performance Liquid Chromatography/Electrospray Ionization Mass Spectrometry

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Abstract: To obtain monosaccharide components information of *Dendrobium moniliforme* polysaccharides, a HPLC-ESI-MS method was proposed. The crude polysaccharides were extracted and purified from *D. moniliforme* as samples (DMP). Then, the polysaccharides were hydrolyzed with trifluoroacetic acid (TFA) and derivated by 1-phenyl-3-methyl-5-pyrazolone (PMP). PMP-labeled mixture of monosaccharides were separated by a reverse-phase high performance liquid chromatography (HPLC) and detected by on-line electrospray ionization mass spectrometry (ESI-MS). With this method, the six monosaccharide derivatives have been well separated and the identification of the monosaccharides composition was carried out. The result showed that *D. moniliforme* polysaccharide consisted of D-mannose, D-ribose, D-glucose, D-galactose and L-arabinose, in a molar ratio of 51.83: 4.01: 38.90: 4.20: 1.65, respectively. Moreover, hydrolysis studies revealed that the monosaccharide concentrations reached the peak value at different time and it suggested that the release of DMP monosaccharides was not hydrolyzed at the same rate. These results indicated that the DMP may be a mixture of multiple polysaccharides. Finally, we used a high performance gel permeation chromatography (HPGPC) method proved that crude polysaccharides were composed by three kinds of polysaccharides (DMP-1, DMP-2 and DMP-3) with molecular weight of 153.1 kDa, 69.28 kDa and 50.14 kDa.

Keywords: *Dendrobium moniliforme* Polysaccharide, Monosaccharide Components, High Performance Gel Permeation Chromatography (HPGPC), High-Performance Liquid Chromatography (HPLC), Electrospray Ionization-Mass Spectroscopy (ESI-MS)

1. Introduction

Dendrobium moniliforme (L.) Sw, is a medical orchid which is widely used in traditional Chinese medicine and folk remedies for ophthalmic, antipyretic, and tonic purpose [1]. In China, *Dendrobium* plants have been used as ingredients for nutraceutical beverages and food products for thousands of years [2]. As for *Dendrobium* species phytochemicals, much research has been carried out on the low molecular compounds, such as bibenzyl [3],

phenanthrene [4], and alkaloids [5]. A previous study has proven that the high-molecular-weight compounds such as polysaccharides were major active constituents in *Dendrobium* species [6]. Recently, studies of Polysaccharides from *Dendrobium moniliforme* have attracted more attention. Bioactivities including anti-oxidation property [7, 8], hypoglycemic effect [9], immunomodulatory activity [10] and antitumor activity against human myeloid leukemia cell line HL-60 [2] have been reported. *Dendrobium* plants are rich in polysaccharides, and the monosaccharide components

analysis of polysaccharides is of fundamental importance for the research on polysaccharide structure, characteristics and its functional properties.

A variety of chromatographic systems such as gas chromatography (GC) [11], thin-layer chromatography (TLC) [12], high-performance liquid chromatography (HPLC) [13–16], high performance liquid chromatography–mass spectrometry (LC–MS) [17] and gas chromatography–mass spectrometry (GC–MS) [18–20] are commonly used methods for identification and quantification of the monosaccharides in polysaccharides. Over the past decades, the development of LC–MS methods dedicated to the analysis of sugars and monosaccharides in particular, has led to significant advances in terms of sensitivity and specificity while maintaining speed and simplicity of implementation. However, LC–MS method is limited by loss of sensitivity owing to the low ionisation efficiency of monosaccharides [21]. Therefore, the derivatization of monosaccharides is indispensable to obtain monosaccharide components information. The reagent 1-phenyl-3-methyl-5-pyrazolone (PMP), which was first developed in 1989 by the Honda's group [22]. Pre-column derivatization with PMP method was first developed for the analysis of carbohydrates by high performance liquid chromatography [23].

Analyses of polysaccharide from some *Dendrobium* species are extensive, such as *Dendrobium huoshanense* [24], *Dendrobium chrysotoxum* Lindl [25] and *Dendrobium denneanum* [26]. However, the methods of PMP derivatization high performance liquid chromatography/electrospray ionization mass spectrometry on *D. moniliforme* have not been reported. In the present study, a precolumn PMP derivatization LC–ESI–MS method for analysis of five monosaccharides was established. The hydrolysis procedure of TFA was evaluated in different time intervals. Furthermore, the high performance gel permeation chromatography was successfully applied in molecular weight distribution analysis of polysaccharides purified from *D. moniliforme*. The information gained in this paper will be valuable for accurate qualitative and quantitative components of polysaccharides in *D. moniliforme*.

2. Experimental

2.1. Materials and Reagents

The plant materials of *D. moniliforme* were collected in April 2016, from Huoshan County, Anhui Province, China. All the plant materials were identified by Professor Yi-Bo Luo, Institute of Botany, the Chinese Academy of Sciences, Beijing, China. D-mannose, D-ribose, D-glucose, D-xylose, D-galactose, and L-arabinose were purchased from Sigma Co. (USA). Trifluoroacetic acid (TFA) was obtained from Merck (Darmstadt, Germany). 1-Phenyl-3-methyl-5-pyrazolone (PMP) was purchased from Beijing Reagent Plant (Beijing, China). Acetonitrile (HPLC-grade) was obtained from Merck (E. Merck, Darmstadt, Germany). Water was purified with a Mili-Q academic water purification system (Millipore,

Bedford, MA, USA). Other chemicals were of analytical reagent grade.

2.2. Extraction and Purification

The polysaccharide was isolated from *Dendrobium moniliforme* by distilled water extraction and ethanol precipitation. The dried *D. moniliforme* (0.3g) were extracted with 200 mL distilled water by heat reflux extraction for 2 h at 100°C. The mixed extracts were concentrated under a reduced pressure and then centrifuged at 5000rpm for 15 min. The proteins in the product of condensation were deproteinized using the Sevag reagent [27]. The supernatant was collected, precipitated by anhydrous alcohol and centrifuged to give polysaccharide precipitation. The precipitation were washed twice with 80% ethanol. Residue was dried and the *D. moniliforme* polysaccharide was obtained. Total polysaccharide content was determined by the phenol-sulfuric acid method, using glucose as the standard reference, and wavelength scanning was presented.

2.3. High Performance Gel Permeation Chromatography

High performance gel permeation chromatography (HPGPC) was performed on a Agilent 1200 liquid chromatography system fitted with an 18-angle light scattering detector (DAWN HELEOS, Wyatt Technology Co., Santa Barbara, CA, USA), using a Shodex SB-806M HQ column (300 × 8.0 mm, Showa Denko KK, Miniato, Japan) operated with column temperature set at 40°C. The column was pre-calibrated with standard dextrans. Sample (3 mg/mL, 20 µL) was injected, eluted with 0.1 M NaNO₃ at a flow rate of 0.5 mL/min.

2.4. Hydrolysis of the Polysaccharide

The polysaccharide (1 mg/mL) was dissolved in 500 µL TFA in an ampoule (5mL). The ampoule was sealed under a nitrogen atmosphere and kept in an oil bath at 110°C for 1.5 h, 2.5 h, 4 h and 5 h periods, respectively. After being cooled to room temperature, the reaction mixture was then centrifuged at 8000rpm for 10 min. The supernatant was collected and dried under a reduced pressure.

2.5. Derivatization Procedure

The procedure employed for the derivatization of monosaccharides was carried out according to the method of Daotian, F *et al.* [28] and modified by us. The six monosaccharides standards or the hydrolysed polysaccharide were dissolved in 0.3 M aqueous NaOH (800 µL) and a 0.3 M methanol solution (800 µL) of PMP was added to each and mixed. Each mixture was allowed to react for 100 min at 70°C, then cooled to room temperature and was neutralized with 0.3 M HCl (1000 µL). The resulting solution was dissolved in chloroform (4 mL each). After shaking vigorously and centrifuging, the chloroform layer was discarded, and the extraction process was repeated four times. The supernatant was filtered through a 0.45 µm membrane and diluted with water for LC/MS analysis.

2.6. Chromatography and Mass Spectrometry Conditions

The analysis of PMP-labeled monosaccharides was carried out on the Agilent 1200 HPLC system (Agilent Technologies, Palo Alto, CA, USA) with a Venusil MP C18 column (4.6×250 mm, 5μm). The wavelength for UV detection was 250 nm. Elution was carried out at a flow rate of 1.0 mL/min at 30°C. The mobile phase consisted of 20 mM ammonium acetate, pH 6.7 (A) and acetonitrile (B), using a gradient elution of 16.9-16.9-19.4-19.4% buffer B by a linear increase from 0-28-38-55 min. Mass spectrometric (MS) analysis was performed on a Shimadzu LCMS-IT-TOF with ESI source. The MS conditions for detection were as follows: positive ion mode; detector voltage, 1.58 kV; CDL temperature, 200°C; heat block temperature, 200°C; 1.5 L/min of nebulizing gas (N₂) flow rate; 1.5×10^{-2} Pa of IT area vacuum; 1.6×10^{-4} Pa of TOF area vacuum; 149 kPa of drying gas pressure. The spectra were recorded from *m/z* 100 to 600.

3. Results and Discussion

3.1. Extraction and Purification Polysaccharides

The polysaccharides, named DMP, were obtained from the stem of *D. moniliforme* by the method of section 2.2. An ultraviolet spectrophotometric method was developed for the quantitative determination of the total polysaccharide content due to strong UV absorption of DMP at 486 nm. The total yield rate of polysaccharides was 42%~48.6% (RSD=7.3%) based on the calibration curve obtained with standard glucose.

3.2. Hydrolysis of Polysaccharide

To investigate the components of DMP, the hydrolysis of polysaccharide were performed. H₂SO₄ is the mostly used hydrolysis agent in the studies of polysaccharide, however, which is difficult to remove and poses negative effect on MS analysis [29]. DMP was hydrolyzed using trifluoroacetic acid (TFA) in this study because TFA can be removed by evaporating the solution in vacuo before samples running in

ESI-MS, so the contamination caused by TFA was avoided. We can see that DMP were composed of mannose, ribose, glucose, galactose, and arabinose with TFA hydrolysis. The effect of the hydrolysis time on concentration of monosaccharides was investigated and the result was shown in Fig. 1. It can be concluded that hydrolysis with TFA at different times seems to have two interesting phenomena. Firstly, with prolonging the hydrolysis time, the concentrations of the mannose and glucose decreased to a minimum and then increased. This result indicated that DMP hydrolysis is a complicated procedure. At the beginning of hydrolysis, Part of the polysaccharide was rapidly hydrolyzed to mannose and glucose. And after that, the concentrations of mannose and glucose decreased due to chemical reaction of monosaccharides under acid condition. Secondly, when the hydrolysis time was about 4 hours later, the new polysaccharide began to be hydrolyzed. So we detected that the rising of the mannose and glucose concentrations. These results illustrated that DMP may be consisted of multiple polysaccharides, which had different hydrolysis rate. We also found that galactose cannot be released until hydrolysis time is 4 hours. This phenomenon also supported previous deduction. It was observed that TFA was suitable to achieve depolymerisation of DMP. Moreover, it was found that the TFA caused less damage to sugars than H₂SO₄ or HCl [30].

In order to confirm the above assumption, high performance gel permeation chromatography (HPGPC) was employed to estimate the homogeneity and molecular weight distribution of DMP. The molecular weight of polysaccharide was also an important factor responsible for biological activities [31]. As presented in Fig. 2, there were three broad, symmetrical peaks on the chromatograms, which means that the molecular weight distribution of DMP from left to right is continuously distributed and gradually decreased. The average molecular weights of the three fractions (DMP-1, DMP-2 and DMP-3) were estimated to be 153.1 kDa, 69.28 kDa and 50.14 kDa, respectively. The result suggested that DMP may be a complex mixture of multiple polysaccharides.

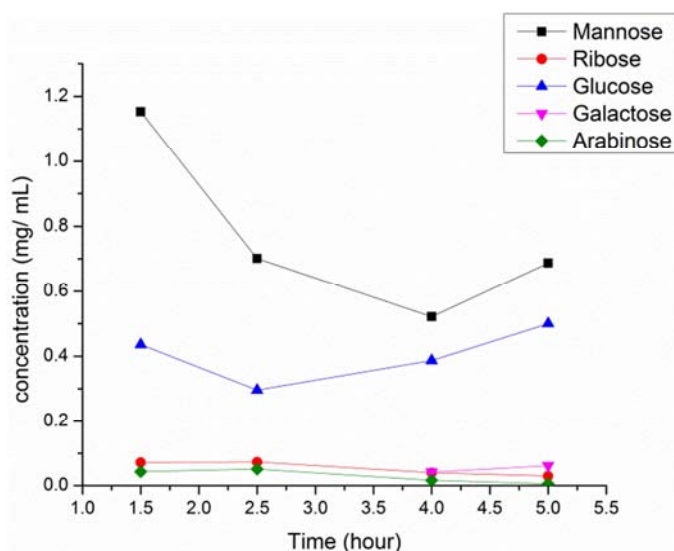


Figure 1. Change trend of different hydrolysis time to peak areas of monosaccharides of DMP pre-column derivation HPLC characteristic spectrum.

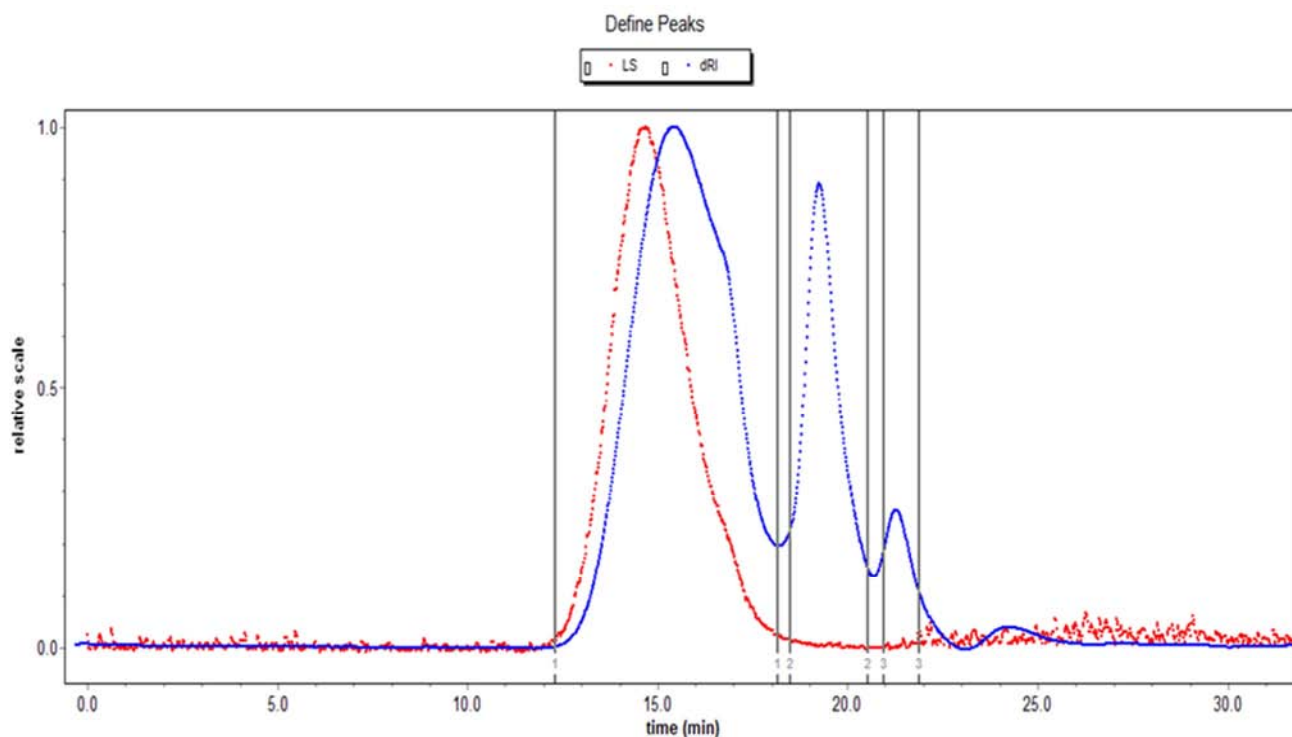


Figure 2. Polysaccharides from crude *Dendrobium moniliforme* extract were isolated by high performance gel permeation chromatography, named as DMP-1, DMP-2 and DMP-3.

3.3. HPLC Separation of PMP-monosaccharides Derivatives

The lack of chromophores or fluorophores in the structure of monosaccharides limits their sensitive detection [30]. Therefore, carbohydrates are generally tagged with a suitable chromophore or fluorophore to obtain highly sensitive detection. The reagent 1-phenyl-3-methyl-5-pyrazolone (PMP) is one of the popular labels that react with reducing carbohydrate under mild condition, requiring no acid catalyst and causing no isomerization [32]. In the process of structural analysis of polysaccharides, the molar ratio of each monosaccharide in polysaccharides is one of the most important parameters.

The PMP-labeled monosaccharide standards were analyzed by HPLC system described above; the chromatogram is suggested in Fig. 3. Six PMP-labeled monosaccharides were separated successfully in the order of peak 1 for mannose, peak 2 for ribose, peak 3 for glucose, peak 4 for galactose, peak 5 for xylose and peak 6 for arabinose. In this study, the good separation of the six monosaccharide derivatives was achieved within 40 min. Table 1 is the summary of calibration curves, linear ranges and limit of detection. The results indicated that mannose and glucose were the major monosaccharides constructing the *D. moniliforme* polysaccharide (Fig. 4). The molar ratio of monosaccharide components was described as follows: mannose: ribose: glucose: galactose: arabinose = 51.83: 4.01: 38.90: 4.20: 1.65. It was clear that the predominantly composition monosaccharide in DMP was mannose up to 51.83%.

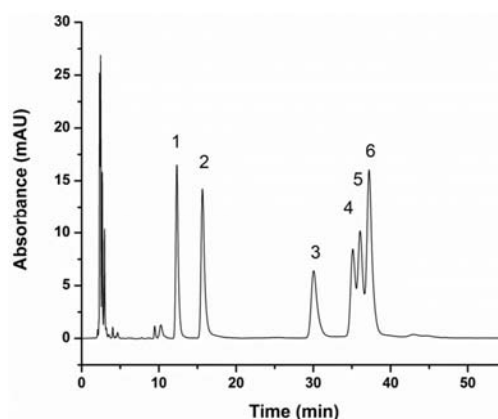


Figure 3. High performance liquid chromatographic (HPLC) separation of PMP-labeled monosaccharide standards.

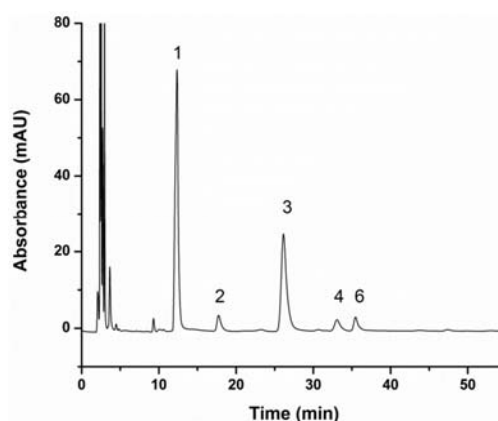


Figure 4. High performance liquid chromatographic (HPLC) separation of PMP-labeled monosaccharide composition of DMP.

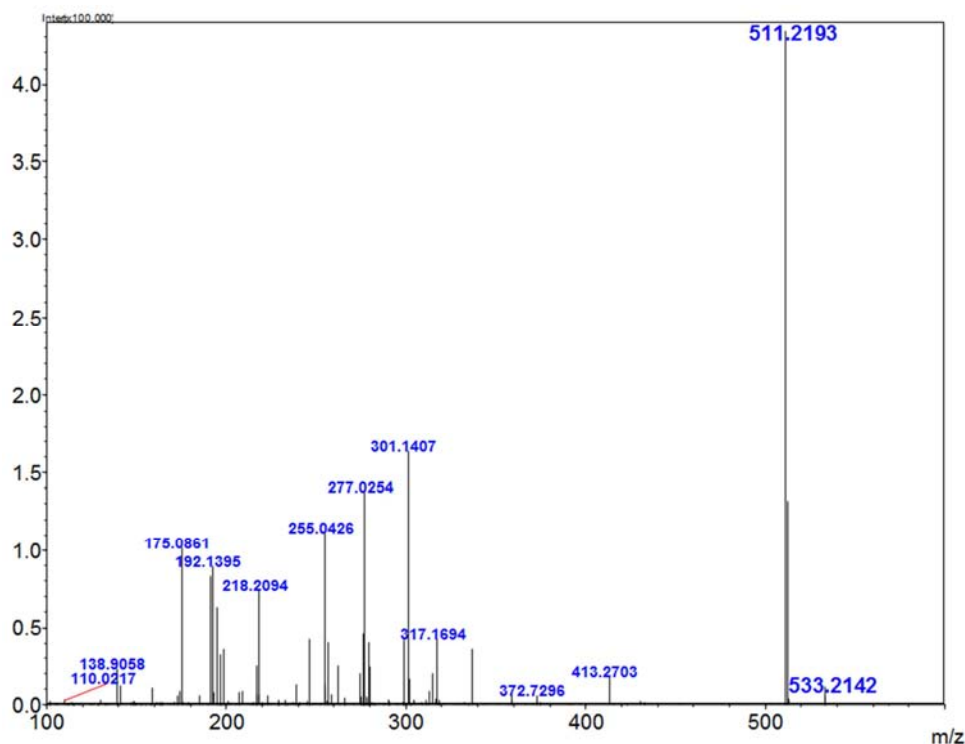


Figure 5. ESI-MS spectrum of PMP-labeled glucose.

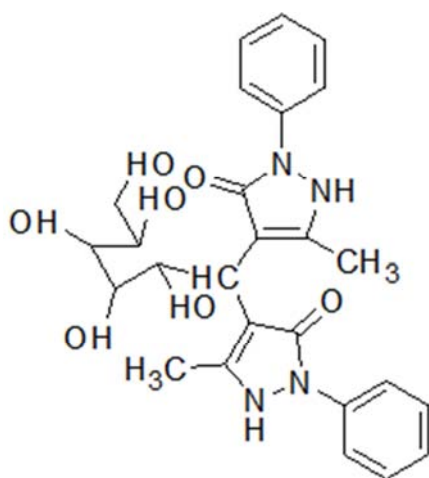


Figure 6. Structure of glucose-PMP.

3.4. LC-ESI-MS Characterization

All PMP-labeled monosaccharides were characterized by LC-ESI-MS method. Carbohydrate derivatives show sufficient ionization information under ESI-MS in positive-ion mode. For example, PMP-labeled glucose

comprises peaks of m/z 511.2193 and 533.2142 correspondent to $[M+H]^+$ and $[M+Na]^+$ by positive-ion mode (Fig. 5), which means that one molecule of glucose reacted with two molecules of PMP. Accordingly glucose-PMP is considered to have the structure given in Fig. 6. Since this compound has conjugated double bonds, it absorbs the UV light strongly, although it does not fluoresce. Other reducing monosaccharides are considered to react with PMP in a similar fashion. An intense protonated molecular ion at m/z $[M+H]^+$ can be used for the accurately qualitative analysis of carbohydrates. When HPLC and MS are combined on-line, six PMP-labeled compounds were separated, among which five PMP-labeled monosaccharides were identified under conditions as described in section 2.6.

Peaks 1, 2, 3, 4 and 6 could be unambiguously identified as PMP-labeled mannose, ribose, glucose, galactose and arabinose based on the comparison of retention times and MS data with those of authentic PMP-labeled monosaccharides. With this method, the carbohydrates from the hydrolyzed DMP can successfully be identified. From above evidence, polysaccharides from *D. moniliforme* mainly compose of mannose, ribose, glucose, galactose and arabinose (Table 2).

Table 1. The calibration ranges, LODs and precision for five monosaccharides.

Analyte	Calibration curve	Range (mg/L)	Correlation coefficient	LODs (mg/L)	Precision (RSD,%)
Mannose	$Y = 3662.5X + 0.8322$	2-1200	0.9999	0.2	0.32
Ribose	$Y = 3695.9X - 2.1978$	2-200	0.9999	0.2	0.37
Glucose	$Y = 3196.3X - 0.8322$	2-1000	0.9999	0.2	0.58
Galactose	$Y = 3524.6X - 0.6457$	2-200	0.9998	0.2	1.65
Arabinose	$Y = 7942.9X - 0.779$	2-200	0.9998	0.2	1.52

Table 2. MS data of double PMP-labeled saccharides.

Saccharides	tR (min)	[M+H] ⁺	[M+Na] ⁺
Mannose	12.330	511.2182	533.1987
Ribose	15.655	481.2086	503.1891
Glucose	30.055	511.2193	533.2142
Galactose	35.137	511.2183	533.1990
Xylose	36.081	481.2084	503.1877
Arabinose	37.240	481.2088	503.1870

4. Conclusion

In the present work, the crude polysaccharides were extracted and purified from the stem of *D. moniliforme*. And the method for monosaccharide analysis with PMP pre-column derivatization by HPLC-ESI-MS demonstrated that DMP consisted of D-mannose, D-ribose, D-glucose, D-galactose and L-arabinose with the molar ratio of 51.83: 4.01: 38.90: 4.20: 1.65. The method reported here is simple, rapid, convenient and reproducible, and proved to be suitable for the analysis of the monosaccharide components of *D. moniliforme* polysaccharides.

Hydrolysis procedure indicated that DMP may be consisted of multiple polysaccharides, which had different hydrolysis rate. For this reason, study on homogeneity and molecular weight distribution of DMP was followed by HPGPC. It was concluded that DMP contained three major polysaccharide fractions with molecular weight of 153.1 kDa, 69.28 kDa and 50.14 kDa. The information gained in this paper will be valuable for accurate qualitativng and quantitativng components of polysaccharides in *D. moniliforme*. In addition, further investigation should be done to better separate three major polysaccharide fractions.

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References

- [1] Chin-Tung W, Keng-Shiang H, Chih-Hui Y, et al. "Inhibitory Effects of Cultured *Dendrobium tosaense* on Atopic Dermatitis Murine Model," *Int. J. Pharm.* 463 (2), pp. 193-200, 2014.
- [2] Zhang J, Xiang LI, Run long LU. "Apoptosis of Human Myeloid Leukemia Cell Line HL-60 Induced by Water Extracts of *Dendrobium Moniliforme*," *Chinese Journal of Cancer*, 20 (9), pp. 956-960, 2001.
- [3] Majumder PL, Guha S, Sen S. "Bibenzyl derivatives from the orchid *Dendrobium amoenum*," *Phytochemistry*, 52 (7), pp. 1365-1369, 1999.
- [4] Yang L, Qin LH, Bligh SW, et al. "A new phenanthrene with a spiro lactone from *Dendrobium chrysanthum* and its anti-inflammatory activities," *Bioorganic & Medicinal Chemistry*, 14 (10), pp. 3496-3501, 2006.
- [5] Wang Q, Gong Q, Wu Q, Shi J. "Neuroprotective effects of *Dendrobium* alkaloids on rat cortical neurons injured by oxygen-glucose deprivation and reperfusion," *Phytomedicine International Journal of Phytotherapy & Phytopharmacology*, 17 (2), pp. 108-115, 2010.
- [6] Fan YJ, Luo AX, Luo AS, Ze C. "Research on the ultrasonic-assisted extraction process for polysaccharide from *D. denneanum*," *Chinese Journal of Hospital Pharmacy*, 29 (22), pp. 1909-1911, 2009.
- [7] Xu C, Chen YL, Zhang M. "Structural characterization of the polysaccharide DMP2a-1 from *Dendrobium moniliforme*," *Chinese Pharmaceutical Journal*, 39 (12), pp. 900-902, 2004.
- [8] Lee W, Eom D, Jung Y, et al. "Dendrobium moniliforme Attenuates High-Fat Diet-Induced Renal Damage in Mice through the Regulation of Lipid-Induced Oxidative Stress," *American Journal of Chinese Medicine*, 40 (6), pp. 1217-1228, 2012.
- [9] Chen Y, He G, Zhang M, Li H. "Hypoglycemic effect of the polysaccharide from *Dendrobium moniliforme* (L.) Sw," *Journal of Zhejiang University*, 30 (6), pp. 693-696, 2003.
- [10] Chen ZH, Chen YL, Wu T, Hua YF, Zhang M. "Study on structural characteristics and immunomodulatory activity of polysaccharide DMP4a-1 from *Dendrobium moniliforme*," *Chinese Pharmaceutical Journal*, 39 (23), pp. 1781-1783, 2005.
- [11] Pradeep GC, Yoo HY, Cho SS, Yun HC, Jin CY. "An Extracellular Chitinase from *Streptomyces* sp. CS147 Releases N-acetyl- d -glucosamine (GlcNAc) as Principal Product," *Appl. Biochem. Biotechnol*, 175 (1), pp. 372-386, 2015.
- [12] Ma Z, Cui F, Gao X, Zhang J, Zheng L, Jia L. "Purification, characterization, antioxidant activity and anti-aging of exopolysaccharides by *Flammulina velutipes* SF-06," *Antonie van Leeuwenhoek*, 107 (1), pp. 73-82, 2015.
- [13] Ikegami T, Horie K, Saad N, Hosoya K, Fiehn O, Tanaka N. "Highly efficient analysis of underivatized carbohydrates using monolithic-silica-based capillary hydrophilic interaction (HILIC) HPLC," *Anal. Bioanal. Chem*, 391 (7), pp. 2533-2542, 2008.
- [14] Lv Y, Yang X, Zhao Y, Yun R, Yang Y, Wang Z. "Separation and quantification of component monosaccharides of the tea polysaccharides from *Gynostemma pentaphyllum* by HPLC with indirect UV detection," *Food Chem*, 112 (3), pp. 742-746, 2009.
- [15] Yang Q, Wang S, Xie Y, Sun J, Wang J. "HPLC analysis of *Ganoderma lucidum* polysaccharides and its effect on antioxidant enzymes activity and Bax, Bcl-2 expression," *Int. J. Biol. Macromol*, 46 (2), pp. 167-172, 2010.
- [16] Dai J, Wu Y, Chen SW, et al. "Sugar compositional determination of polysaccharides from *Dunaliella salina* by modified RP-HPLC method of precolumn derivatization with 1-phenyl-3-methyl-5-pyrazolone," *Carbohydr. Polym*, 82 (3), pp. 629-635, 2010.
- [17] Jmeian YR. "Quantification of monosaccharides through multiple-reaction monitoring liquid chromatography/mass spectrometry using an aminopropyl column," *Rapid Commun. Mass Spectrom*, 24 (11), pp. 1565-1574, 2010.

- [18] Akiyama T, Yamazaki T, Tanamoto K. "Analysis of thickening polysaccharides by the improved diethyldithioacetal derivatization method," *Shokuhinseigaku Zasshi Journal of the Food Hygienic Society of Japan*, 52 (1), pp. 40-46, 2011.
- [19] Li B, Dobruchowska JM, Gerwig GJ, Dijkhuizen L, Kamerling JP. "Structural investigation of water-soluble polysaccharides extracted from the fruit bodies of *Coprinus comatus*," *Carbohydr. Polym.*, 91 (1), pp. 314-321, 2013.
- [20] Liang Y, Si T, Ang EL, Zhao H. "Engineered pentafunctional minicellulosome for simultaneous saccharification and ethanol fermentation in *Saccharomyces cerevisiae*," *Applied & Environmental Microbiology*, 80 (21), pp. 6677-6684, 2014.
- [21] Wu X, Jiang W, Lu J, Yu Y, Wu B. "Analysis of the monosaccharide composition of water-soluble polysaccharides from *Sargassum fusiforme* by high performance liquid chromatography/electrospray ionisation mass spectrometry," *Food Chem.*, 145 (7), pp. 976-983, 2014.
- [22] Honda S, Akao E, Suzuki S, Okuda M, Kakehi K, Nakamura J. "High-performance liquid chromatography of reducing carbohydrates as strongly ultraviolet-absorbing and electrochemically sensitive 1-phenyl-3-methyl-5-pyrazolone derivatives," *Anal. Biochem.*, 180 (2), pp. 351-357, 1989.
- [23] Shen X, Perreault H. "Characterization of carbohydrates using a combination of derivatization, high-performance liquid chromatography and mass spectrometry," *J. Chromatogr. A*, 811 (1-2), pp. 47-59, 1998.
- [24] Hsieh SY, Cheng C, Liao KS, et al. "Structure and bioactivity of the polysaccharides in medicinal plant *Dendrobium huoshanense*," *Bioorganic & Medicinal Chemistry*, 16 (11), pp. 6054-6068, 2008.
- [25] Zhao Y, Son YO, Kim SS, Jang YS, Lee JC. "Antioxidant and anti-hyperglycemic activity of polysaccharide isolated from *Dendrobium chrysotoxum* Lindl," *Journal of Biochemistry & Molecular Biology*, 40 (5), pp. 670-677, 2007.
- [26] Fan Y, He X, Zhou S, Luo A, He T, Chun Z. "Composition analysis and antioxidant activity of polysaccharide from *Dendrobium denneanum*," *Int. J. Biol. Macromol.*, 45 (2), pp. 169-173, 2009.
- [27] Navarini L, Gilli R, Gombac V, Abatangelo A, Bosco M, Toffanin R. "Polysaccharides from hot water extracts of roasted *Coffea arabica* beans: isolation and characterization," *Carbohydr. Polym.*, 40 (1), pp. 71-81, 1999.
- [28] Fu DT, Oneill RA. "Monosaccharide Composition Analysis of Oligosaccharides and Glycoproteins by High-Performance Liquid Chromatography," *Anal. Biochem.*, 227 (2), pp. 377-384, 1995.
- [29] Zhou J, Hu N, Wu Y, Pan Y, Sun C. "Preliminary studies on the chemical characterization and antioxidant properties of acidic polysaccharides from *Sargassum fusiforme*," *Journal of Zhejiang University-SCIENCE B*, 9 (9), pp. 721-727, 2008.
- [30] Emaga TH, Rabetafika N, Blecker CS, Paquot M. "Kinetics of the hydrolysis of polysaccharide galacturonic acid and neutral sugars chains from flaxseed mucilage," *Biotechnology Agronomy Society & Environment*, 16 (2), pp. 139-147, 2012.
- [31] Luo A, Fan Y. "In vitro Antioxidant of a Water-Soluble Polysaccharide from *Dendrobium fimbriatum* Hook.var.*oculatum* Hook," *Int. J. Mol. Sci.*, 12 (6), pp. 4068-4079, 2011.
- [32] You L, Yang XB, Yan Z, Yun R, Ying Y, Wang ZZ. "Separation and quantification of component monosaccharides of the tea polysaccharides from *Gynostemma pentaphyllum* by HPLC with indirect UV detection," *Food Chem.*, 112 (3), pp. 742-746, 2009.