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Fingerprint analysis of polysaccharides from different *Ganoderma* by HPLC combined with chemometrics methods

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ABSTRACT

A fingerprint analysis method has been developed for characterization and discrimination of polysaccharides from different *Ganoderma* by high performance liquid chromatography (HPLC) coupled with chemometrics means. The polysaccharides were extracted under ultrasonic-assisted condition, and then partly hydrolyzed with trifluoroacetic acid. Monosaccharides and oligosaccharides in the hydrolyzates were subjected to pre-column derivatization with 1-phenyl-3-methyl-5-pyrazolone and HPLC analysis, which will generate unique fingerprint information related to chemical composition and structure of polysaccharides. The peak data were imported to professional software in order to obtain standard fingerprint profiles and evaluate similarity of different samples. Meanwhile, the data were further processed by hierarchical cluster analysis and principal component analysis. Polysaccharides from different parts or species of *Ganoderma* or polysaccharides from the same parts of *Ganoderma* but from different geographical regions or different strains could be differentiated clearly. This fingerprint analysis method can be applied to identification and quality control of different *Ganoderma* and their products.

1. Introduction

As early as in 100 B.C., Lingzhi (*Ganoderma*) was cited in the Shen Nong's Herbal Classic (widely considered as the oldest book on oriental herbal medicine and the foundation of traditional Chinese medicine) for enhancing "vital energy" and promoting "longevity" (Huie & Di, 2004). Now, *Ganoderma* has been widely known as a tonic and a functional food in China. So far, more than 200 species of *Ganoderma* have been found in the world, and *Ganoderma lucidum* (*G. lucidum*) and *Ganoderma sinense* (*G. sinense*) are the most well-known two species used most widely, which are being cultivated in many countries due to their healthcare function and commercial value. In this study, the samples used to test are also *G. lucidum* (it is now called *Ganoderma* Lingzhi (Cao, Dai, & Wu, 2012), but the previous name was still used in our study) and *G. sinense*, which were permitted to use in health food according to the prescript of China Food and Drug Administration.

In recent years, many researches have shown that *Ganoderma* contains a wide variety of bioactive components, including polysaccharides, triterpenes, adenosine, sterols, alkaloids, proteins, and so on (Da et al., 2012; Ferreira, Vaz, Vasconcelos, & Martins, 2010; Heleno et al., 2012). Among these components, polysaccharides and triterpenes were normally thought to be major active substances (Da et al., 2012). Many researches have shown that *Ganoderma* polysaccharides had the abilities of immuno-enhancing (Bao, Wang, Dong, Fang, & Li, 2002; Huang & Ning, 2010), anti-tumor (Xu, Chen, Zhong, Chen, & Wang, 2011), anti-hepatitis (Li, Fang, & Zhang, 2007), anti-oxidant (Chen, Xie, Nie, Li, & Wang, 2008; Chen, Yan, et al., 2008; Liu, Wang, Pang, Yao, & Gao, 2010), and anti-diabeties (Seto et al., 2009). So, now more and more *Ganoderma* products and *Ganoderma* polysaccharides products as health foods or medicines have appeared at the market. However, it is hard to say whether its quality is good or bad, and it is also difficult to identify whether the raw materials were authentic or adulterant. On the other hand, even though the products were qualified according to the enterprise standards, it is not certain that the actual effects were stable enough because most of the standards have been behind the times (only the content of polysaccharides and triterpenes was prescribed as main quality index) and modern analytical technology was still not applied to quality inspection of *Ganoderma*. So, effective and reliable analytical methods and quality standards were urgent need to be developed for evaluating the quality of *Ganoderma* scientifically.

With the development of modern analytical technology, fingerprint profiling has become internationally recognized as a

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convenient and efficient technology of quality inspection. Many scholars have also made a lot of exploratory work on the fingerprint of *Ganoderma* products. The studies about the fingerprint profiling of *Ganoderma* triterpenes by high performance liquid chromatography (HPLC) have been reported frequently (Chen, Xie, et al., 2008; Chen, Yan, et al., 2008; Da et al., 2012). But few studies have been done on the fingerprint profiling of *Ganoderma* polysaccharides, because the polysaccharides were complex biomacromolecule mixtures (Bao et al., 2002; Li et al., 2007; Liu et al., 2010; Zhang et al., 2012), and their separation and identification were difficult and highly challenging. For now, about 200 kinds of polysaccharides have been separated from different *Ganoderma* samples and identified as α- or β- (1-3)-, (1-6)-glucans and heteropolysaccharides with different combinations of glucose, mannose, galactose, xylose, fucose, as well as arabinose (Bao et al., 2002; Li et al., 2007; Nie, Zhang, Li, & Xie, 2013). So based on the diversity of *Ganoderma* polysaccharides, it is feasible that HPLC analysis coupled with the partial acid hydrolysis of the different *Ganoderma* polysaccharides with unique structural features and chemical compositions would yield a different fingerprinting profile that could be used for authentication.

In our present work, the polysaccharides were extracted from different *Ganoderma* samples with hot water under ultrasonic-assisted condition and partly hydrolyzed with trifluoroacetic acid (TFA), and then analyzed by RP-HPLC with PMP pre-column derivatization. The chromatographic data with high resolution were submitted into the professional software for establishing standard fingerprint chromatogram and evaluating similarity based on the matched data of relative retention time and area of common peaks of the hydrolyzates, which can reveal the differences of polysaccharide samples from different parts or species of *Ganoderma*. Further, *G. lucidum* samples from different geographical regions or strains could be distinguished by hierarchical cluster analysis (HCA) and principal component analysis (PCA) of fingerprint data of polysaccharide hydrolyzates.

2. Experimental

2.1. Materials and chemicals

Eleven batches of dried *G. lucidum spores* and eleven batches of *G. lucidum* fruiting bodies were collected from different geographical regions, and numbered them from 1 to 22, respectively (as shown in Table 1). Ten batches of dried *G. sinense* fruiting bodies and five of *G. sinense* spores were purchased from Jilin, Jiangxi, and Shandong, and so on, and numbered from 23 to 37, respectively.

Glucose (Glc), galactose (Gal), mannose (Man), arabinose (Ara), ribose (Rib), xylose (Xyl), fucose (Fuc), rhamnose (Rha), glucuronic acid (GlcA), and galacturonic acid (GalA) were purchased from Sigma-Aldrich Co. (St. Louis, USA). Glucosamine (GlcN) and galactosamine (GalN) were purchased from Acros Organics. Dextran standards were offered by National Institute for the Control of Pharmaceutical and Biological Products. 1-Phenyl-3-methyl-5-pyrazolone (PMP) was obtained from Acros (Glee, Belgium). HPLC-grade acetonitrile was purchased from America Tedia Co. Trifluoroacetic acid (TFA) was purchased from Sinopharm Chemical Reagent Co., Ltd. The water was purified by a Milli-Q water purification system (Millipore Inc., Milford, MA, USA). All the other reagents were of analytical-reagent grade or HPLC-grade.

2.2. Extraction of polysaccharides

Fruiting bodies and spores of *Ganoderma* were dried at 35 °C for 12 h in electric vacuum drying oven. The fruiting bodies were broken into small pieces, and the non-broken spores were ground to break wall. Then 2.0 g of fruiting bodies or spores were extracted with water in three kinds of auxiliary conditions, respectively (fruiting bodies need to be soaked overnight in water prior to extraction). The extracting solution was concentrated, and then precipitated by the addition of ethanol to final concentration of 75% (v/v). After keeping it overnight (>12 h) under 4 °C, the mixture was centrifuged (8000 r/min, 10 min). Then, the precipitates were washed with 10 mL of 95% ethanol twice and then the residual ethanol was removed on water bath at 60 °C. The dried residues were dissolved in 5 mL hot water (60 °C). After centrifugation, the supernatant was used for subsequent analysis or freeze–dried for standby application.

2.3. Optimization of extraction conditions

Three methods of ultrasonic-assisted extraction (UAE), microwave-assisted extraction (MAE), and accelerated solvent extraction (ASE) were employed for extracting the polysaccharides of *G. lucidum* spores. Based on single factor experiments, the conditions of three extraction methods were optimized using orthogonal design (Sun et al., 2009). The extraction yield and molecular weight distribution associated with different extract methods were taken as evaluating criterions. The extraction yield of polysaccharides was determined using phenol-sulfuric acid colorimetric method (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956) with glucose as reference standard at 490 nm. Then, the molecular weight distribution was determined using high performance size-exclusion chromatography (HPSEC) equipped with a refractive index detector (RID). The sample solution was injected on three of serially linked Shodex OHpak SB-803 HQ, SB-804 HQ, and SB-805 HQ (8.0 mmID × 300 mmL × 3). The injection volume was 20 μL, and the flow rate of 0.1 M sodium nitrate eluent was 0.8 mL/min at 30 °C.

2.4. Hydrolysis procedures for polysaccharides

Five hundred microliters of above-mentioned polysaccharide supernatant was mixed with 500 μL of the different concentration of TFA in a screw-cap vial. The mixture was hydrolyzed under nitrogen atmosphere according to single factor and orthogonal experiments. After cooling, 1 mL of methanol was added into the hydrolyzates, and then the mixture was dried by blowing nitrogen stream and heating of water bath. Then the same amount of methanol was again added and dried by the same method as above, and the procedure was repeated thrice for TFA to be removed. The dried hydrolyzates were redissolved in 1 mL of water for subsequent derivatization. Similarly, water treated as mentioned above was used as blank control.

2.5. Derivatization of hydrolyzates with PMP

Fifty microliters of the hydrolyzates were mixed with the same volume of 0.6 M sodium hydroxide in a small sample tube with lid. Then, 100 μL of 0.5 M methanolic solution of PMP was added to the mixture and mixed thoroughly by a vortex mixer, and kept at 70 °C water-bath for 100 min. After cooling, the mixture was neutralized with 120 μL of 0.3 M hydrochloric acid and diluted to 1 mL with water. Then, 1 mL of chloroform was added. After vigorous shaking and layering, organic phase was discarded. The operation was performed in triplicate, and finally the solution was passed through a 0.45 μm syringe filter for HPLC analysis. A standard solution, containing 12 kinds of monosaccharide (Rha, Ara, Xyl, Man, Glc, Gal, Fuc, Rib, GlcA, GalA, GlcN, and GalN, about 0.33 mg/mL for each), was treated as mentioned above.

Table 1
Samples information in this study.

Number	Strains	Cultivation method	Geographical region
1	*Ganoderma lucidum* (*Hanzhi*) spores	Log cultivated	Anhui
2	*Ganoderma lucidum* (*Hanzhi*) spores	Log cultivated	Hubei
3	*Ganoderma lucidum* (*Hanzhi*) spores	Log cultivated	Anhui
4	*Ganoderma lucidum* (*Meizhi*) spores	Log cultivated	Anhui
5	*Ganoderma lucidum* (*Meizhi*) spores	Bag cultivated	Shandong
6	*Ganoderma lucidum* (*Hanzhi*) spores	Log cultivated	Shandong
7	*Ganoderma lucidum* (*Hanzhi*) spores	Log cultivated	Shandong
8	*Ganoderma lucidum* (*Hanzhi*) spores	Log cultivated	Zhejiang
9	*Ganoderma lucidum* (*Meizhi*) spores	Bag cultivated	Heilongjiang
10	*Ganoderma lucidum* (*Hanzhi*) spores	Log cultivated	Shandong
11	*Ganoderma lucidum* (*Hanzhi*) spores	Log cultivated	Shandong
12	*Ganoderma lucidum* (*Meizhi*) fruiting bodies	Bag cultivated	Shandong
13	*Ganoderma lucidum* (*Meizhi*) fruiting bodies	Bag cultivated	Shandong
14	*Ganoderma lucidum* (*Meizhi*) fruiting bodies	Log cultivated	Liaoning
15	*Ganoderma lucidum* (*Hanzhi*) fruiting bodies	Log cultivated	Liaoning
16	*Ganoderma lucidum* (*Hanzhi*) fruiting bodies	Log cultivated	Zhejiang
17	*Ganoderma lucidum* (*Meizhi*) fruiting bodies	Log cultivated	Zhejiang
18	*Ganoderma lucidum* (*Rizhi*) fruiting bodies	Log cultivated	Fujian
19	*Ganoderma lucidum* (*Meizhi*) fruiting bodies	Log cultivated	Fujian
20	*Ganoderma lucidum* (*Meizhi*) fruiting bodies	Log cultivated	Yunnan
21	*Ganoderma lucidum* (*Hanzhi*) fruiting bodies	Log cultivated	Zhejiang
22	*Ganoderma lucidum* (*Meizhi*) fruiting bodies	Log cultivated	Liaoning

2.6. PMP-RP-HPLC-DAD analysis

The analysis of PMP derivatives prepared in Section 2.5 was performed on an Agilent 1100 series HPLC system consisted of a G1379A vacuum degasser, G1311A Quaternary pump, G1313A auto-sampler, and G1315B diode array detector. A 20 μL of derivatives was injected onto a ZORBAX Eclipse XDB-C18 HPLC column (250 mm × 4.6 mm i.d., 5 μm) (Agilent, USA) operated at 30 °C, and eluted with a mixture of 0.1 M phosphate buffer (pH 6.7) and acetonitrile (83:17, v/v) at a flow rate of 0.8 mL/min. UV detection wavelength was set at 245 nm.

2.7. Fingerprint profiling and similarity evaluation

RP-HPLC-DAD chromatogram data of all samples were submitted for analysis by the professional software named "Similarity Evaluation System for Chromatographic Fingerprint of traditional Chinese medicine (TCM)" published by China Pharmacopoeia Committee (Version 2004A). Then, the reference chromatogram was chosen and the peaks would be matched. Subsequently, the standard chromatogram was produced, and the similarity value of all input chromatograms relative to standard chromatogram would be calculated by using the cosine value of the angle (Xu et al., 2009; Zhou et al., 2011).

2.8. LC-ESI-MS analysis of PMP-derivatives

LC-ESI-MS analysis of PMP derivatives of saccharides was performed on an Agilent1200 series-6460 QQQ Mass Spectrometer. The system was controlled with MassHunter Workstation. The condition of HPLC separation was the same with Section 2.6 except the mobile phase consisted of 20 mM ammonium acetate buffer-acetonitrile (84:16, v/v). The ESI-MS experiment was carried out in positive ion mode. The drying gas flow rate was 10 L/min and nebulizer pressure 50 psi, capillary voltage: 4.0 kV; drying gas temperature: 300 °C; ion resource temperature: 100 °C. The scan mass range was set from m/z 100 to 1500.

2.9. HCA and PCA

HCA and PCA were carried out based on the data matrix, which were formed by relative peak areas of monosaccharide and oligosaccharide common peaks in fingerprint chromatograms using IBM SPSS statistics software.

3. Results and discussion

3.1. Extraction of polysaccharides

In our study, *G. lucidum* spores (sample 9) was selected for optimizing extraction conditions. The optimal extraction condition of each extraction method was as follows: UAE was at 70 °C for 50 min at 250 W and the ratio of solid to water was 1:20; The MAE program was performed at 600 W and 110 °C for 20 min, and the extraction by ASE was performed in a 34 mL stainless steel cell, kept at 130 °C and 1500 psi with 5 min preheating time, then 10 min static time and 120 s purge time for a total of two cycles.

Under the optimal extraction condition, extraction yield of polysaccharides increased obviously as follows: MAE (0.86%) < UAE (1.15%) < ASE (1.23%). Simultaneously, the result of molecular weight distribution by HPSEC-RID showed that the ratio of polysaccharides in which molecular weight was more than 10 kDa was evidently different: ASE (65.01%) > UAE (63.85%) > MAE (53.11%). The results were revealed in Table 2. Molecular weight distributions of polysaccharides from the same *G. lucidum* spores were different due to the different extraction methods. To my knowledge, this may be mainly due to the degradation of polysaccharide structure to some extent under the huge power of ultrasonic or microwave (Tao and Xu, 2008; Wang et al., 2010). The results showed that the degradation effect of microwave was obviously higher than ultrasonic. The molecular weight distributions by UAE and ASE were very similar, but UAE was more simple and efficient in practice. So, ultrasonic assisted extraction (UAE) was employed for getting polysaccharides for subsequent HPLC analysis.

3.2. Hydrolysis of polysaccharides

In the present study, polysaccharides of *G. lucidum* spores were chosen to optimize partial acid hydrolysis conditions. Under the hydrolysis procedure in Section 2.4, the sum of common peak areas in HPLC fingerprints related to composition and structure of polysaccharides was selected as a criterion for characterizing the polysaccharides comprehensively. The effect of the concentration of TFA, temperature and time on the hydrolysis was investigated

Table 2
Comparision of extraction yield and molecular weight distribution of polysaccarides extracted by three methods.

Extraction method	Extraction yield (%)	Molecular weight distribution (area %)				
		>1000 kDa	100–1000 kDa	10–100 kDa	2–10 kDa	<2 kDa
UAE	1.15	6.98	21.42	35.45	22.65	13.51
MAE	0.86	6.08	13.01	34.02	26.53	20.36
ASE	1.23	7.70	17.22	40.09	19.93	15.06

through single factor experiments. Then, based on single factor experiments, the conditions of hydrolysis procedure were optimized by orthogonal test. Results showed that the optimal conditions for partial acid hydrolysis were 1.0 M TFA for 70 min at 110 °C. So, in this study, this hydrolysis condition would be used for characterizing the polysaccharides from different *Ganoderma*.

3.3. Selection of chromatography methods

High sensitivity analysis methods of monosaccharide and oligosaccharide mainly include HPLC with pre-column derivatization (Honda et al., 1989; Meyer, Raba, & Fischer, 2001; Yang, Chang, Weyers, Sterner, & Linhardt, 2012; Zhang, Wang, Xie, Nie, & Huang, 2010), high performance thin layer chromatography (HPTLC) (Di, Chan, Leung, & Huie, 2003), high performance anion exchange chromatography (HPAEC) (Cordella, Militão, Clément, Drajnudel, & Bass, 2005; Xie et al., 2013), gas chromatography (GC) (Chen, Xie, Wang, Nie, & Li, 2009; Li, Fan, & Ding, 2011), and so on. Di et al. have researched on the fingerprints of *Ganoderma* polysaccharides hydrolyzates exploringly by HPTLC and found that the differences between the two important *Ganoderma* (*Ganoderma applanatum* and *G. lucidum*) polysaccharides fingerprints were so obvious as to be differentiated. However, the resolution of many components in hydrolyzates was not perfect and for the two related species of *Ganoderma* (*G. lucidum* and *Ganoderma nigrolucidum*) and different parts of *G. lucidum* (fruiting bodies and spores), the differences of their fingerprints were not apparent, and it cannot effectively tell the differences between samples. In addition, the hydrolyzates of *Ganoderma* polysaccharides may be analyzed using HPAEC, but it needed two column systems (for example, CarboPac PA 200 and CarPac PA 20 column) to analyze monosaccharide and oligosaccharide, respectively, and also the data fusion in two systems was relatively troublesome. So the method of RP-HPLC with PMP pre-column derivatization (Dai et al., 2010), which is used more for analyzing polysaccharides in our lab, was still used in this study, and this method can separate and detect monosaccharides and oligosaccharides in polysaccharide hydrolyzates simultaneously.

3.4. Standard fingerprint profiling

According to the methods discussed in Section 2.7, 11 of *G. lucidum* spores polysaccharides and 11 of *G. lucidum* fruiting bodies polysaccharides were extracted, hydrolyzed, and analyzed by RP-HPLC. Fingerprints of polysaccharides from different parts of *G. lucidum* are shown in Fig. 1(a) and (c), and there were 16 and 18 common peaks, respectively. The total area proportion of all common peaks was more than 95%. Fingerprint information of the components in hydrolyzates was abundant, and the resolution of peaks was suitable to the demand of qualitative analysis. In addition, the fingerprint of 10 of *G. sinense* fruiting bodies polysaccharides was also established (as shown in Fig. 2(a) and (b)).

For polysaccharides from *G. lucidum* spores and fruiting bodies, the standard fingerprints were established by the software "Similarity Evaluation System for Chromatographic Fingerprint of

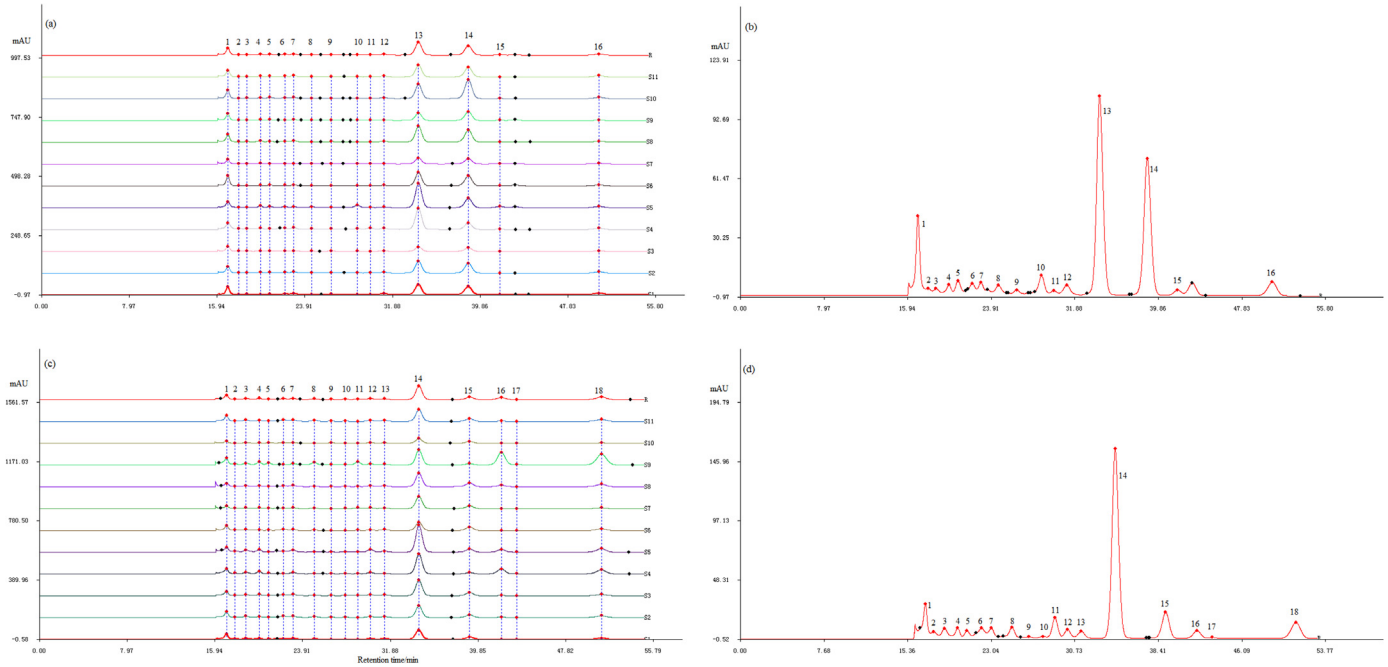

Fig. 1. Fingerprint of *G. lucidum* spores polysaccharides (a) (S1 to S11 is samples 1 to 11) and *G. lucidum* fruiting bodies polysaccharides (c) (S1 to S11 is sample 12 to 22). The chromatogram "R" of (a) was standard fingerprint of *G. lucidum* spores polysaccharides, (b) was an enlarged chromatogram "R" of (a) and the monosaccharide peak number 1, 5, 6, 8, 12, 13, 14, 15, 16 was Man, GlcN, Rib, GlcA, GalN, Glc, Gal, Xyl, Fuc, respectively. The chromatogram "R" of (c) was standard fingerprint of *G. lucidum* fruiting bodies (S1 to S11 is samples 12 to 22) polysaccharides, (d) was an enlarged chromatogram "R" of (c) and the monosaccharide peak number 1, 5, 6, 8, 13, 14, 15, 16, 17, and 18 is Man, GlcN, Rib, GlcA, GalN, Glc, Gal, Xyl, Ara, and Fuc, respectively.

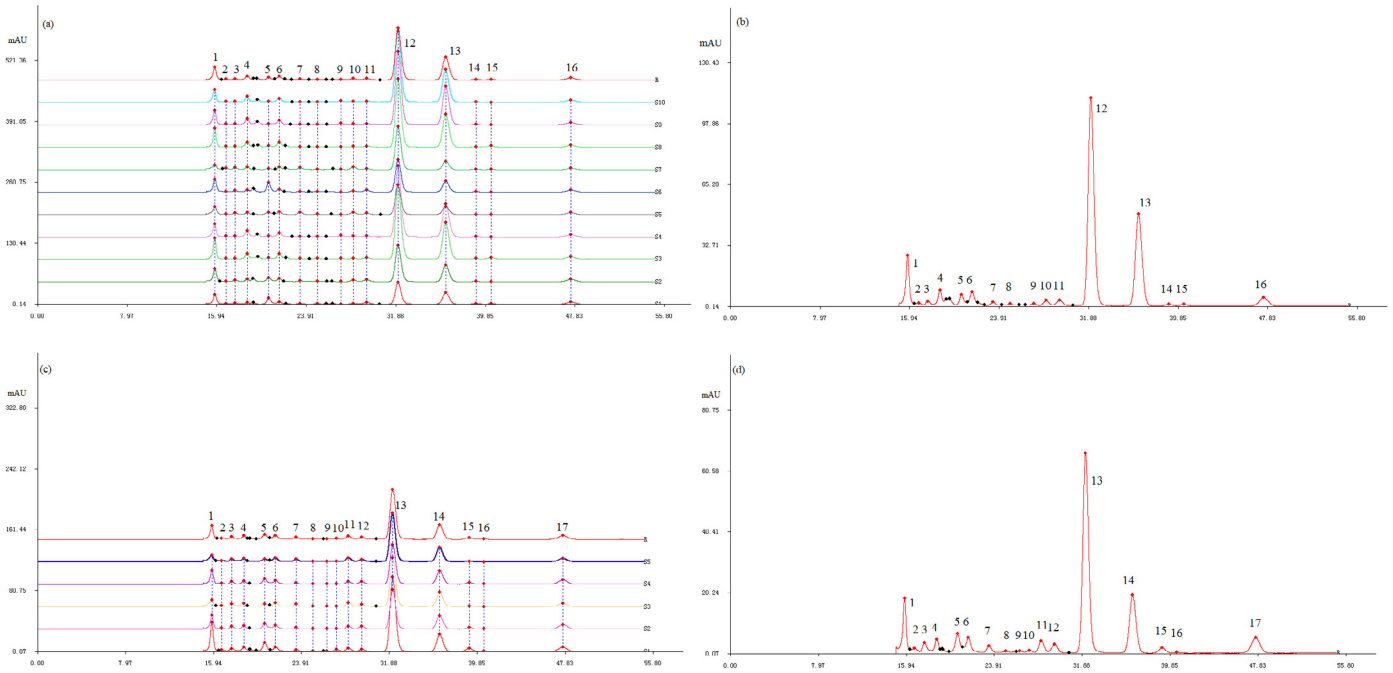

Fig. 2. Fingerprint of G. sinense fruiting bodies polysaccharides (a) and G. sinense spores polysaccharides (c). The chromatogram "R" of (a) was standard fingerprint of G. sinense fruiting bodies polysaccharides, and (b) was an enlarged chromatogram "R" of (a). The chromatogram "R" of (c) was standard fingerprint of G. sinense spores polysaccharides, and (d) was an enlarged chromatogram "R" of (c).

traditional Chinese medicine (TCM)" published by China Pharmacopoeia Committee (Version 2004A) as shown in Fig. 1(b) and (d), respectively. The mixed standard sample of 12 kinds of monosaccharide in Fig. 3 was used for qualitative analysis of monosaccharide peaks of *Ganoderma* samples in the same chromatographic conditions. According to the relative retention time of each fingerprint peak (the retention time of glucose as the reference), there were nine kinds of monosaccharide in all common peaks of *G. lucidum* spores polysaccharides and 10 kinds of monosaccharide in *G. lucidum* fruiting bodies polysaccharides as shown in Fig. 1(b) and (d), respectively. The other peaks were mainly oligosaccharides (as described in Section 3.8).

Based on the standard fingerprints, the similarity of fingerprints was calculated by the Similarity Evaluation System for Chromatographic Fingerprint of TCM (Version 2004A). The results showed that all the similarity values of each of *G. lucidum* spores polysaccharide with the standard fingerprint of *G. lucidum* spores polysaccharides were larger than 0.946. Likewise, all the similarity values of each of *G. lucidum* fruiting bodies polysaccharide with the standard fingerprint of *G. lucidum* fruiting bodies polysaccharides were larger than 0.936. It is clear that fingerprint characteristics of the polysaccharides of same part of *G. lucidum* from different geographical regions or strains were highly similar.

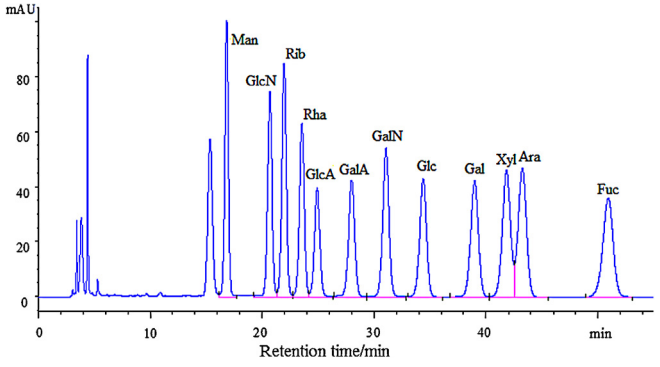

Fig. 3. Chromatogram of PMP derivatives of 12 kinds of monosaccharide by RP-HPLC.

3.5. Methodology validation

Precision of chromatographic separation and detection was investigated by injecting sample 9 in succession for 5 times under the condition of Section 2.6. The results showed that the relative standard deviations (RSDs) of relative retention time of common peaks were less than 0.23% and RSDs of relative peak area of common peaks were less than 1.86%, and precisions of chromatographic separation and detection were satisfactory. Meanwhile, sample 9 in quintuplicate was subjected to extraction, hydrolysis, derivatization reaction and HPLC determination according to the methods discussed in Section 2.2–2.6 for testing the method repeatability. The results revealed that RSDs of relative retention time were less than 0.73% and RSDs of relative peak area were less than 3.72%, and so the methods of sample preparation and chromatography analysis had good repeatability. Moreover, derivatives of acid hydrolyzates from sample 9 were stored at room temperature, and analyzed to evaluate stability of the derivative solution in 0, 5, 10, 15, 20, and 25 h, respectively. The results indicated that derivatives of hydrolyzates were stable, and RSDs of relative retention time were less than 0.33% and RSDs of relative peak area were less than 2.48%. All results indicated that the analysis method was able to meet the requirement of fingerprint analysis.

3.6. Comparison of fingerprint features of polysaccharides from different parts of G. lucidum

According to the above method, all the similarity values of each of *G. lucidum* fruiting bodies polysaccharides with the standard fingerprint of *G. lucidum* spores polysacchsrides were smaller than 0.823. Likewise, all the similarity values of each of *G. lucidum* spores polysaccharides with the standard fingerprint of *G. lucidum* fruiting bodies polysaccharides were smaller than 0.781. As known

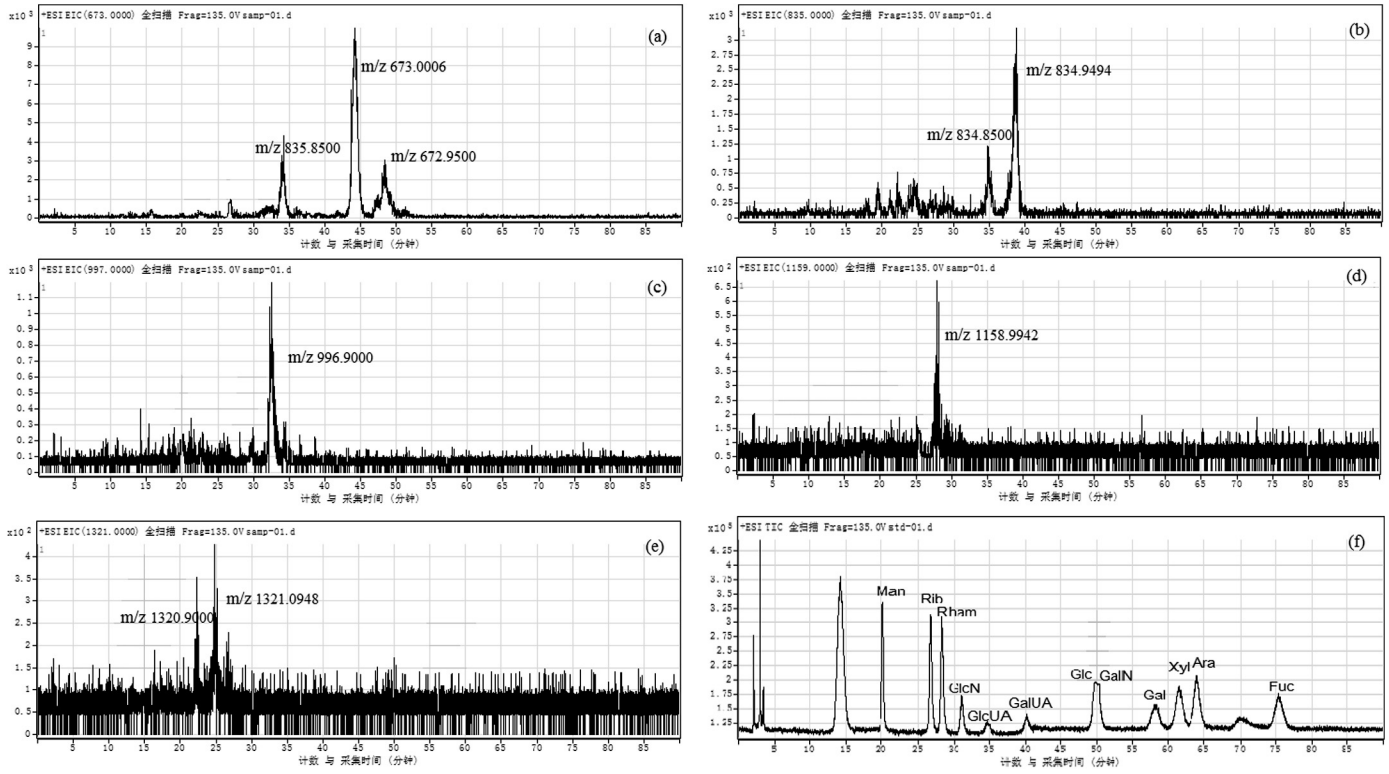

Fig. 4. The SIC of polysaccharides hydrolyzates in sample 19 and the TIC of PMP derivatives of 12 kinds of monosaccharide by LC-MS. (a) to (e) show the SIC of m/z 673.0, m/z 835.0, m/z 997.0, m/z 1159.0, m/z 1321.0, successively. (f) shows the TIC of PMP derivatives of 12 kinds of monosaccharide.

from the differences between the primary common characteristic peaks in the standard fingerprints of *G. lucidum* polysaccharides from the two parts in Fig. 1(b) and (d), the relative content of Gal and Man in *G. lucidum* spores polysaccharides were higher than in the *G. lucidum* fruiting bodies, but the relative content of Xyl in *G. lucidum* spores polysaccharides was lower. Meanwhile, by comparing the standard fingerprint of *G. sinense* fruiting bodies polysaccharides with *G. sinense* spores polysaccharides in the same chromatographic conditions as shown in Fig. 2 (c) and (d), the differences between them were significant, the relative content of Xyl in *G. sinense* spores polysaccharides was higher but the relative content of Gal were lower. The above results indicated that both similarity value and fingerprint characteristics of polysaccharides from different parts of *Ganoderma* had greater differences, and these differences and similarities can be the main basis of authenticity identification and quality control of different *Ganoderma* materials used for preparations of TCM and their products.

3.7. Fingerprint characteristics of polysaccharides from different species of Ganoderma

Comparing the fingerprint of *G. sinense* spores polysaccharides with the standard fingerprint of *G. lucidum* spores or the fingerprint of *G. sinense* fruiting bodies with the standard fingerprint of *G. lucidum* fruiting bodies polysaccharides, all the similarity values were smaller than 0.816 and 0.831, respectively. Comparing the differences between the main fingerprint characteristic peaks, it was clear that the relative content of Gal in *G. sinense* spores polysaccharides was lower as shown in Fig. 1(b) and Fig. 2 (d), and the relative content of Xyl in *G. lucidum* fruiting bodies was higher in Fig. 1 (d) and Fig. 2 (b). It indicated that the distinctions between fingerprint characteristic peaks of polysaccharides from different species *Ganoderma* samples were more obvious, and these differences made it easier to distinguish them.

3.8. Qualitative judgment of oligosaccharides peaks by LC-ESI-MS

For qualitative analysis of oligosaccharide peaks in the fingerprints of partial acid hydrolyzates, several unknown peaks except monosaccharide peaks were identified by LC-ESI-MS. The analytical condition was described in Section 2.8, and only the order of aminosugar peaks (GlcN relative to Rha, GalN relative to Glc) was different. The others were still consistent, and the relative peak's height was not changed (Fig. 3 and Fig. 4(f)). Taking sample 19 as an example, Fig. 4(a) to (e) was selective ion chromatogram (SIC) of oligosaccharides in LC-MS, respectively. The information of each peak in mass spectrogram (the mass-to-charge ratio of single-charged ion of PMP derivatives of monosaccharide and oligosaccharide) was shown in Table 3. Then, the corresponding unknown peaks 2, 3, 4, 7, 9, 10, 11, and 12 in Fig. 1(d) were hexasaccharide, another hexasaccharide isomer, pentasaccharide, tetrasaccharide, trisaccharide, another trisaccharide isomer, disaccharide, and another disaccharide isomer, respectively. Likewise, the corresponding peaks 2, 3, 4, 7, 9, 10, and 11 in Fig. 1(b) were hexasaccharide, another hexasaccharide isomer, pentasaccharide, tetrasaccharide, trisaccharide, disaccharide, and another disaccharide isomer, respectively. In conclusion, the information of monosaccharide and oligosaccharide in hydrolyzates could be characterized simultaneously by RP-HPLC using PMP pre-column derivatization, and the analysis method aimed at partial acid hydrolyzates may be used to identify *Ganoderma* polysaccharides with different chemical composition and structure.

3.9. HCA of Ganoderma polysaccharides

The relative areas of monosaccharide and oligosaccharide common peaks of all fingerprint chromatograms of polysaccharides hydrolyzates from *Ganoderma* formed the data matrix. Then, the data matrix was imported into IBM SPSS Statistics software for HCA.

Table 3
Peak identification of the monosaccharide and oligosaccharide of derivatives of polysaccharide hydrolyzates in sample 19 by LC-MS.

Peaks	$[M+H]^+$	Saccharides
1	510.9500	Man
2	1320.9000	Hexasaccharide
3	1321.0948	Hexasaccharide (isomer)
4	1158.9942	Pentasaccharide
5	480.8500	Rib
6	510.0000	GlcN
7	996.7500	Tetrasccharide
8	525.1002	GlcA
9	834.9000	Trisaccharide
10	834.9494	Trisaccharide (isomer)
11	673.0006	Disaccharide
12	672.9500	Disaccharide (isomer)
13	510.9500	Glc
14	510.1000	GalN
15	511.0000	Gal
16	480.9500	Xyl
17	480.9500	Ara
18	495.0000	Fuc

The dendrogram was shown in Fig. 5(a) and reflected the whole process of clustering intuitively. Samples from 1 to 11 were classified as a major category, namely *G. lucidum* spores; samples from 12 to 22 were classified as another major category, namely *G. lucidum* fruiting bodies. Samples from 23 to 32 were *G. sinense* fruiting bodies, and samples from 33 to 37 were *G. sinense* spores.

3.10. PCA of Ganoderma polysaccharides

The data matrix as mentioned in Section 3.8 was submitted into the software for PCA. The results revealed that on the basis of eigenvalues >1, the five principal components made up 86.817% of the total variance. Namely, the five principal components had contained most information of all variables. The score plot of the first three principal components PC1, PC2, and PC3 (Fig. 5(b)) visually showed the obvious differentiation between polysaccharides from different *Ganoderma*. That is, it can also be easy to distinguish between *Ganoderma* polysaccharides from different species or different parts by PCA of chromatographic fingerprint data.

In addition, though the results of similarity evaluation showed similarity value between different *G. lucidum* spores polysaccharides or different *G. lucidum* fruiting bodies polysaccharides were higher, *Ganoderma* polysaccharides from the same species and parts, but different origins had some differences as shown in Fig. 5. The influence of strains, cultivation ways and geographical regions on the similarities of polysaccharide fingerprint characteristics decreased in turn. Samples 5 and 9 were bag cultivated *G. lucidum* spores (the strains were both *Meizhi*) from Shandong and Heilongjiang province in China, respectively, the two were very similar. And sample 4 was log cultivated *G. lucidum* spores (the strains were also *Meizhi*) collected from Anhui province, it was only slightly different with the above two samples (sample 5 and sample 9). The strains of other spore samples were *Hanzhi* and they were cultured in log cultivation way but come from different geographical areas, so the similarities among these eight samples were larger but they were different with the former three obviously. Likewise, strains, cultivation ways and geographical regions had the similar effects on fingerprints of *G. lucidum* fruiting bodies polysaccharides, and the differences of polysaccharide fingerprint characteristics can be used for identifying different *Ganoderma* and their products. Samples 15 and 21 were *Hanzhi* from Anshan in Liaoning and Longquan in Zhejiang in log cultivation way. Sample 16 was also *Hanzhi* in log cultivation from Qingyuan in Zhejiang. The three strains were the same, and the similarities were higher. Samples 12 and 13 were different batches of bag cultivated *Meizhi* from Liangxian in Shandong,

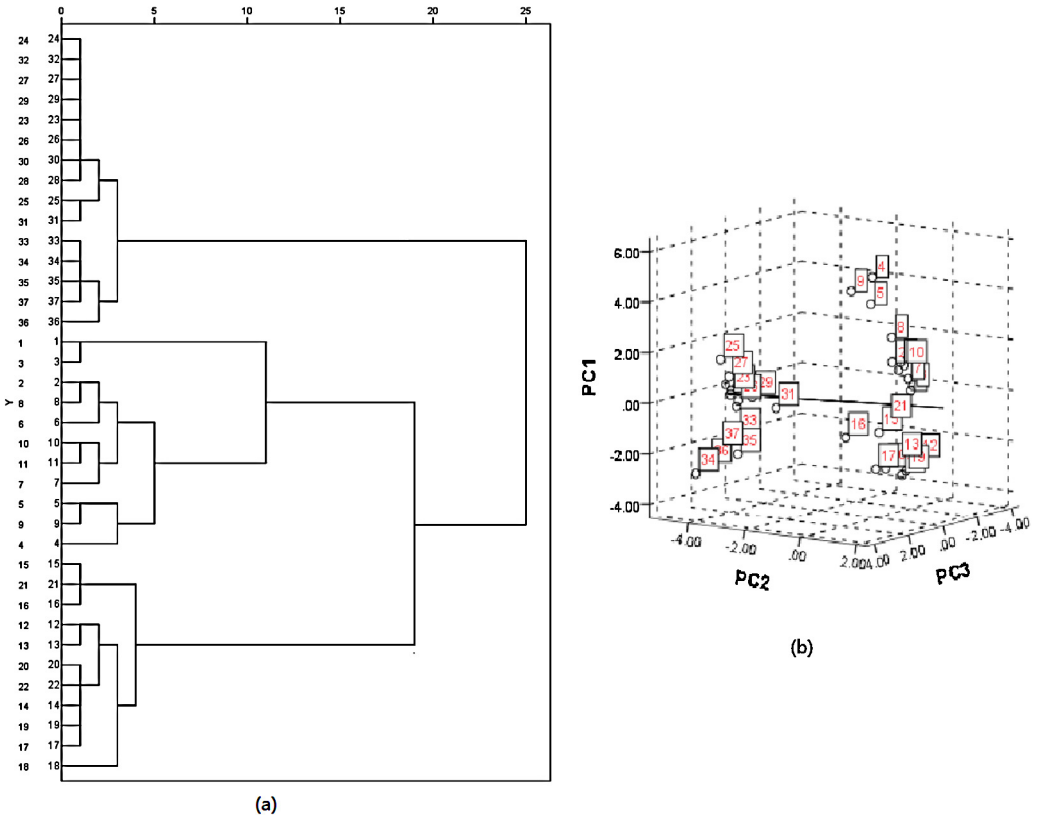

Fig. 5. The dendrogram of polysaccharides of different *Ganoderma* (a) and the 3D score plot of polysaccharides of different *Ganoderma* on the first three principal components (b).

so their differences were very small. Samples 14, 17, 19, 20, and 22 were *Meizhi* in log cultivation way from different geographical regions, so they were very similar. Sample 18 was from Fujian in log cultivation way and the strains were *Rizhi*. But for *G. sinense*, different samples were quite similar because we only collected samples with the same strains but different geographical regions or only in different harvest date.

4. Conclusions

Fingerprint analysis is a quantifiable, comprehensive, and effective analysis technique to evaluate the authenticity and quality stability of TCM and natural products. In this paper, the fingerprint profiles of *Ganoderma* polysaccharides were successfully established by RP-HPLC with PMP pre-column derivatization of partial acid hydrolyzates of the polysaccharides. The results of similarity evaluation, HCA and PCA of the fingerprints showed that *Ganoderma* polysaccharides from different parts or different species had their own chromatographic fingerprint characteristics, and the strains, cultivation ways and geographical regions had some influences on the similarities and differences of fingerprints. Therefore, the differences and similarities may be applied to identification and quality control of *Ganoderma* materials from different origins used for the preparation of TCM and *Ganoderma* polysaccharides products as health foods. The fingerprint analysis method established in this research had good repeatability and high chromatographic resolution of the hydrolyzates related to unique structural features and chemical compositions of the polysaccharides, so it possesses practical value as a kind of important means of quality monitoring of *Ganoderma* products.

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