Quality Assessment of *Curcuma longa* L. by Gas Chromatography-Mass Spectrometry Fingerprint, Principle Components Analysis and Hierarchical Clustering Analysis

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Gas Chromatography-Mass Spectrometry (GC-MS) fingerprint analysis, Principle Components Analysis (PCA), and Hierarchical Cluster Analysis (HCA) were introduced for quality assessment of *Curcuma longa* L. (*C. longa*). The GC-MS fingerprint method was developed and validated by analyzing 33 batches of samples of *C. longa* from different geographic locations. 18 chromatographic peaks were selected as characteristic peaks and their relative peak areas (RPA) were calculated for quantitative expression. Two principal components (PCs) were extracted by PCA. *C. longa* collected from Guizhou and Fujian were separated from other samples by PC1, capturing 71.83% of variance. While, PC2 contributed for their further separation, capturing 11.13% of variance. HCA confirmed the result of PCA analysis. Therefore, GC-MS fingerprint study with chemometric techniques provides a very flexible and reliable method for quality assessment of *C. longa*.

Key Words: Fingerprint, *Curcuma longa* L., GC-MS, Principle components analysis, Hierachical clustering analysis

Introduction

Traditional Chinese Medicines (TCMs) have a long history in medical practice in China, which emphasizes the importance of multi-compound, multi-ingredient as being responsible for the activity of the herbal drug, in contrast to modern pharmacology and drug development that often focus on a single chemical entity. Because of variation in plant species, geographical origin, cultivation practice, harvest time, and storage or processing conditions, the qualities of TCMs vary greatly. Therefore, fingerprint technology was introduced to achieve quality control of herbal medicines in recent years.

The chemometric methods, especially PCA and HCA which can extract chemical information objectively, have been well performed for classification and discrimination chemical profiles of herbal medicines.

C. longa, a perennial herb, is a member of the *Zingiberaceae* (ginger) family, which has been listed in the Chinese Pharmacopoeia (2005 edition).[1] It has been used as anti-inflammatory, anti-bacterial, anti-oxidant, anti-fungal, anti-schistosomal, anti-depressant agent.[2-6] and for the treatment of hemorrhage, diabetes and nephropathy.[7,8] It was reported that *C. longa* contained groups of such chemical components as essential oil and curcuminoids.[9]

Research results indicated that the essential oil of the *C. longa* had anti-bacterial, anti-inflammatory, anti-arthritic, anti-oxidant and anti-mutagenicity activities.[10-12] A large number of reports about its essential oil have been published. For example, Hu *et al.* studied the chemical constituents of the essential oil from *C. longa*.[13] Lee reported that ar-turmerone could be useful as a leading compound for inhibiting platelet aggregation induced by collagen and arachidonic acid.[14] and Rathore *et al.* reported that C. oil appeared to be a promising agent not only for the treatment of cerebral stroke, but also for other disorders associated with oxidative stress.[15] Fang *et al.* used high-performance liquid chromatography (HPLC) to study the chemical components between the chromatographic fingerprints of essential oil of Rhizoma Curcuma longa and Rhizoma Wenyujin Concisum, but only one component was identified.[16] As we know, it is more suitable to use GC-MS to analyze and identify the chemical components of essential oil according to their mass spectrums. Furthermore, although there were so many publications associated with *C. longa* oil multicomponent determination or fingerprints, none of the extracted information is objective in evaluating the similarities or differences in the fingerprints. Due to this, GC-MS fingerprint in combination with multivariate statistical techniques seems to be suitable for the quality assessment of *C. longa*.

The purpose of this paper is to establish an effective GC-MS fingerprint method that can be better used to assess the quality of *C. longa*. Fingerprint method was developed and validated by the analysis of 33 samples using computer aided similarity evaluation system recommended by the State Food and Drug Administration. PCA and HCA were further performed according the data to generate a visual plot for evaluation of the resemblance and differences between tested samples.

Experimental

Plant Material and Standards. 33 batches of *C. longa* crude

Table 1. A summary of the test samples.

Sample no.	Origins	Collecting Time
1	Wangmo,Guizhou (arvensis)	Dec., 2006
2	Wangmo,Guizhou (arvensis)	Jan., 2007
3	Zhenfeng,Guizhou (cultivated; introduced from Wangmo of Guizhou)	Dec., 2006
4	Zhenfeng,Guizhou (cultivated; introduced from Wangmo of Guizhou)	Jan., 2007
5	Zhenfeng,Guizhou (cultivated; introduced from Wangmo of Guizhou)	Dec., 2007
6	Ningde,Fujian (commercial)	Dec., 2006
7	Zhenfeng,Guizhou (cultivated; introduced from Guangxi)	Jan., 2007
8	Zhenfeng,Guizhou (cultivated; introduced from Guangxi)	Dec., 2007
9	Zhenfeng,Guizhou (cultivated; introduced from Guangxi Sub-tropical Crops Research Institute)	Jan., 2007
10	Zhenfeng,Guizhou (cultivated; introduced from Guangxi Sub-tropical Crops Research Institute)	Dec., 2007
11	Zhenfeng,Guizhou (cultivated; introduced from Tianlin of Guangxi)	Jan., 2007
12	Zhenfeng,Guizhou (cultivated; introduced from Tianlin of Guangxi)	Oct., 2007
13	Zhenfeng,Guizhou (cultivated; introduced from Tianlin of Guangxi)	Nov., 2007
14	Zhenfeng,Guizhou (cultivated; introduced from Tianlin of Guangxi)	Dec., 2007
15	Zhenfeng,Guizhou (cultivated; introduced from Guangxi Botanical Garden of Medicine Plants)	Jan., 2007
16	Zhenfeng,Guizhou (cultivated; introduced from Guangxi Botanical Garden of Medicine Plants)	Oct., 2007
17	Zhenfeng,Guizhou (cultivated; introduced from Guangxi Botanical Garden of Medicine Plants)	Nov., 2007
18	Zhenfeng,Guizhou (cultivated; introduced from Guangxi Botanical Garden of Medicinal Plants)	Dec., 2007
19	Zhenfeng,Guizhou (cultivated; introduced from Daxin of Guangxi)	Jan., 2007
20	Zhenfeng,Guizhou (cultivated; introduced from Daxin of Guangxi)	Oct., 2007
21	Zhenfeng,Guizhou (cultivated; introduced from Daxin of Guangxi)	Nov., 2007
22	Zhenfeng,Guizhou (cultivated; introduced from Daxin of Guangxi)	Dec., 2007
23	Leshan,Sichuan (commercial)	Mar., 2006
24	Leshan,Sichuan (commercial)	Dec., 2003
25	Shuangliu,Sichuan (commercial)	Mar., 2006
26	Shuangliu,Sichuan (cultivated)	Dec., 2003
27	Guilin,Guangxi (commercial)	Feb., 2007
28	Xianseng,Sichuan (commercial)	Mar., 2003
29	Wenzhou,Zhejiang (commercial)	Feb., 2007
30	Zhengzhou,Henna (commercial)	Feb., 2007
31	Linyi,Shandong (commercial)	Feb., 2007
32	Shanglou,Shanxi (commercial)	Feb., 2007
33	Chenzhou,Hunan (commercial)	Feb., 2007

samples were collected from different geographical locations in China (Table 1). A standard *C. longa* was obtained from National Institute for the Control of Pharmaceutical and Biological Products, China. All samples were authenticated by Professor Qinfu Chen (Guizhou Normal University, Guiyang, China) and were ground to fine powder with particle size of 40 mesh.

Sample Preparation. The essential oil was extracted according to the Chinese Pharmacopoeia.[17] Dry *C. longa* (100 g) and distilled water (1000 mL) were placed into an extraction apparatus and subjected to hydrodistillation for 8 h at 100 °C. The essential oil was dried over anhydrous magnesium sulphate and stored at 4 °C in the dark.

GC-MS Analysis. The chromatographic separation was carried out in a HP6890/HP5973 GC/MS (Agilent Technologies, USA) system with a HP-5MS 5% Phenyl Methyl Siloxane (30 m × 0.25 mm i.d., 0.25 μm film thickness) resilience quartz capillary column. Split injection was conducted with a split ratio of 40:1. The injector temperature was 250 °C. The column temperature was programmed as follows: initially, 50 °C for 2 min. 10 °C/min to 100 °C. 4 °C/min to 200 °C, 10 °C/min to 290 °C and 290 °C for 2 min. Mass conditions were as follows: electron impact ionization (EI); interface temperature, 150 °C; ion source temperature, 230 °C; the detector voltage, 1.4 kV; solvent delay, 3 min. Helium was used as the carrier gas at a flow rate of 1.0 mL/min. All data were obtained by collecting the full-scan mass spectra within the scan range of 10 - 400 amu.

Statistical Software Package. The correlation coefficients and the similarities of entire chromatographic patterns among tested samples, and the simulative mean chromatogram were calculated and generated using the Computer Aided Similarity Evaluation System (CASES, MATLAB 5.3 based, developed by Research Center for Modernization of Chinese Medicine, Central South University, China). SPSS 15.0 for windows (SPSS Inc., USA) was specially coded for analyzing a series of chromatographic data and evaluation the similarities of different chromatograms by PCA and HCA.

Results and Discussion

Identification of Components in *C. longa*. The gas chromatographic method is used almost exclusively for the qualitative analysis of the volatiles. Retention time is utilized as primary criterion for the peak identification. However, the identification is practically difficult because the analyte may be eluted at the same time with other compounds. The mass spectrometer used as chromatographic detector offers additional data for the identification of the separated compounds. The most frequent identification method is the comparison of the recorded spectra with the MS library.

Total ion chromatograms of *C. longa* were shown in Fig. 1. All the main components were separated well. By similarity match using the libraries (Wiley 257, Nist 98), a majority of the chemical components were identified (Fig. 2).

Validation. To validate this method, the tests of precision and repeatability were performed based on RRT and RPA. Method precision was investigated by repeatedly analyzing the same sample, with the values of relative standard deviations (RSDs) for RRT and RPA, respectively, reported as less than 0.37% and

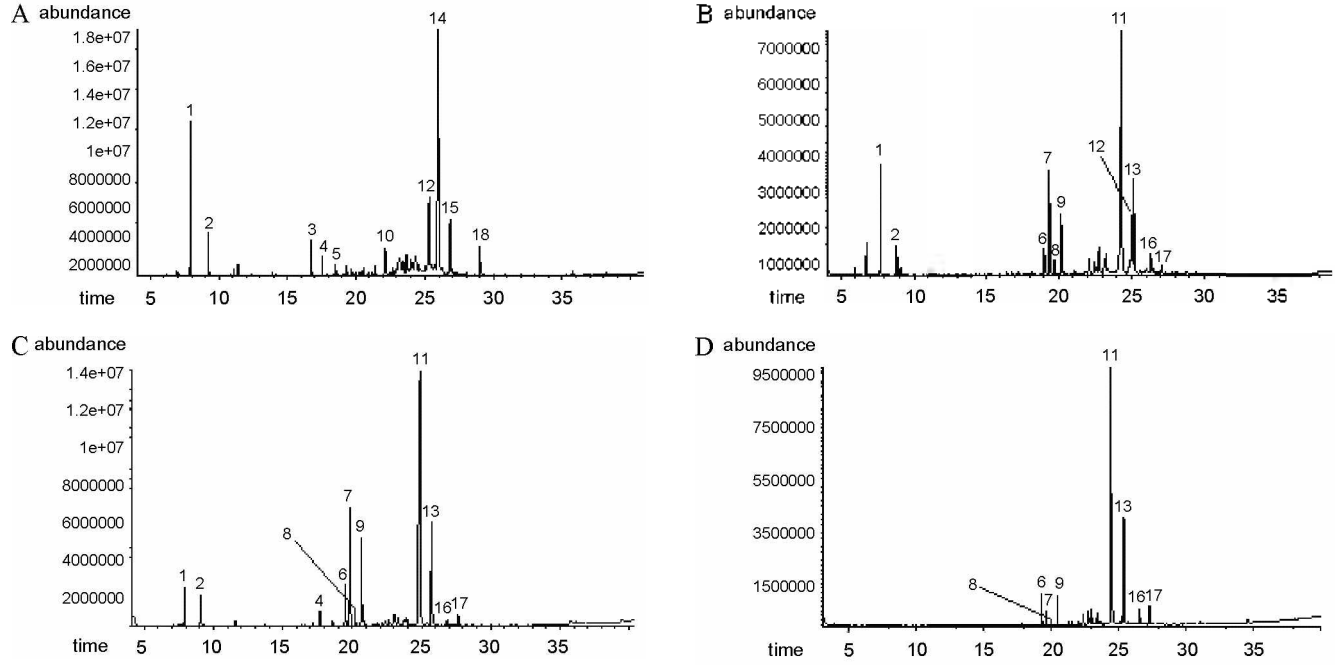
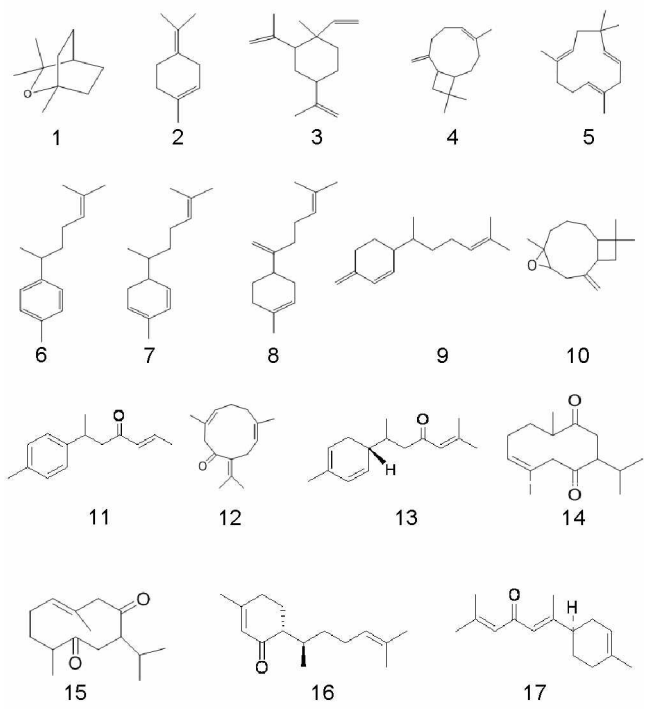


Figure 1. Total ion chromatograms for different samples. A. sample No. 14; B. sample No.19; C. sample No.23; D. standard material of *C. longa* obtained from National Institute for the Control of Pharmaceutical and Biological Products, China.

4.23% (n = 6). Method reproducibility was evaluated by analyzing six individual samples over the period of investigations.



(1) 1,8-cineole (7.71 min), (2) α-terpinolene (9.08 min), (3) β-elemene (16.47 min), (4) β-caryophyllene (17.27 min), (5) α-humulene (18.25 min), (6) ar-curcumene (19.02 min), (7) α-zingiberene (19.37 min), (8) β-bisabolene (19.73 min), (9) β-sesquiphellandrene (20.19 min), (10) caryophyllene oxide (21.92 min), (11) ar-turmerone (24.37 min), (12) germacrone (25.02 min), (13) α-turmerone (25.19 min), (14) curdione (25.76 min), (15) neocurdione (26.58 min), (16) (6R,1'R)-6-(1',5'-dimethylhex-4'-enyl)-3-methylcyclohex-2-enone (26.33 min), (17) (+)-α-atlantone (27.08 min), (18) unknown (28.78 min).

Figure 2. The structures of the identified compounds in *C. longa*.

The RSDs of RRT and RPA were found to be less than 0.41% and 4.71% (n = 6), respectively. Stability testing was performed with a freshly prepared methanolic solution of *C. longa* over a period of 0, 1, 2, 4, 8, 12 and 24 h. The reported RSDs of the RRT and RPA were found to be less than 0.74% and 4.63 (n = 6), respectively. All the results above indicate that the developed methodology is applicable for establishing a GC-MS fingerprint of *C. longa*.

GC-MS Fingerprint of *C. longa*. 33 batches of *C. longa* collected from different geographical locations were analyzed. The chromatograms were shown in Fig. 3. The correlation coefficients of each chromatogram to the simulative mean chromatogram was within 0.105 ~ 0.949. The correlation coefficients of some samples were higher than 0.8 while those of their chromatograms to other sample's simulative mean chromatogram were lower than 0.2. The results indicated that their chromatographic patterns were very inconsistent.

According to the differences of the chemical constituents of *C. longa* samples collected from different geographical locations, two types of standard fingerprints were established. The simulative mean chromatograms (Fig. 4) were generated by computer aided similarity evaluation system.

Samples of the first type (samples No. 1 - 14) which collected from Guizhou and Fujian were consistent to one another, the correlation coefficient of each chromatogram to the simulative mean chromatogram was within 0.934 ± 0.042 (mean ± SD). These results indicated that their chromatographic patterns were generally consistent, although the abundance intensity of some peaks was different.

Sample Nos. 15 - 22 which were introduced from Guangxi Botanical Garden of Medicine Plants, Daxin of Guangxi, and sample Nos. 23 - 33 which were collected from market, belonged to the second type of standard fingerprint. The correlation coe-

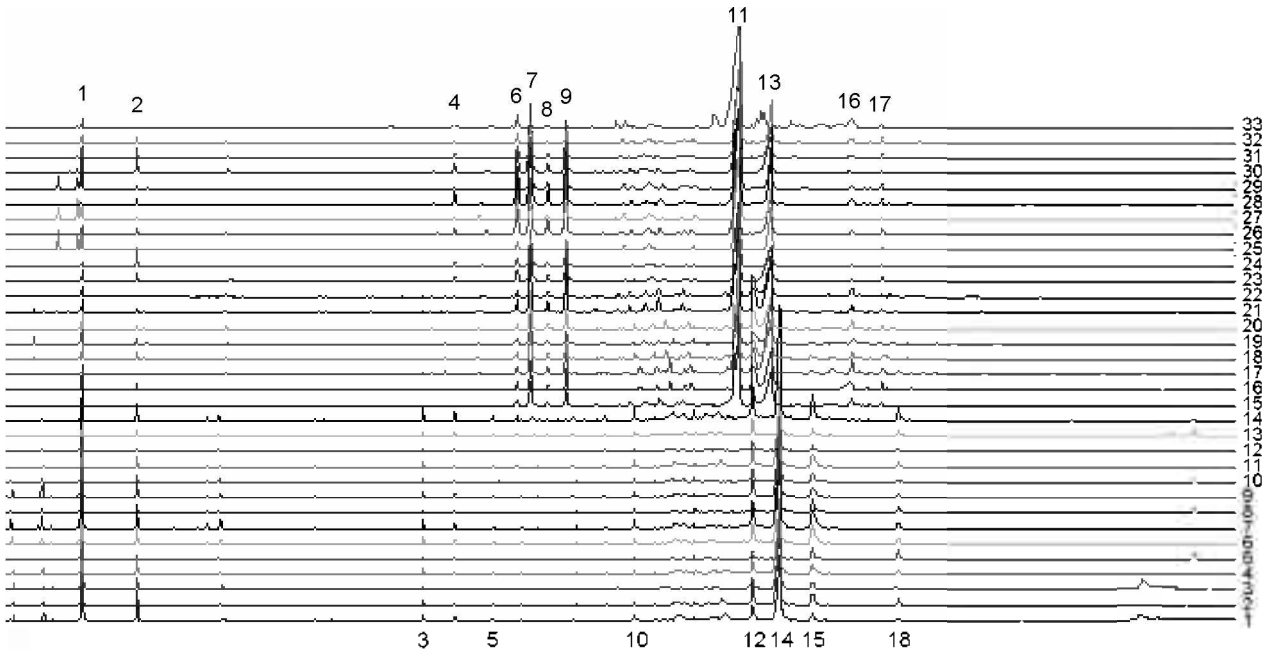


Figure 3. The overlapped chromatograms of the 33 samples.

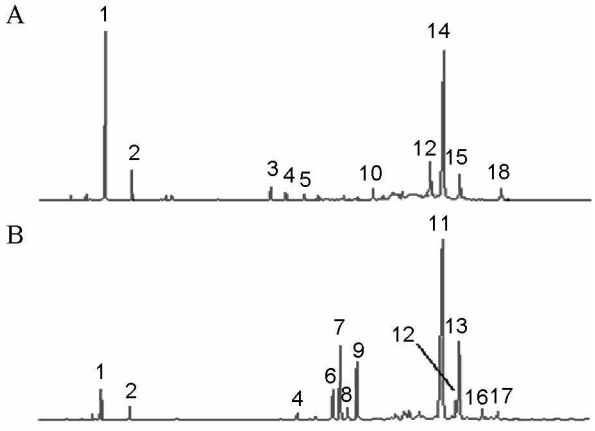


Figure 4. The simulative mean chromatogram of *C. longa*. A. the first type; B. the second type.

fficient of each chromatogram to the simulative mean chromatogram was within 0.935 ± 0.054 (mean ± SD).

The RRT and RPA of each characteristic peak to reference peak (curdione), which was the highest peak and the major compound in the volatile components in samples collected from Guizhou and Fujian (sample Nos. 1 - 14), were calculated (Table 2a and Fig. 5a). The data indicated that the content ratio of curdione was similar in these samples and different in others. The RRT and RPA of each characteristic peak to reference peak (ar-tumerone), which was the highest peak and the major compound in the volatile components in samples collected from other geographical locations of China (samples Nos. 15 - 33), were also calculated (Table 2b and Fig. 5b). Fig. 5 revealed the distribution of each peak in different samples.

PCA. When measuring only two variables, it is easy to plot this data and to visually assess the correlation between these two factors. But in many circumstances, lots of variables need to be analyzed to give a comprehensive evaluation. Therefore, it becomes impossible to make a visual inspection of the relationship between these factors in such a multi-dimensional matrix. Several data decomposition techniques are available for this purpose. PCA is among these techniques, which is used abundantly in all forms of analysis from neuroscience to computer graphics by reducing multidimensional data sets to lower dimensions. This process of throwing out the less important axes can help reveal hidden, simplified dynamics in high dimensional data.

The statistical software SPSS 15.0 was used to evaluate the differences among the 33 samples by analyzing the ratio of peak area of the 18 common peaks to the total peak area of the standard material of *C. longa*, respectively as variables. The full 33 × 18 autoscaled data matrix was submitted to PCA analysis. The first and second PCs described 71.83 and 11.13% of the variability in the original observations respectively, and both PCs accounted for 82.96% of the total variance. Thus, the first two PCs concentrated the multidimensional information into a 2-D dataset to classify the samples.

Examination of the plot (Fig. 6) obtained by measuring the GC-MS data of 33 samples showed good effect on quality assessment of *C. longa*. The scores clustered samples into three groups. Sample Nos. 1 - 14 were in group A, sample Nos. 15 - 25, 27, 29 and Nos. 31 - 33 were in group B, sample Nos. 26, 28 and 30 were in group C.

The main differences among three groups were that group A didn't contain peaks 6, 7, 8 and 9, and group B and C didn't contain peaks 3, 5, 10, 14, 15 and 18. And the ratio sum of the peak area of peaks 6, 7, 8 and 9 to the total peak area of the standard material of *C. longa* was the main difference between group B and C. The values of the ratio sum of peak areas of peaks 6, 7, 8 and 9 to the total peak area of the standard material of

Table 2. RRT of the investigated components in *C. longa*. (a). the RRT of characteristic peaks in sample Nos.1-14 using curdione as reference compound; (b). the RRT of characteristic peaks in sample Nos.15-33 using ar-tumerone as reference compound.

Sample No.	Peak No.																	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1	0.30	0.36	0.64	0.67	0.71	–	–	–	–	0.85	–	0.97	–	1.00	1.03	–	–	1.12
2	0.30	0.35	0.64	0.67	0.71	–	–	–	–	0.85	–	0.97	–	1.00	1.03	–	–	1.12
3	0.30	0.36	0.64	0.67	–	–	–	–	–	0.85	–	0.97	–	1.00	1.03	–	–	1.12
4	0.30	0.35	0.64	0.67	0.71	–	–	–	–	0.85	–	0.97	–	1.00	1.03	–	–	1.12
5	0.31	0.36	0.65	0.68	0.72	–	–	–	–	0.86	–	0.98	–	1.00	1.03	–	–	1.12
6	0.30	0.35	0.64	0.67	0.71	–	–	–	–	0.85	–	0.97	–	1.00	1.03	–	–	1.12
7	0.30	0.35	0.64	0.67	0.71	–	–	–	–	0.85	–	0.97	–	1.00	1.03	–	–	1.12
8	0.31	0.36	0.65	0.68	0.72	–	–	–	–	0.86	–	0.98	–	1.00	1.03	–	–	1.12
9	0.30	0.35	0.64	0.67	0.71	–	–	–	–	0.85	–	0.97	–	1.00	1.03	–	–	1.12
10	0.31	0.36	0.65	0.68	0.72	–	–	–	–	0.86	–	0.98	–	1.00	1.03	–	–	1.12
11	0.30	0.35	0.64	0.67	0.71	–	–	–	–	0.85	–	0.97	–	1.00	1.03	–	–	1.12
12	0.31	0.36	0.65	0.68	0.72	–	–	–	–	0.86	–	0.98	–	1.00	1.03	–	–	1.12
13	0.31	0.36	0.65	0.68	0.72	–	–	–	–	0.86	–	0.98	–	1.00	1.03	–	–	1.12
14	0.30	0.36	0.65	0.68	0.71	–	–	–	–	0.86	–	0.97	–	1.00	1.03	–	–	1.12
Average	0.30	0.36	0.64	0.67	0.71	–	–	–	–	0.85	–	0.97	–	1.00	1.03	–	–	1.12
RSD(%)	1.64	1.44	0.80	0.76	0.71	–	–	–	–	0.60	–	0.51	–	–	0	–	–	0

Sample No.	Peak No.																	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
15	0.32	–	–	–	–	0.78	0.79	0.81	0.83	–	1.00	1.03	1.04	–	–	1.08	1.11	–
16	0.32	0.37	–	0.72	–	0.79	0.80	0.82	0.83	–	1.00	1.03	1.04	–	–	1.08	1.11	–
17	0.32	0.37	–	0.71	–	0.78	0.80	0.81	0.83	–	1.00	1.03	1.04	–	–	1.08	1.11	–
18	0.32	0.37	–	0.72	–	0.79	0.80	0.81	0.83	–	1.00	1.03	1.04	–	–	1.08	–	–
19	0.32	0.36	–	–	–	0.78	0.80	0.81	0.83	–	1.00	1.03	1.04	–	–	1.08	1.11	–
20	0.32	0.37	–	–	–	0.79	0.80	0.82	0.83	–	1.00	1.03	1.04	–	–	1.08	1.11	–
21	0.32	0.37	–	–	–	0.78	0.80	0.81	0.83	–	1.00	1.03	1.04	–	–	1.08	1.11	–
22	0.32	0.37	–	–	–	0.79	0.80	0.82	0.83	–	1.00	1.03	1.04	–	–	1.08	1.11	–
23	0.32	0.36	–	0.71	–	0.78	0.80	0.81	0.83	–	1.00	–	1.04	–	–	1.08	1.11	–
24	0.32	0.36	–	0.71	–	0.78	0.80	0.81	0.83	–	1.00	–	1.04	–	–	1.08	1.11	–
25	0.32	0.36	–	0.71	–	0.78	0.80	0.81	0.83	–	1.00	–	1.04	–	–	–	1.11	–
26	0.32	0.36	–	0.71	–	0.78	0.80	0.81	0.83	–	1.00	–	1.04	–	–	1.08	1.11	–
27	0.32	0.36	–	0.71	–	0.79	0.80	0.81	0.83	–	1.00	–	1.04	–	–	–	1.11	–
28	0.32	0.36	–	0.71	–	0.78	0.80	0.81	0.83	–	1.00	–	1.04	–	–	1.08	1.11	–
29	0.32	0.36	–	0.71	–	0.78	0.80	0.81	0.83	–	1.00	–	1.04	–	–	1.08	1.11	–
30	0.32	0.36	–	0.71	–	0.78	0.80	0.81	0.83	–	1.00	–	1.04	–	–	1.08	1.11	–
31	0.32	0.36	–	0.71	–	0.78	0.80	0.81	0.83	–	1.00	–	1.04	–	–	1.08	1.11	–
32	0.32	0.36	–	0.71	–	0.78	0.80	0.81	0.83	–	1.00	–	1.04	–	–	1.08	1.11	–
33	0.32	–	–	–	–	0.78	0.80	0.81	0.83	–	1.00	–	–	–	–	1.07	1.11	–
Average	0.32	0.36	–	0.71	–	0.78	0.80	0.81	0.83	–	1.00	1.03	1.04	–	–	1.08	1.11	–
RSD(%)	0	1.36	–	0.53	–	0.59	0.29	0.46	0	–	–	0	0	–	–	0.22	0	–

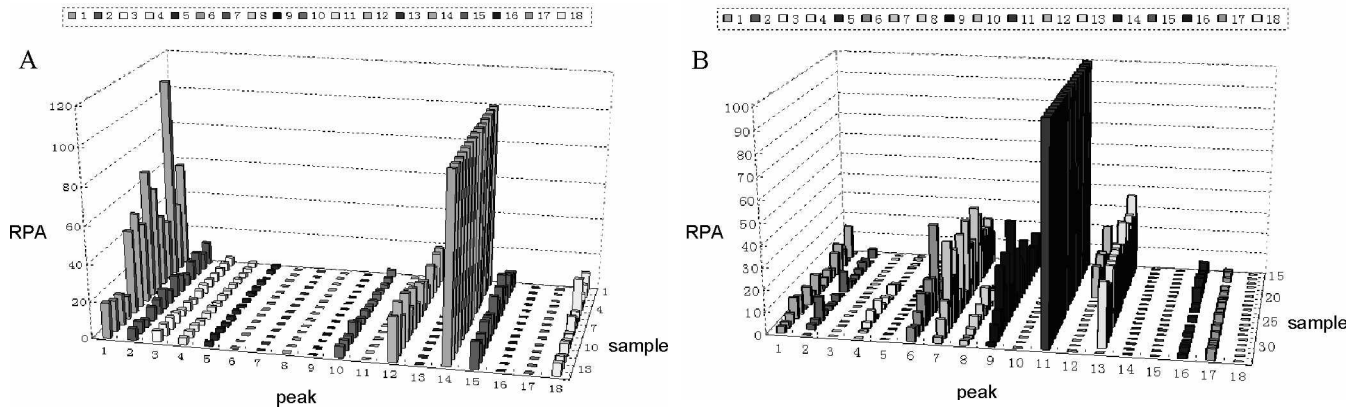


Figure 5. RPA of the investigated components in *C. longa*. (a) the RPA of characteristic peaks in sample Nos.1-14 using curdione as reference compound; (b) the RPA of characteristic peaks in sample Nos.15-33 using ar-tumerone as reference compound.

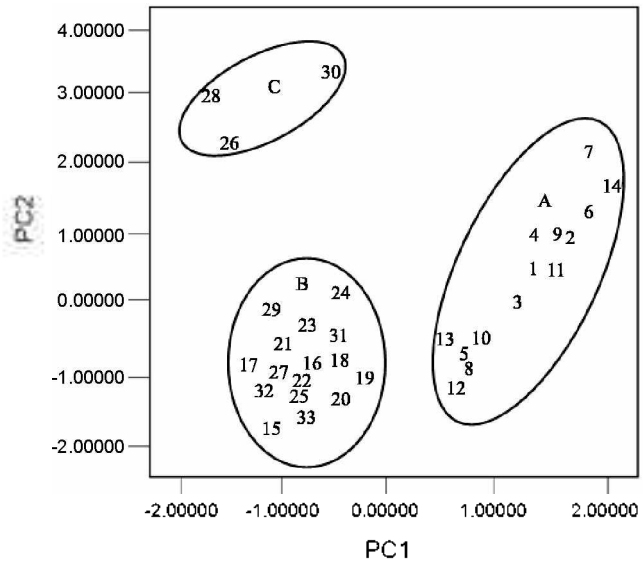


Figure 6. The result of PCA of fingerprints of *C. longa* samples.

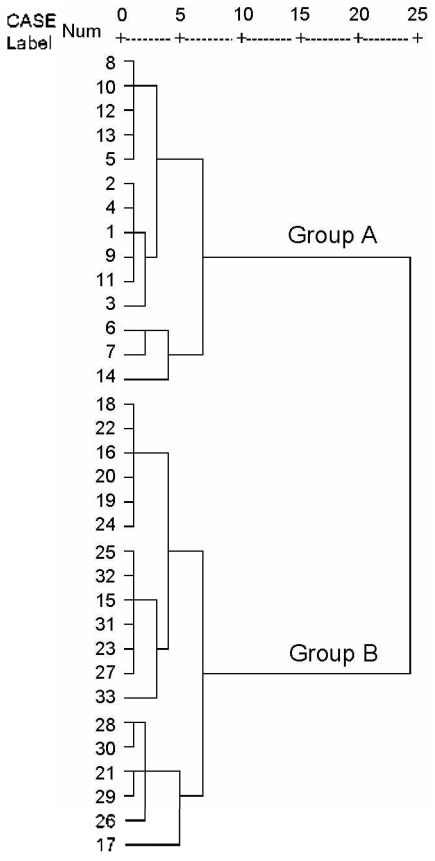


Figure 7. Dendrogram resulting from within linkage HCA.

C. longa were lower in group B (0.18 ~ 0.96). In contrast, they were higher in group C (1.25 ~ 1.51). The results indicated that PCA could reveal the slight differences of the contents of chemical components among the samples.

HCA. Cluster analysis was made as an attempt to derive groups from the autoscaled dataset and to compare them with the previous ones from PCA.

In the present study, average linkage was used and Euclidean distances were calculated. Fig. 7 showed the results of HCA based on the 18 peaks characteristics from GC profiles of the tested 33 samples, which were divided into two main clusters. In group A, samples from Guizhou and Fujian clustered together. In group B, samples from other places clustered in the same subgroup. High similarity was observed between the results of PCA and HCA.

TCMs emphasize the importance of multi-compound, multi ingredient preparations as being responsible for the activities in contrast to modern pharmacology and drug development that often focus on a single chemical entity. Quality control of herbs faces a number of severe challenges in the standardization and modernization of TCMs. Chromatographic fingerprint, a comprehensive and quantifiable identification method, is able to reveal chemical information with chromatograms, spectrograms and other graphs by analytical and chemical techniques. SFDA has required that all the injections made from herbal medicines be standardized by chromatographic fingerprinting, and all of the herbal chromatograms should be evaluated by their similarities, a commonly employed approach based on calculating the correlative coefficient and/or cosine value of vectorial angle of original data. Therefore, chromatographic fingerprint technique, as a more meaningful formulation for controlling the quality of herbs or their products, has been attracting more and more attention because of its emphasizing on the systematic characterization of the components and focusing on identifying and assessing the stability of the plants. Chemometric analysis such as PCA and HCA were also performed in our study using RRAs as parameters to reveal the similarities and differences among the samples, which has a significant effect in evaluating the stability and diversity of the samples from different regions.

Conclusion

In the present study, a developed GC-MS fingerprint method together with chemometric analysis was used for the quality assessment of *C. longa* collected from different geographical locations. The advantages of the newly established approach over conventional methods for quality control were as follows: Firstly, 18 peaks were regarded as the characteristic peaks of *C. longa*, two types of standard fingerprints were validated, and thus, can be used effectively for the screening analysis or quality assessment of *C. longa*. Secondly, PCA and HCA were performed on the basis of GC-MS fingerprints. Sample grouping based on PCA and HCA coincided nicely with their geographical origin. Thirdly, the study confirmed a great potential of GC-MS fingerprints in combination with chemometric methods for quality assessment of TCMs.

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