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Development of a chromatographic fingerprint for the chloroform extracts of *Ganoderma lucidum* by HPLC and LC–MS

Yi Chen, Yan Yan, Ming-Yong Xie *, Shao-Ping Nie, Wei Liu, Xiao-Feng Gong, Yuan-Xing Wang

State Key Laboratory of Food Science and Technology, Nanchang University, Nanchang 330047, People's Republic of China

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Abstract

A new high-performance liquid chromatographic (HPLC) fingerprinting method was developed for the quality control of *Ganoderma lucidum*. Twenty-nine batches obtained from three different origins in China were used to establish the fingerprint. The constituents of these samples were separated with a Kromasil C_{18} column (250 mm × 4.6 mm, 5 μm) by linear gradient elution using water–acetic acid (100:0.1, v/v) and acetonitrile as mobile phase components at a flow rate of 0.8 ml/min and detector wavelength at 254 nm. Mean chromatograms and correlation coefficients of samples were calculated by the software "Similarity Evaluation System for Chromatographic Fingerprint of TCM". There were 19 common peaks in this fingerprint. Eleven of these common peaks were tentatively identified with reference to literature data based on their liquid chromatography–electrospray ionization-tandem mass spectrometry (LC–ESI-MS) and UV data. This profile was then successfully used to identify and assess the differences among samples from various origins with the aid of similarity analysis. The diverse similarities among different samples indicated that the quality of *G. lucidum* was not stable and the products from different areas were inconsistent. All results showed that the developed fingerprint assay was specific and could further serve for quality identification and comprehensive evaluation of *G. lucidum*.

Keywords: High-performance liquid chromatography; Fingerprint analysis; *Ganoderma lucidum*; Mass spectrometry; Similarity analysis

1. Introduction

Ganoderma lucidum, a traditional Chinese medicine called Lingzhi, is one of the genuine *Ganoderma* in Chinese Pharmacopoeia [1], whose fruiting bodies, mycelia, and spores were traditionally used as a folk medicine for treatment of debility and weakness, insomnia, hepatitis, cardiovascular diseases, cancer, etc. [2–9]. During the past two decades, modern research has revealed that *G. lucidum* contains a variety of triterpenes, polysaccharides, nucleosides, steroids, fatty acids, alkaloids, proteins, peptides, amino acids, and inorganic elements [10–13]. The herb is widely distributed and used in China. The content of each component varies significantly due to difference in geographic origin, climate condition, environment and other factors. Therefore, controlling the quality of the herbal medicines and their derivatives is difficult. Besides, it is well known that the therapeutic effects of the traditional Chinese medicine (TCM) are based on the synergic effect of its bioactive compounds, which are totally different from that of chemical drugs. Determination of merely one or a few components is not adequately representative [14–16]. Therefore, a holistic approach for quality control is necessary.

Recently, the chromatographic fingerprint technique was regarded as a useful method to control the quality of the herbal medicines and their derivatives because this technique emphasizes the systemic characterization of compositions of samples and focuses on the identification and assessment of the stability of the components [17]. Accordingly, fingerprint technology was introduced and accepted by WHO and Chinese State Food and Drug Administration (SFDA), as a strategy for the quality evaluation for identification and quality control of TCM [18,19]. The chromatographic methods include high-performance liquid chromatography (HPLC), capillary electrophoresis (CE), gas chromatography (GC), X-ray and thin-layer chromatography (TLC) [20,21]. It is worth noting that the authentication of commercial samples of *G. lucidum* was generally carried

* Corresponding author. Tel.: +86 791 3969009; fax: +86 791 3969069.
E-mail address: myxie@ncu.edu.cn (M.-Y. Xie).

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out by applying classical TLC procedures [22]. However, it is known that although TLC is fast and easy to operate, its precision is poor. Comparatively, HPLC fingerprint analysis has been regarded as the first choice [23,24] due to precision, sensitivity and reproducibility [17,25].

Up to now, HPLC method had been widely used in the analysis of triterpenes and related compounds in *G. lucidum*. Cai et al. [26] reported the effective use of RP-HPLC (YWG-C_{18} column) for the determination of ganoderic acids contents, i.e., ganoderic acid A, ganoderic acid C, ganoderic acid D and ganoderic acid E, in cultured and wild *G. lucidum*, and this method was claimed to be highly suitable for the quality control of herbal formulations containing Lingzhi. Most recently, a binary gradient HPLC method for the simultaneous quantitative analysis of six triterpenoids, namely ganoderic acids C2, B, AM1, K, H and D in *G. lucidum* and its related species has been reported by Wang et al. [27]. Similarly, Gao et al. [28] developed an analytical system using HPLC with an ODS column for quantitative determination of 19 triterpene constituents, including 6 ganoderma alcohols (1–6) and 13 ganoderma acids (7–19) in the products of *G. lucidum*. Although many quantitative studies of the major triterpenoids in *G. lucidum* by HPLC have been reported [29–34], none of them involved the comparison among the *G. lucidum* from the main cultivation areas in China using HPLC fingerprinting technique and chromatographic patterns analysis based on chemometrics. Thus it is necessary to establish a universal and systematic approach to develop a convenient, and reliable chromatographic fingerprinting method for quality control of *G. lucidum*.

In the present study, high-performance liquid chromatography-photodiode array detection (HPLC-PAD) was used for this purpose. By using the professional software named computer-aided similarity-evaluation (CASE), multi-sample batches of samples were examined to generate a mean global chromatogram as the representative standard fingerprint chromatogram and the similarity of each chromatogram against the mean global chromatogram was also calculated. The chemical constituents were identified in the fingerprints based on the on-line liquid chromatography–electrospray ionization-tandem mass spectrometry (LC–ESI-MS) and UV techniques. The method provides useful information for quality control of *G. lucidum* crude drugs.

2. Experimental

2.1. Plant materials and reagents

29 samples of *G. lucidum* from Zhejiang, Shandong, and Anhui province were collected from their original cultivation places (Table 1). These raw herbs were labeled according to their sources. All of them were identified by Dr. Zhi-hong Fu (Jiangxi University of Traditional Chinese Medicine). Representative samples (the whole fruiting bodies of the fungus) were dried after collection to preserve and then were cut into smaller pieces and further ground into powder, passed through a 20-mesh (0.9 mm) sieve before analysis.

Table 1
Genoderma lucidum samples studied

Sample no.	Origin	Harvesting time
1–9	Jiaxiang, Shandong	July 2006
10–14	Taishan, Shandong	August 2006
15–19	Huangshan, Anhui	August 2006
20–24	Jinzhai, Anhui	August 2006
25–29	Longquan, Zhejing	September 2006

HPLC grade methanol and acetic acid from Merck (Darmstadt, Germany) were used for HPLC analyses. Deionized water was purified by a Milli-Q system from Millipore (Bedford, MA, USA). Analytical grade methanol from Huada Chemical Reagent Co. Inc. (Guangdong, China) was used for sample preparation. Other chemicals and solvents purchased from Tianjin Chemical Reagent Co. Inc. (Tianjin, China) were of analytical grade.

2.2. Preparation of sample solutions

An accurately weighed sample of 1.0 g ground powder was introduced into the flask, refluxed with 25 ml chloroform at a constant temperature of 70 °C for 2 h in a water bath (model HH-4, Guohua Electric Co., Jiangsu, China). The extraction process was repeated twice. The extracts were combined, filtered while hot and evaporated on a vacuum concentrator system (Rongsheng, Shanghai, China), and then diluted to volume with methanol in a 10-ml volumetric flask. A volume of 2 ml of the solution was filtered through a 0.45-μm syringe filter before analysis. An aliquot of 10 μl solution was injected for HPLC analysis.

2.3. HPLC instrumentation and chromatographic conditions

The HPLC analyses were performed on a Waters HPLC system (Milford, MA, USA) equipped with a Model 515 HPLC pumping system, a column oven, and a photodiode array detector coupled with a Millennium 32 Workstation software. The samples were separated on a reversed-phase column (Kromasil C_{18}, 250 mm × 4.6 mm i.d.) with a guard column (5 μm, 10 mm × 4.6 mm i.d.). The mobile phase consisted of 0.1% aqueous acetic acid (v/v, A) and acetonitrile (B) using a linear gradient program of 30–32% B in 0–40 min, 32–40% B in 40–60 min, 40% B in 60–65 min, 40–82% B in 65–70 min, 82–100% B in 70–85 min. The flow rate was 0.8 ml/min and column temperature 35 °C. PAD detector was set at 254 nm for acquiring chromatograms, UV spectra and 3D-plots were recorded between 200 and 400 nm.

2.4. LC–MS instrumentation and chromatographic conditions

The LC effluent was introduced into the ESI source at a post-column splitting ratio of 2:1. The mass spectra were acquired using a Finnigan LCQ Advantage ion trap instrument with an

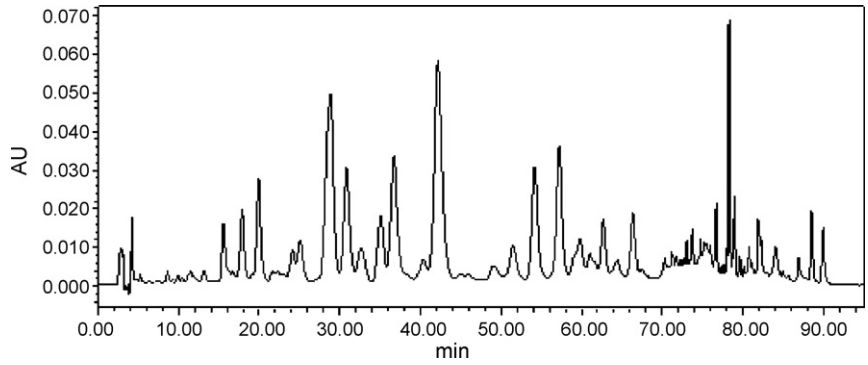


Fig. 1. Chromatograms of *Ganoderma lucidum* sample Kromasil C_{18} column (250 mm × 4.6 mm i.d. 5 μm); other experimental conditions are the same as in text.

ESI source (ThermoFinnigan, San Jose, CA, USA). Nitrogen was used as the sheath and auxiliary gas and helium was used as the collision gas. The ESI MS spectra were acquired in both positive and negative ion modes, and the interface and MSD parameters were as follows: sheath gas, 40 arbitrary units; auxiliary gas, 10 units; spray voltage, 4.0 kV; capillary temperature, 350 °C; capillary voltage, −10 V; tube lens offset, −30 V. For full scan MS analysis, the spectra were recorded in the range of *m/z* 50–1000.

2.5. Data analysis

Data analysis was performed by professional software named *Similarity Evaluation System for Chromatographic Fingerprint of Traditional Chinese Medicine* (Version 2004 A), which was developed and recommended by Chinese State Food and Drug Administration. In this study, the software was employed to synchronize and do quantitative comparison among different samples, as well as to compute and generate the mean chromatogram as a representative standard fingerprint chromatogram for a group of chromatograms. Then the correlation coefficients of samples with mean chromatogram could be provided.

Furthermore, the relative retention time (RRT) and relative peak area (RPA) of each characteristic peak related to the reference peak were calculated for quantitative expression of the chemical properties in the chromatographic pattern of herbs.

3. Results and discussion

3.1. Selection of suitable chromatographic conditions

In the course of optimizing the conditions of separation, the influence of the stationary and mobile phases were firstly investigated. Considering the presence of triterpenoids in the herbal extraction, a little amount of acetic acid was added to the mobile phase to reduce the ionization of these compounds. Different elution conditions with methanol–water, acetonitrile–water and different concentrations of acetic acid in water were compared to get the most suitable mobile phase. The results showed that best resolution and shortest analysis time were achieved when the acetonitrile–water/acetic acid (100:0.1, v/v) system was used. This system used in the further study differs from the earlier systems in the literature by the addition of acetic acid [31]. Because in the isocratic elution mode, some of the components in the sample have too long retention times and not all the compounds in the sample can be eluted, we used linear gradient elution (see Section 2.3). As seen in Fig. 1, not all the peaks but most of the main peaks could be well separated by the optimum gradient elution. It is not necessary (nor practically feasible) to strive for baseline separation of all components in one run, to fulfill the identification objectives of fingerprinting of herbal medicines. So the resolution under the optimized conditions was acceptable.

Two columns (Waters Symmetry Shield C_{18}, 250 mm × 4.6 mm i.d. 5 μm; Kromasil C_{18} column 250 mm × 4.6 mm i.d. 5 μm) were screened. The best selectivity and resolution can be observed on the Kromasil C_{18} column as shown in Fig. 1. Over 29 sharp and symmetrical peaks were obtained.

Column temperature was an important parameter influencing retention, selectivity, system pressure and column stability. 15, 25 and 35 °C were screened and the results indicated that 35 °C was the optimum temperature.

Selection of detection wavelength was one of the key factors contributing to a reliable and reproducible HPLC fingerprint of *G. lucidum*. Photodiode array detector (PAD) was applied to select the optimum wavelength. It was observed that the UV absorption maximum for compound 10 was at 254 nm where most of other compounds in the chromatogram possessed strong UV absorbance (Table 2). Hence, 254 nm was selected as the detection wavelength.

3.2. Method validation

3.2.1. Precision test

Injection precision was assessed by repetitive injections of the same sample solution six times in 1 day. The R.S.D. of relative retention time and relative peak area were lower than 0.43 and 2.8%, respectively.

3.2.2. Repeatability test

Repeatability was determined by analyzing six independently prepared samples of *G. lucidum* using the same method. We could find that R.S.D. of relative retention time and relative peak area were not more than 0.56 and 2.9%, respectively.

Table 2
HPLC-PAD and ESI-MS data and identification of constituents from the fruits of *Ganoderma lucidum*

Peak no.	t_R (min)[a]	Mass data	λ_{max} (nm)	Identification
1	15.71	531 $[M-H]^-$, 513 $[M-H-CO]^-$	256	Unknown
2	17.47	515 $[M-H]^-$, 497 $[M-H-H_2O]^-$	252	Unknown
3	19.99	517 $[M-H]^-$, 519 $[M+H]^+$	258	Ganoderic acid C_2
4	24.22	459 $[M-H]^-$, 415 $[M-H-CO_2]^-$	253	Lucidenic acid N
5	25.07	511 $[M-H]^-$, 513 $[M+H]^+$	259	Unknown
6	28.41	513 $[M-H]^-$, 495 $[M-H-H_2O]^-$	250	Unknown
7	30.78	515 $[M-H]^-$, 497 $[M-H-H_2O]^-$	256	Ganoderic acid B
8	32.83	513 $[M-H]^-$, 495 $[M-H-H_2O]^-$	260	Ganoderic acid AM_1
9	35.12	571 $[M-H]^-$,553 $[M-H-H_2O]^-$, 511 $[M-H-H_2O-CH_2=CO]^-$	249	Ganoderenic acid K
10	36.76	555 $[M-H]^-$, 513 $[M-H-CH_2=CO]^-$, 499 $[M-H-CH_2=CO-CH_2]^-$	253	Unknown
11	42.09	571 $[M-H]^-$, 553 $[M-H-H_2O]^-$, 573 $[M+H]^+$	258	Ganoderic acid H
12	51.30	511 $[M-H]^-$, 513 $[M+H]^+$	253	Ganoderenic acid D
13	54.16	511 $[M-H]^-$, 513 $[M+H]^+$, 493 $[M-H-H_2O]^-$, 495 $[M+H-H_2O]^+$	247	Unknown
14	57.16	513 $[M-H]^-$, 495 $[M-H-H_2O]^-$	256	Ganoderic acid D
15	59.93	–	250	Unknown
16	60.91	511 $[M-H]^-$, 513 $[M+H]^+$	259	Ganoderic acid F
17	62.65	499 $[M-H]^-$, 501 $[M+H]^+$	259	Ganolucidic acid D
18	64.33	–	250	Unknown
19	66.33	569 $[M-H]^-$, 551 $[M-H-H_2O]^-$, 571 $[M+H]^+$	256	12-Acetoxyganoderic acid F
20	73.74	–	254	Unknown
21	75.23	–	253	Unknown
22	76.68	–	253	Unknown
23	78.26	–	253	Unknown
24	78.76	–	254	Unknown
25	79.86	–	253	Unknown
26	81.14	–	280	Unknown
27	83.91	–	254	Unknown
28	88.53	–	253	Unknown
29	89.53	–	254	Unknown

[a] The retention time refer to the data in Fig. 2.

3.2.3. Sample stability test

Sample stability test was determined with one sample at regular intervals of 3 h in 24 h at 4 °C. The R.S.D. of relative retention time and relative peak area were found below 0.83 and 2.9%, respectively. The results indicated that the sample remained stable for 24 h.

3.3. Establishment of chromatographic fingerprint of cultured G. lucidum

3.3.1. Selection of the samples

Altogether 29 *G. lucidum* samples were analyzed (Table 1). These samples were collected from a variety of locations and conditions to ensure that the reference fingerprint thus developed was geographically representative and authentic.

3.3.2. Selection of reference substance

To calculate the RRT and RPA, a reference substance should be chosen [24]. There are two kinds of reference substances: one is an internal reference substance which belongs to common peaks and the other is an external reference substance which is added to the sample. In this study, the peak no. 11 (RT = 42.1 min, ganoderic acid H, Fig. 2) was chosen as the internal reference substance because this peak, which was present at the middle of the chromatogram with maximum content, existed in all chromatograms. The RRT and RPA of common peaks in the simulative mean chromatograms of *G. lucidum* from different origins were calculated, and the data of RPA are shown in Table 3.

3.3.3. Analysis of the chromatographic fingerprint of all the cultured G. lucidum

29 batches of samples from different areas in China were analyzed (see Tables 1, 3 and 4). The average chromatogram from the 29 batches was regarded as the standardized characteristic fingerprint of *G. lucidum*. Peaks existing in all chromatograms of the 29 samples were assigned as "common peaks". The chromatograms of *G. lucidum* from the 29 samples consisted of 29 distinct common peaks within 90 min, more than that in previous report, shown in Fig. 2. For convenience of recognition, the total fingerprint was divided into four sections; section 1 contains peaks 1–5 (retention time region from 13 to 26 min); section 2 contains peaks 6–11 (retention time region from 27 to 43 min); section 3 contains peaks 12–19 (retention time region from 45 to 67 min); section 4 contains peaks 20–29 (retention time region from 69 to 92 min). Reviewing the line chart generated from the original HPLC fingerprint (the bottom of Fig. 2) it is very easy to recognize the chromatographic patterns of the various sections. This can be considered as a characteristic HPLC fingerprint for *G. lucidum*.

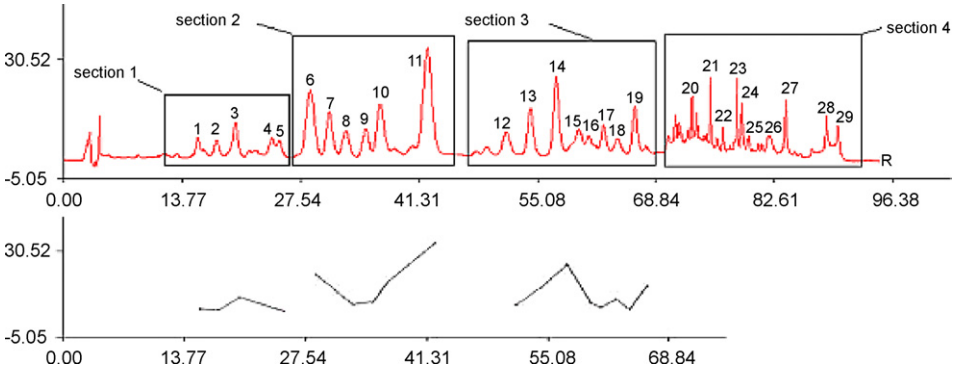


Fig. 2. HPLC fingerprints and line chart of 29 commercial samples of *Ganoderma lucidum* from different sources derived from computer-aided similarity-evaluation (CASE) software.

Table 3
The relative peak area (RPA) of characteristic peaks in simulative median chromatograms of *Ganoderma lucidum* from different origins

Peak no.	Huangshan		Jiaxiang		Jinzhai		Jingdangpu		Taishan		Longquan	
	RRT	RPA	RRT	RPA	RRT	RPA	RRT	RPA	RRT	RPA	RRT	RPA
1	0.369	0.145	0.375	0.231	0.370	0.099	0.372	0.117	0.368	0.190	0.373	0.111
2	0.423	0.114	0.433	0.121	0.425	0.145	0.426	0.070	0.423	0.136	0.415	0.078
3	0.472	0.289	0.464	0.294	0.476	0.256	0.475	0.187	0.471	0.220	0.475	0.169
4	0.569	0.061	0.579	0.151	0.576	0.046	0.573	0.151	0.571	0.268	0.580	0.132
5	0.587	0.055	0.598	0.208	0.597	0.112	0.594	0.080	0.592	0.143	0.601	0.107
6	0.684	0.639	0.682	0.681	0.686	0.386	0.681	0.645	0.678	0.698	0.675	0.495
7	0.731	0.415	0.736	0.286	0.735	0.296	0.733	0.290	0.731	0.238	0.731	0.185
8	0.774	0.161	0.779	0.257	0.774	0.120	0.777	0.211	0.775	0.298	0.780	0.205
9	0.830	0.136	0.835	0.273	0.834	0.086	0.832	0.216	0.830	0.313	0.834	0.117
10	0.871	0.417	0.872	0.290	0.871	0.484	0.870	0.351	0.869	0.301	0.873	0.304
11	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
12	1.218	0.109	1.217	0.296	1.218	0.114	1.210	0.277	1.209	0.253	1.219	0.240
13	1.277	0.411	1.282	0.300	1.280	0.268	1.276	0.320	1.276	0.254	1.287	0.184
14	1.354	0.367	1.354	0.473	1.352	0.393	1.348	0.563	1.347	0.462	1.358	0.581
15	1.414	0.208	1.418	0.268	1.389	0.181	1.387	0.280	1.383	0.252	1.424	0.218
16	1.445	0.146	1.442	0.119	1.438	0.118	1.435	0.147	1.430	0.094	1.447	0.173
17	1.484	0.210	1.486	0.083	1.484	0.237	1.477	0.123	1.472	0.107	1.488	0.156
18	1.521	0.338	1.520	0.061	1.520	0.084	1.514	0.065	1.508	0.041	1.528	0.070
19	1.571	0.352	1.566	0.256	1.566	0.127	1.560	0.306	1.557	0.241	1.576	0.265
TPA	13549.38		14909.5		23343.03		12411.51		7041.56		14001.35	

3.4. HPLC–MS analysis

3.4.1. General characters in MS patters and UV absorption

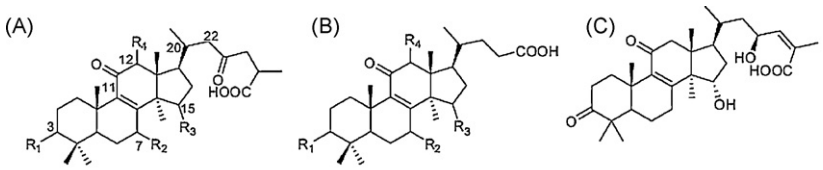
The triterpenoids compounds in *G. lucidum* could be classified into five groups according to their chemical structures and MS fragmentation patterns. The dominant fragmentation pathways of the compounds studied are losses of H_2O and CO_2; however, the cleavages of C- and D-ring, which produce a–e ions, etc., are characteristic features [31].

Characteristic UV absorption: according to literature data, UV spectra of the chromatographic fractions with their absorption maxima at 240–255 nm are characteristic of the

Table 4
The area proportion of characteristic peaks in fingerprints

Origin	Section 1			Section 2	Section 3		
	Peak 2/peak 1	Peak 4/peak 3	Peak 5/peak 4	Peak 8/peak 7	Peak 14/peak 13	Peak 16/peak 15	Peak 18/peak 17
Huangshan	0.78	0.21	0.90	0.39	*0.89*[a]	0.70	*1.61*[a]
Jiaxiang	0.52	0.51	*1.38*[a]	0.90	1.58	0.44	0.73
Jinzhai	*1.46*[a]	0.18	*2.43*[a]	0.41	1.47	0.65	0.35
Jingdangpu	0.60	0.81	0.53	0.73	1.76	0.53	0.53
Taishan	0.72	*1.22*[a]	0.53	*1.25*[a]	1.82	0.37	0.38
Longquan	0.70	0.78	0.81	*1.11*[a]	3.16	*0.79*[a]	0.45

[a] Characteristic value of peak ratios.



No.[a]	Compound name	Type	R_1	R_2	R_3	R_4	Double bond	MW
3	Ganoderic acid C_2	A	β-OH	β-OH	α-OH	H	---	518
4	Lucidenic acid N	B	β-OH	β-OH	=O	H	---	460
7	Ganoderic acid B	A	β-OH	β-OH	=O	H	---	516
8	Ganoderic acid AM1	A	β-OH	=O	=O	H	---	514
9	Ganoderenic acid K	A	β-OH	β-OH	=O	β-OAc	Δ20,22	572
11	Ganoderic acid H	A	β-OH	=O	=O	β-OAc	---	572
12	Ganoderenic acid D	A	=O	β-OH	=O	H	Δ20,22	512
14	Ganoderic acid D	A	=O	β-OH	=O	H	---	514
16	Ganoderic acid F	A	=O	=O	=O	H	---	512
17	Ganolucidic acid D	C	---	---	---	---	---	500
19	12-acetoxyganoderic acid F	A	=O	=O	=O	β-OAc	---	570

[a] the No. refers to the the peak No. in fig.2

Fig. 3. Chemical structures of the triterpenoids identified from *Ganoderma lucidum*.

20,22-unsaturated carbonyl group. Ganoderenic acids exhibit maximum absorption at 240–250 nm, and ganoderic acids at 250–256 nm [35–38].

3.4.2. Analysis of MS data of the main peaks

LC–MS was adopted to identify the chemical constituents of *G. lucidum*. ESI in both negative and positive modes was used for the detection of triterpenic acids. Negative mode ESI was found to be sensitive for triterpenic acids.

In the ESI-MS experiment, the molecular weight of each separated constituent was obtained. Then on the basis of the comparison of UV spectrum, HPLC retention time and mass spectra with reference to literature data [31] and a MS library (NIST), we tentatively identified and deduced the possible structures of 11 main constituents (see Table 2 and Fig. 3). However, owing to the unavailability of authentic compounds, the peaks could only be tentatively assigned. For unambiguous identification further studies are required by using authentic compounds.

3.5. Comparison of chromatographic fingerprints of G. lucidum from different origins

Samples from more than 20 sources were investigated (see Tables 1, 3 and 4). The "common peak" in each sample from different areas was generally similar. However, the concentration, distribution, and proportion of triterpenoids differ significantly depending on the location and climate, each presenting a unique fingerprint pattern as shown in Fig. 4.

When comparing the cumulative fingerprint and line chart of 29 samples with those from different origins we found that the samples from Jinzhai had the most similar patterns; all of the others were more or less different, as shown in Fig. 4. Although it

Table 5
The similarities of 29 chromatograms of *Ganoderma lucidum*

No.	Similarities[a]
1	0.962
2	0.959
3	0.961
4	0.964
5	0.968
6	0.921
7	0.824
8	0.902
9	0.789
10	0.949
11	0.938
12	0.929
13	0.875
14	0.935
15	0.964
16	0.88
17	0.864
18	0.756
19	0.956
20	0.942
21	0.961
22	0.968
23	0.901
24	0.941
25	0.892
26	0.94
27	0.957
28	0.946
29	0.93

[a] The reference fingerprint was developed with the median of all chromatograms.

is possible to visually differentiate the different chromatograms, however, the process is subjective and not quantitative. In addition, minor differences between very similar chromatograms might be missed. Hence, the HPLC chromatograms of *G. lucidum* samples were further quantitatively expressed in terms of RRT and RPA. In all the 29 samples, the total area of the former 19 peaks in sections 1–3 represented about 31.4–43.7% of the total area of all peaks in each chromatogram. They can serve as characteristic peaks for identification of "unknown" samples. Thus the RRT and RPA of the 19 characteristic peaks with respect to the reference peak were calculated (Table 3). The data clearly indicated that the relative amounts of these compounds in samples from different origins were different.

In order to further compare the constituents in the fingerprint of the samples from different origins, the area proportion of several characteristic peaks were calculated as shown in Table 4. In the line chart, for sections 1–3, the great differences among the peak intensities in the chromatograms were resulted from peaks

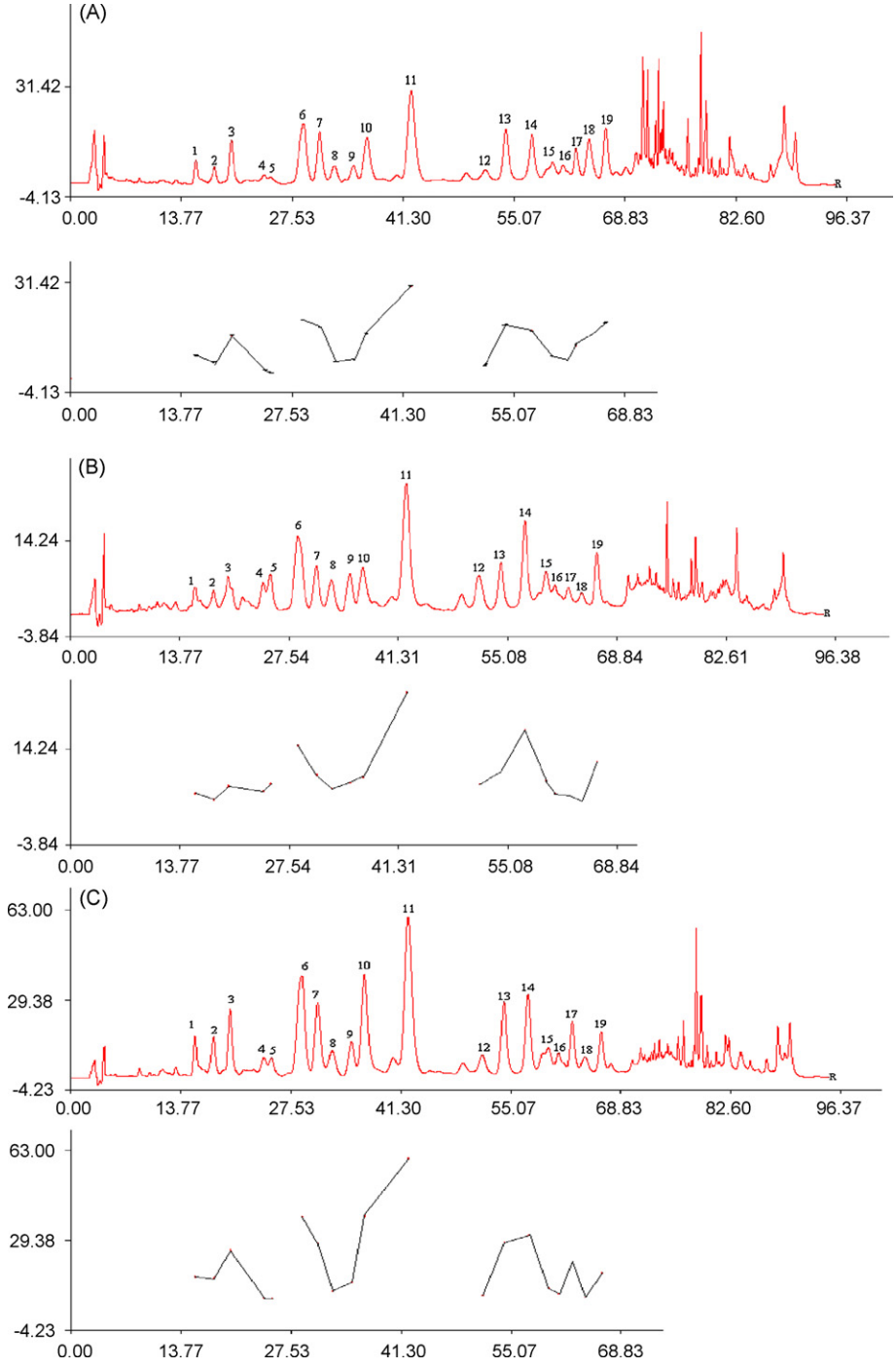


Fig. 4. HPLC fingerprint and the line chart of the *Ganoderma lucidum* from different origins: (A) five samples from Huangshan; (B) four samples from Jiaxiang; (C) five samples from Jinzhai; (D) five samples from Jingdangpu; (E) five samples from Taishan; (F) five samples from Longquan.

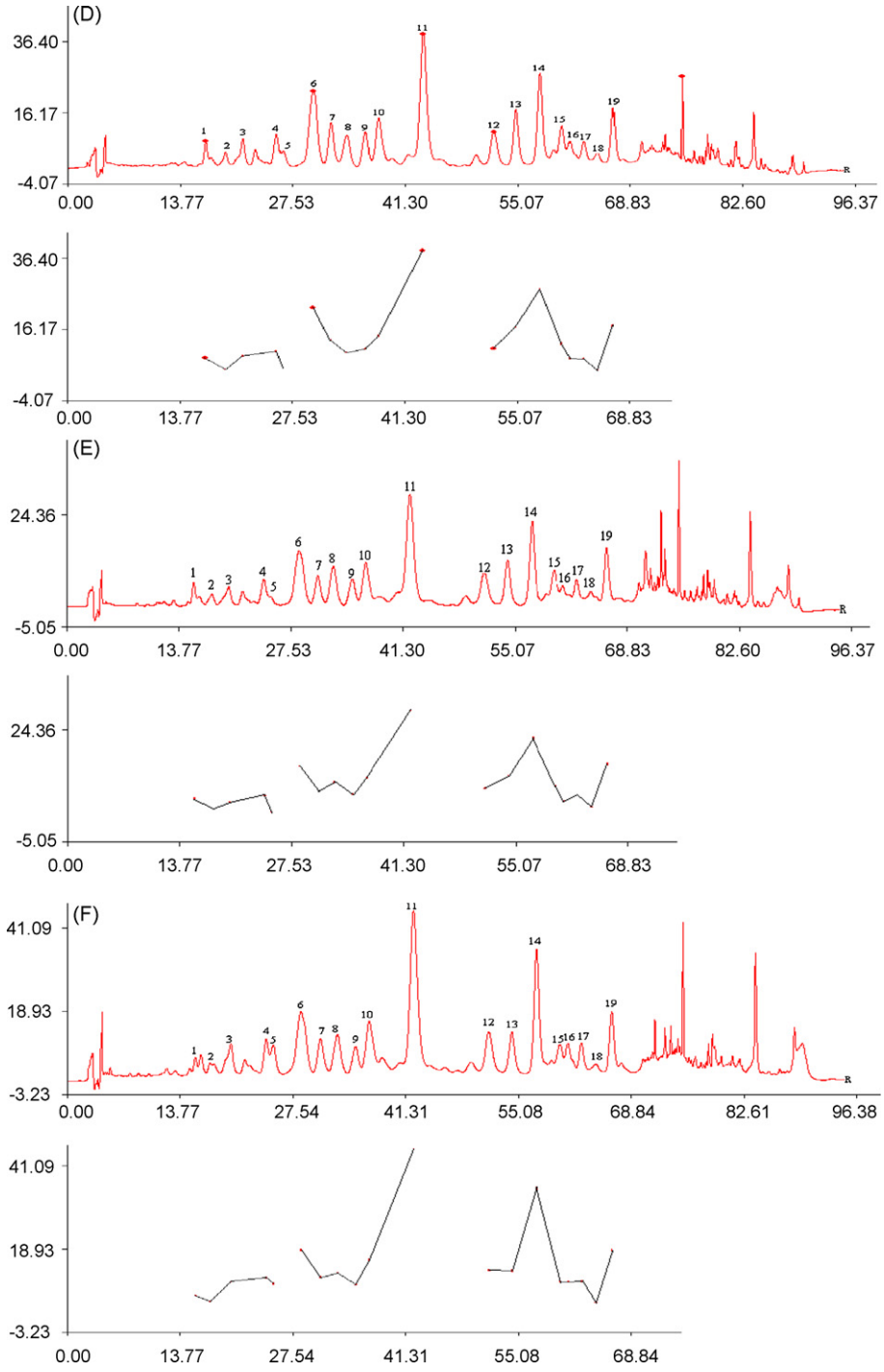


Fig. 4. (*Continued*).

2–5; 7 and 8; 14–16 and 18, respectively as shown in Fig. 4. Thus the area proportions of those peaks were calculated and the highest or the lowest value of each peak ratio was regarded as characteristic values. From the data in italics in Table 4, we found that one or two characteristic values of peak area ratios could be obtained for each fingerprint to discriminate itself from others.

Besides RRT and RPA, total peaks area (TPA) was employed to evaluate the total quantity of triterpenoids in each fingerprint of different origins. The values of TPA were found in the range of 7041–23,343 as shown in Table 3. The result indicated that there was a significant variability in the content of total triterpenoids among different *G. lucidum* samples along with their inconsistent chromatographic patterns.

In this case, the fingerprint pattern of the different samples was considerably different. However, using this information, it would be helpful to distinguish between them by comparing some of the unique characteristic peaks.

3.6. Similarity analysis

SFDA suggested that all of herbal chromatograms should be evaluated by their similarities, which come from the calculation on the correlation coefficient and/or angle cosine value of original data [39–41]. According to the relative peak areas of 29 common peaks in the chromatograms of 29 samples, the similarity analysis was conducted based on the standard fingerprints, and the results are shown in Table 5. The closer the cosine values are to 1, the more similar the two chromatograms are. All the similarity values of 29 samples are more than 0.80, except for samples 9, 18 but all were above 0.75. In practice, the herbs which have the smallest similarity values, or are below certain value, for example 0.8 can be regarded as not qualified. Therefore, if 0.80 is set as an appropriate threshold, it is easy to find that samples 9, 18 are unacceptable based on the chromatographic fingerprint.

4. Conclusions

The validity and advantage of applying HPLC chromatographic fingerprint for the quality evaluation of *G. lucidum* were established by systematically comparing chromatograms of samples from different origins. The fingerprint of the herb showing 19 "common peaks" represents the characteristics of this herb's constituents. However, the concentration, distribution, and proportion of these common peaks differ significantly depending on the origin, which was reflected by the diversity of similarities among the samples. It indicated that the quality of *G. lucidum* was not stable and the products from different areas were inconsistent. So in order to get the consistent raw materials of *G. lucidum*, the location of the collection should be fixed. These results have demonstrated that the chromatographic fingerprint combined with the similarity analysis may be acceptable in the general quality control of herbal medicines in the future.

In order to assess the quality of *G. lucidum* more adequately and comprehensively, may be more than one fingerprint will be needed due to the complexity of ingredients in herbal drugs. However, it could be acceptable as a first step to create a representative fingerprint, as presented here, and more fingerprints as new evidence will be studied later.

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