Establishment of GC-MS Fingerprint of Fresh *Houttuynia cordata*

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Fresh *Houttuynia cordata* THUNB. is a Chinese materia medica generally used in Chinese medicine therapy. It possesses the actions of clearing heat, eliminating toxins, reducing swelling, discharging pus and relieving stagnation. However, dry *H. cordata* has traditionally been used in clinical application instead of the fresh counterpart. In this paper, the chemical profiles of *H. cordata* were established using fingerprinting techniques. A modified GC-MS method was developed in the comparison of fingerprints among fresh and dry herbs of *H. cordata*. It was shown that the varieties, as well as relative levels of chemical components, in the fresh herb were more abundant than in the dry counterpart. Fingerprinting profiles were found to be consistent for fresh herbs acquired from various production areas, but the relative abundance of peaks were varied. Besides, the chemical components among different medicinal portions of fresh herbs were found to be inconsistent. The developed fingerprint can be successfully applied to distinguish between fresh and dry herbs, as well as determining differentiation among different medicinal portions.

Key words fingerprint; fresh *Houttuynia cordata*; GC-MS

A well-established quality assessment tool for Chinese materia medica (CMM) has always been a key issue for the rapid development of Chinese medicine. Among other analytical techniques, HPLC, capillary electrophoresis (CE), TLC, NMR and IR have been widely used in the quality evaluation of medicinal herbs.[1—3] The rapid development of fingerprinting methods using these ranges of analytical tools for authentication and quality assessment of CMM has recently been accepted by the World Health Organization (WHO) as a strategy for the assessment of herbal medicines.[1] Besides, it is also required by the Drug Administration Bureau of China as a quality assessment tool for standardizing all medicinal injections manufactured from traditional Chinese medicines and their raw materials.[2] Among these techniques, GC-MS is commonly used for characterization and is a particularly useful approach in the identification of volatile organic compounds in CMM.

Fresh *H. cordata* is the entire herb of fresh *Houttuynia cordata* THUNB., which is a well known traditionally used Chinese medicinal material in China and Japan and is listed in the Chinese Pharmacopoeia.[4] It possesses a variety of pharmacological activities including anti-platelet aggregation, antibacterial, antitumor, antimicrobial, anti-inflammatory, immunomodulatory and recently, it has demonstrated considerable efficacy in anti-SARS.[5—8] It was reported that *H. cordata* contained groups of such chemical components as flavones, fatty acids, essential oil and alkaloids.[9] In modern pharmacological terms, a volatile oil is regarded as the major bioactive compound possessing antibacterial, antifungal, anti-histamine and anti-viral activities.[6,10] The steam distillate of fresh *H. cordata* can inhibit herpes simplex virus type 1 (HSV-1), influenza virus, and human immunodeficiency virus type 1 (HIV-1).[11,12]

However, it is worth noting that the components of volatile oil are generally unstable and liable to decompose during the drying process.[11,13] Besides, some previous related research has stated that variation in medicinal parts, production areas and processing methods are also influential factors affecting the therapeutic efficacy of herbs.[14,15] In this regard, the present study seeks to develop a characteristic fingerprint of fresh *H. cordata* for authentication, with respect to the dry counterpart, and then apply it in quality assessment.

Herb samples were analyzed with GC-MS. The chemical components were identified by matching against the standard mass spectral database of National Institute of Standards and Technology (NIST147, NIST27). A combination of mathematics and statistical approaches were then applied to study the relationship of the acquired chromatographic profiles.

The Computer Aided Similarity Evaluation System is computer software recently developed by the Research Center of Modernization of Traditional Chinese Medicines (Central South University, Changsha, China), and is mainly applied in the similarity study of chromatographic and spectral patterns.[16—18] In this study, this software was used to synchronize the chromatographic peaks and calculate the correlation coefficients between entire chromatographic profiles, and to do quantitative comparison among different samples, as well as to compute and generate the simulative mean chromatogram as a representative standard fingerprint chromatogram for a group of chromatograms. Besides, the relative retention time (RRT) and relative peak area (RPA) of each characteristic peak related to the reference peak were calculated for the quantitative expression of chemical properties in the chromatographic pattern of herbs. The generated data provided valuable insights into the application of the fingerprint in the analysis/quality control of herbs. In this study, a total of 31 *H. cordata* samples were analyzed (Table 1). Sixteen common chemical compounds were identified in the GC-MS fingerprint. A reproducible GC-MS fingerprint technique was developed for the authentication of fresh and dry herbs. The methodology was found to differentiate between fresh and drug herbs, as well as different portions of the herb.

Experimental

Materials and Reagents Acetic ether was purchased from E. Merck (Darmstadt, Germany). Water was obtained from a Mili-Q (Millipore, Bedford, MA, U.S.A.) water purification system. Table 1 summarizes the information from the 31 test samples. All the samples were authenticated by Prof. Zhongzhen Zhao (School of Chinese Medicine, Hong Kong Baptist Univer-

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Table 1. A Summary of the Test Samples

No.	Sample code	Source	Sampling part	Time (year. month)
1	YA-1	YaAn, Sichun, China	Fresh entire herb	2004.8
2	YA-2	YaAn, Sichun, China	Fresh entire herb	2004.8
3	YA-3	YaAn, Sichun, China	Fresh entire herb	2004.8
4	YA-4	YaAn, Sichun, China	Fresh entire herb	2004.8
5	YA-5	YaAn, Sichun, China	Fresh entire herb	2004.8
6	YA-6	YaAn, Sichun, China	Fresh entire herb	2004.8
7	YA-7	YaAn, Sichun, China	Fresh entire herb	2004.8
8	YA-8	YaAn, Sichun, China	Fresh entire herb	2004.8
9	YA-9	YaAn, Sichun, China	Fresh root	2004.8
10	YA-10	YaAn, Sichun, China	Fresh root	2004.8
11	YA-11	YaAn, Sichun, China	Fresh leaf	2004.8
12	YA-12	YaAn, Sichun, China	Fresh leaf	2004.8
13	YA-13	YaAn, Sichun, China	Fresh leaf	2004.8
14	YA-14	YaAn, Sichun, China	Fresh stem	2004.8
15	YA-15	YaAn, Sichun, China	Fresh stem	2004.8
16	YA-16	YaAn, Sichun, China	Dry entire herb	2004.8
17	YA-17	YaAn, Sichun, China	Dry entire herb	2004.8
18	EM-1	Emei, Sichuan, China	Fresh entire herb	2004.8
19	EM-2	Emei, Sichuan, China	Fresh entire herb	2004.8
20	EM-3	Emei, Sichuan, China	Fresh entire herb	2004.8
21	EM-4	Emei, Sichuan, China	Fresh entire herb	2004.8
22	EM-5	Emei, Sichuan, China	Fresh entire herb	2004.8
23	EM-6	Emei, Sichuan, China	Fresh entire herb	2004.8
24	EM-7	Emei, Sichuan, China	Fresh entire herb	2004.8
25	EM-8	Emei, Sichuan, China	Fresh entire herb	2004.8
26	EM-9	Emei, Sichuan, China	Fresh entire herb	2004.8
27	EM-10	Emei, Sichuan, China	Fresh entire herb	2004.8
28	GD-1	Zhaoqing, Guangdong, China	Fresh entire herb	2004.8
29	GD-2	Zhaoqing, Guangdong, China	Fresh entire herb	2004.8
30	HK-1	HongKong, China	Fresh entire herb	2004.8
31	HK-2	HongKong, China	Fresh entire herb	2004.8

sity, Hong Kong, P. R. China). Freeze-dried processes were as follows: soon after collection, samples were placed in a −20 °C freezer. After pre-cooling for at least 12 h, samples were placed in the freeze-drying system for another 48 h, and finally sealed in airtight containers.

Samples were prepared by two methods: lyophilization (cryochem) for the fresh, and air-dried for the dry.[4] Some herb samples collected from YaAn were analyzed according to the different medicinal portions (leaf, stem, root and herb). All samples were crushed and passed through a 40-mesh sieve. Ground powders were stored at about 4 °C before use.

Instrumentation and Conditions Chromatographic separation was carried out on a SHIMADZU GC/MS-2010. The freeze-dryer (Model 77530) was purchased from Labconco Corporation (Switzerland).

The software Computer Aided Similarity Evaluation System, which was coded in MATTLAB 6.1 for Windows and run on a Pentium III 850 (Intel) personal computer, was employed to calculate correlation coefficients, then to generate simulative mean chromatograms.

Sample Preparation The essential oil was prepared according to the Chinese Pharmacopoeia.[19] Fresh *H. cordata* (100 g) and distilled water (1000 ml) were placed into an extraction apparatus and subjected to hydrodistillation for 6 h at 100 °C.

Analytical Condition Column: high resolution capillary column DM-1 (Dikma Technologies. Film thickness: 0.25 μm; column: 0.25 mm ID× 30 m). Temperature: initially, 60 °C for 10 min; then, increased to 100 °C at a flow rate of 1 °C/min, after that, a further increase at 3 °C/min to 280 °C, which was maintained for 5 min. Split injection was conducted with a split ratio of 30 : 1, and helium was used as the carrier gas at a rate of 0.8 ml/min, with the volume of injection as 1 μl. The mass spectrometer was operated in electron-impact (EI) mode, the scan range was 40—400 *m*/*z*, and the scan rate was 0.5 s/scan. Pre-column pressure: 70 kPa. Injection temperature: 250 °C. Ion source: EI (200 °C). Transfer line temperature: 280 °C. Electron energy: 70 eV. Solvent delay: 7 min.

Data Analysis of Chromatogram The correlation coefficients of entire chromatographic patterns among samples were calculated, and the simulative mean chromatogram was calculated and generated using the Computer Aided Similarity Evaluation System. Similarities of the entire chromatographic profiles were analyzed among tested samples. The RRT and RPA of each characteristic peak to reference peak were also calculated in the chromatograms.

Table 2. Results of the Method's Reproducibility, Precision and Stability

Peak no.	Reproducibility (n=5)		Precisions (n=5)		Stability (n=5)	
	RRT (%)	RPA (%)	RRT (%)	RPA (%)	RRT (%)	RPA (%)
1	0.05	2.84	0.07	2.86	0.27	2.70
2	0.05	2.85	0.06	2.33	0	1.18
3	0.05	2.41	0.07	2.68	0	2.17
4	0.05	2.99	0.06	2.28	0.25	2.04
5	0.04	2.79	0.05	2.15	0.25	1.21
6	0.03	2.90	0.06	2.12	0.22	2.08
7	0.03	2.04	0.06	1.85	0.16	2.02
8	0.04	2.11	0.06	1.81	0	1.73
9	0.03	1.75	0.06	2.37	0	2.95
10	0.03	2.19	0.06	1.72	0.12	1.48
11	0.01	1.33	0.03	0.84	0	1.22
12	0.04	1.32	0.04	2.95	0.05	2.54
13	0	0	0	0	0	0
14	0.03	0.65	0.03	0.70	0.04	1.82
15	0.06	2.71	0.05	2.72	0.04	2.64
16	0.06	1.82	0.05	2.53	0.05	1.55

Results and Discussion

Method Validation In order to achieve a stable and reproducible chromatographic fingerprint of *H. cordata* for the purpose of quality assessment, a comprehensive method validation on the developed GC-MS fingerprint analysis was conducted. Method precision was investigated by repeatedly analyzing the same set of samples, with the values of relative standard deviations (RSDs) for RRT and RPA, respectively, reported as less than 0.07% and 2.95% (n=5). Method reproducibility was evaluated by analyzing five individual samples over the period of investigations. The RSDs of RRT and RPA were found to be less than 0.06% and 2.99% (n=5), respectively. Stability testing was performed with a freshly prepared methanolic solution of fresh *H. cordata* over a period of 24 h. The reported RSDs of the RRT and RPA were found to be less than 0.27% and 2.95% (n=5), respectively. All of the above findings indicate that the developed methodology is applicable for establishing a GC-MS fingerprint of fresh *H. cordata* (Table 2).

GC-MS Fingerprint of Fresh *H. cordata* To establish a representative chromatographic fingerprint for fresh *H. cordata*, 10 authentic batches of fresh *H. cordata* acquired from Emei (major production area in Sichun Province, P. R. China) (EM-1 to EM-10) were analyzed using the established GC-MS method. By similarity match using the mass spectrometric libraries (NIST147, NIST27), a majority of the chemical components were identified. Individual results indicated that their chromatographic patterns were generally consistent to one another, although there existed some variations in peak abundance (Fig. 1). The correlation coefficient of each chromatogram to their simulative mean chromatogram was found to be 0.962±0.034 (mean±S.D., n=10).

Among the 10 acquired chromatograms, peaks having matched RRT with a reasonable abundance of RPA were chosen and assigned as common peaks for representing the characteristics pattern of fresh *H. cordata*. Altogether, 16

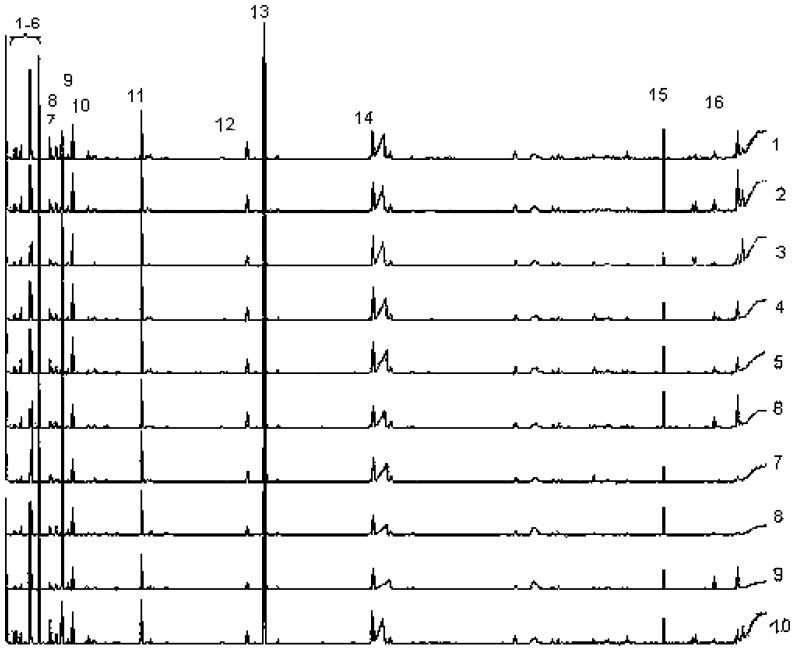

Fig. 1. GC-MS Fingerprint of 10 Batches of Fresh *H. cordata* Acquired from Emei

Table 3. The Relative Retention Time (RRT) and Relative Peak Area (RPA) of Sixteen Identified Peaks of Samples from Various Sources

Peak	Chemical compound	YA (n=8)		GD (n=2)		HK (n=2)		EM (n=10)	
		RRT	RPA	RRT	RPA	RRT	RPA	RRT	RPA
1	α-Phellandrene (**1**)	0.16	0.03±0.008	0.16	0.01±0.001	—	—	0.16	0.01±0.004
2	α-Pinene (**2**)	0.17	0.30±0.062	0.17	0.18±0.009	0.17	0.24±0.012	0.17	0.11±0.014
3	Camphene (**3**)	0.18	0.04±0.005	0.18	0.02±0.001	0.18	0.05±0.002	0.18	0.02±0.005
4	3-Carene (**4**)	0.22	1.04±0.366	0.21	0.14±0.006	0.21	0.01±0.001	0.21	0.27±0.043
5	β-Pinene (**5**)	0.22	0.40±0.064	0.22	0.37±0.018	0.22	0.39±0.016	0.22	0.14±0.020
6	β-Myrcene (**6**)	0.25	1.27±0.354	0.24	1.06±0.056	0.24	1.79±0.467	0.24	0.30±0.044
7	Terpinolene (**7**)	0.28	0.14±0.090	0.28	0.02±0.001	0.28	0.01±0.001	0.28	0.07±0.012
8	Limonene (**8**)	0.31	0.10±0.008	0.30	0.04±0.001	0.30	0.14±0.004	0.30	0.03±0.008
9	β-Ocimene (**9**)	0.32	0.45±0.144	0.32	0.02±0.004	0.32	0.68±0.011	0.32	0.40±0.118
10	γ-Terpinene (**10**)	0.35	0.32±0.070	0.35	0.03±0.001	0.34	0.03±0.001	0.35	0.10±0.015
11	Terpinen-4-ol (**11**)	0.59	0.68±0.160	0.58	0.06±0.001	0.58	0.01±0.001	0.59	0.22±0.028
12	Bornyl acetate (**12**)	0.94	0.05±0.016	0.93	0.07±0.001	0.94	0.16±0.001	0.94	0.08±0.018
13	2-Undecanone (**13**)	1	1	1	1	1	1	1	1
14	Geraniol acetate (**14**)	1.35	0.04±0.016	1.35	0.02±0.001	1.35	0.14±0.013	1.35	0.20±0.035
15	Isopentyl decanoate (**15**)	2.32	0.02±0.008	2.31	0.03±0.002	2.31	0.02±0.001	2.32	0.07±0.022
16	n-Hexadecanoic acid (**16**)	2.49	0.12±0.102	2.47	0.01±0.003	2.47	0.01±0.001	2.48	0.06±0.035

RRT and RPA are the ratio of retention time and peak area of each characteristic peak with reference to peak 13 (reference peak). The values of RPA are expressed as mean±S.D.

common peaks were located in the chromatograms (Table 3). Peak 13 (2-undecanone) was assigned as the reference peak because of its highest content and definite pharmacological actions.[11,20] RRT and RPA were used as the parameters for the analysis between each characteristic peak and the reference peak.

Comparative Analysis of Chromatographic Fingerprints of Fresh *H. cordata* among Various Sources Samples collected from Emei (EM-1 to EM-10), YaAn (YA-1 to YA-8), GuangDong (GD-1 to GD-2) and Hong Kong (HK-1 to HK-2) of P. R. China were analyzed under the same experimental conditions. The correlation coefficient between each chromatogram and respective simulative mean chromatogram were as follows: 0.970±0.022 (n=8, from YA), 0.999 (n=2, from HK), 0.900±0.004 (n=2, from GD). In general, their chromatographic fingerprinting profiles were highly similar to each other within the same source. However, the correlation coefficients among various sources were significantly different. Their correlation coefficients with respect to the simulative mean chromatogram were evaluated as 0.742 (EM *vs.* YA), 0.6192 (EM *vs.* GD), 0.5472 (EM *vs.* HK). A summary of similarity match is given in Table 4. Comparing the chemical components in various production areas, the contents of compounds **7**, **10** and **16** were found to be highest in YA. The peak area of compound **7**, for example, in YA was 7 times higher than that of GD, and about 14 times higher than that of HK and EM. On the other hand, the contents of compounds **14**, **15** were found to be highest in the sample from EM. It was observed that the RPA of compound **15** in the EM sample was 4 times higher than that

from HK and YA, and 2 times higher than that of GD. In contrast, the content of compound **12** was found to be highest in the HK samples, with the peak area generally doubled that from other sources. In this regard, fresh *H. cordata* of different production areas can be distinguished by comparing the relative contents of compounds **7**, **12** and **15**. The result of quantitative analysis of those 16 chemical components is given in Table 3.

Comparison of Chromatographic Fingerprint among Various Medicinal Parts of Fresh *H. cordata* Chromatographic fingerprinting can be applied in the differentiation of various medicinal parts of fresh *H. cordata*. A batch of samples from YaAn was divided into various medicinal parts, namely: entire herb, leaf, stem and root. Representative fingerprints and a list of the corresponding chemical compounds are given in Fig. 2 and Table 5, respectively. The correlation coefficients of the simulative mean chromatogram were found to be 0.834 (entire herb *vs.* leaf), 0.617 (entire herb *vs.* root), 0.559 (entire herb *vs.* stem), 0.191 (leaf *vs.* root), 0.163 (leaf *vs.* stem), 0.221 (root *vs.* stem). Comparing the chemical compounds observed in various medicinal parts (Table 5), the contents of compounds **2**, **5**, **9**, **12**, **14** and **10** were found highest in the leaf portion. Among these compounds, the RPA of compound **14** was almost 10 times more abundant than that in other medicinal parts. On the other hand, the level of compound **15** was found to be highest in the root. The corresponding RPA of compound **15** in the root was about 3 times higher than that in the stem and entire herb. Contrarily, the contents of compounds **4**, **9** and **16** were found to be highest in the entire herb. The RPA of compound **16** in entire herb was several times higher than that solely from the leaf and root regions.

The results indicated that the entire herb and leaf possessed high similarity in terms of overall chemical components. In contrast, the stem and root regions possessed low similarity with the entire herb.

Distinguishing between Fresh and Dry *H. cordata* In general, dry *H. cordata* was used as a medicinal material, and this practice was recorded in previous editions of Chinese Pharmacopoeia. However, the fresh counterpart has been added in the latest 2005 edition. In this regard, the corresponding fingerprint between these two herbs of different processing methods was compared. 8 fresh (YA-1 to YA-8) and 2 dry (YA-16 to YA-17) samples were compared together with their fingerprinting profiles. The correlation coefficient of each chromatogram to their simulative mean chromatogram was found to be 0.970±0.022 (n=8) for fresh and 0.962±0.031 (n=2) for dry herbs, respectively. The correlation coefficient of the simulative mean chromatogram between the fresh and the dry form was found to be 0.691. Comparing the chemical components between the fresh and dry herbs (Table 6), the contents of compounds **2**, **5** and **6** were higher for the former, with the RPA generally 10 times higher than that of the dry counterpart. In contrast, the contents of compounds **12** and **14** were higher in the dry form with the RPA of compound **14** almost 10 times higher than that in the fresh. It is worth noting that compounds **1**, **3**, **7**, **10** and **15** were not observed in the dry herbs, but were present in considerable abundance in the fresh counterpart. To sum up, the fresh and dry herb of *H. cordata* from YA possessed similar fingerprinting profiles. However, there existed drastic differences in terms of chemical composition and the corre-

Table 4. Similarity Comparison of Chromatographic Pattern for Fresh *H. cordata* from Various Sources

Sample	Entire herb (n=8)	Leaf (n=2)	Root (n=2)	Stem (n=2)
Entire herb	0.970±0.022[a)]			
Leaf	0.834[b)]	0.914±0.011[a)]		
Root	0.617[b)]	0.190843[b)]	0.966±0.017[a)]	
Stem	0.559[b)]	0.163081[b)]	0.22071[b)]	0.900±0.010[a)]

a) The correlation coefficient of each chromatogram to the simulative mean chromatogram, mean±S.D. *b*) The correlation coefficient between simulative mean chromatograms.

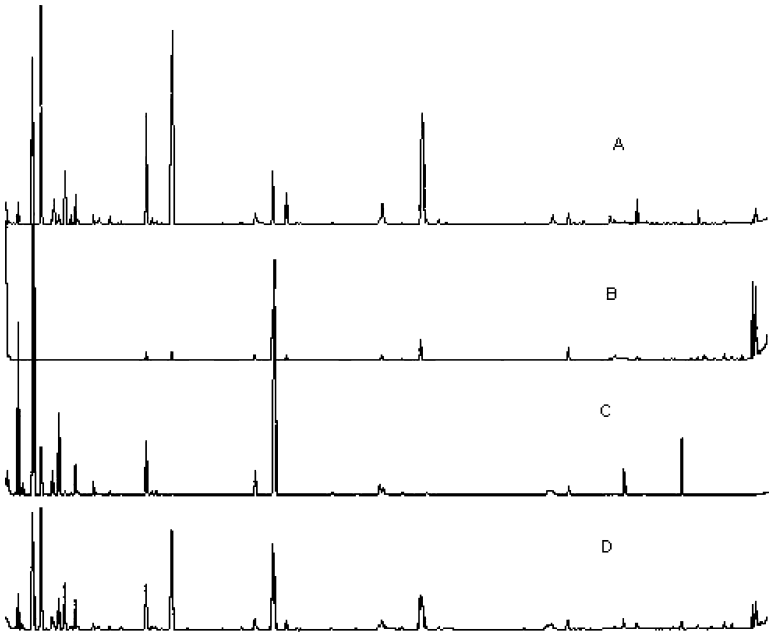

Fig. 2. The Total Ion Chromatogram (TIC) Obtained from the Essential Oil of Different Parts of *H. cordata*

A: leaf, B: stem, C: root, D: entire herb.

Table 5. The Relative Retention Time (RRT) and Relative Peak Area (RPA) of Characteristic Peaks for Samples among Different Medicinal Parts of *H. cordata* in YaAn

Peak no.	Leaf (n=3)		Root (n=2)		Stem (n=2)		Entire herb (n=8)	
	RRT	RPA	RRT	RPA	RRT	RPA	RRT	RPA
1	0.16	0.07±0.001	0.16	0.01±0.001			0.16	0.03±0.008
2	0.17	0.24±0.147	0.17	0.17±0.009			0.17	0.30±0.062
3	0.18	0.02±0.002	0.18	0.02±0.008			0.18	0.04±0.005
4	0.21	3.55±0.779	0.21	0.44±0.022			0.21	1.04±0.366
5	0.22	0.54±0.307	0.22	0.41±0.157			0.22	0.40±0.064
6	0.24	2.96±0.881	0.24	0.16±0.006			0.24	1.27±0.354
7	0.28	0.10±0.052	0.28	0.03±0.008			0.28	0.14±0.090
8	0.30	0.05±0.030	0.29	0.01±0.001			0.30	0.10±0.008
9	0.32	1.65±0.611	0.32	0.01±0.002			0.32	0.45±0.144
10	0.35	0.39±0.132	0.35	0.05±0.002			0.35	0.32±0.070
11	0.59	1.05±1.094	0.58	0.11±0.001	0.59	0.05±0.009	0.59	0.68±0.160
12	0.94	0.20±0.130	0.93	0.06±0.003	0.94	0.06±0.005	0.94	0.05±0.016
13	1	1	1	1	1	1	1	1
14	1.35	0.34±0.302	1.34	0.02±0.002	1.35	0.01±0.002	1.35	0.04±0.016
15	2.32	0.02±0.013	2.30	0.07±0.005			2.32	0.02±0.008
16	2.48	0.02±0.016	0.16	0.01±0.001			2.49	0.12±0.102

RRT and RPA are the ratio of retention time and peak area of each characteristic peak with reference to peak 13 (reference peak). The values of RPA are expressed as mean±S.D.

Table 6. The Relative Retention Time (RRT) and Relative Peak Area (RPA) of Characteristic Peaks for Samples among Dry and Fresh Herbs of *H. cordata* in YaAn

Peak no.	Dry YA (n=2)		Fresh YA (n=8)	
	RRT	RPA	RRT	RPA
1			0.16	0.03±0.008
2	0.17	0.01±0.012	0.17	0.30±0.062
3			0.18	0.04±0.005
4	0.21	0.16±0.009	0.21	1.04±0.366
5	0.22	0.04±0.045	0.22	0.40±0.064
6	0.24	0.15±0.032	0.24	1.27±0.354
7			0.28	0.14±0.090
8	0.30	0.02±0.019	0.30	0.10±0.008
9	0.32	0.05±0.380	0.32	0.45±0.144
10			0.35	0.32±0.070
11	0.59	0.06±0.026	0.59	0.68±0.160
12	0.94	0.13±0.021	0.94	0.05±0.016
13	1	1	1	1
14	1.35	0.33±0.057	1.35	0.04±0.016
15			2.32	0.02±0.008
16			2.49	0.12±0.102

RRT and RPA are the ratio of retention time and peak area of each characteristic peak with reference to peak 13 (reference peak). The values of RPA are expressed as mean±S.D.

sponding quantities. These differences can probably be attributed to the loss of or decomposition of various components during the drying process.[11,13]

Conclusion

The established GC-MS fingerprinting technique of fresh *H. cordata* provided a facile means to evaluate the variety of chemical constituents and thus the quality of this traditionally used medicinal material. By comparing the fingerprints, fresh *H. cordata* acquired from different production areas possessed drastic differences in chemical components. This implies that the choice of production area should be carefully considered in order to maintain a consistent production of quality herb. Besides, the fingerprinting profiles among different parts of the fresh herbs possessed limited similarities to each other. It also revealed that the distribution of chemical compounds varied among those medicinal parts of fresh *H. cordata*. Traditionally, the aerial portion was used as the medicinal part. However, the root portion has also been included as an additional usable part in the 2005 Chinese Pharmacopoeia. In literature, the root portion of *H. cordata* was reported to have an antifungal effect.[21] Nowadays, a variety of tonic drinks with roots as a component have been proven effective in the removal of toxic substances from various organs and tissues, and in preventing various diseases.[22] Comparing the fingerprints of dry and fresh *H. cordate*, the number of chemical components for the latter was always more abundant than the former. Some bioactive components in particular compounds **2**, **5** and **6** are always higher in content. Previous studies also reported that the fresh herb was proven comparable in quality and therapeutic effectiveness with the dry counterpart. Using the fresh herb for treating a variety of diseases such as fevers, acute and exterior syndromes were not uncommon.[23] Although, practically, it is difficult to use fresh *H. cordata* in clinical application, preservation methods should nonetheless be developed with an aim to facilitate and popularize the use of these fresh herbs.

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