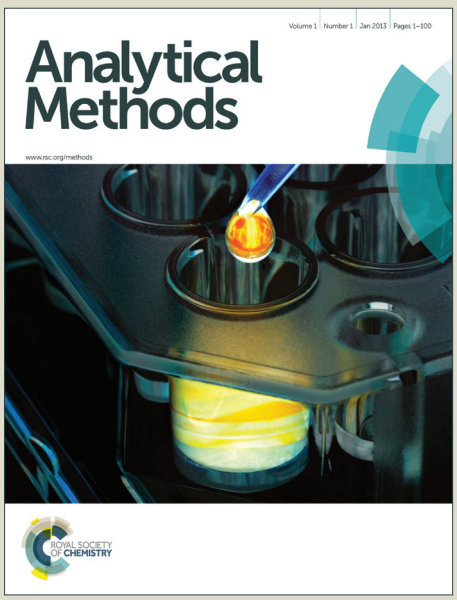
# Analytical Methods

Accepted Manuscript





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# Development of the fingerprints for the quality evaluation of *Cyathula officinalis* Kuan by LC-DAD-ESI-Q-TOF MS/MS coupled with multivariate statistical analysis

Qiao-Jia Fan [a], Jin-Liang Liu [b, c], Lei Sun [d], Shun-Lin Zheng [b, c], Ji-Chao Yuan [b, c,*], Meng-Liang Tian [b], Fan-Lei Kong [b, c]

[a] College of Veterinary Medicine, Sichuan Agricultural University, Ya'an 625014, China,

[b] College of Agronomy, Sichuan Agricultural University, Chengdu 611130, China;

[c] Key Laboratory of Crop Ecophysiology and Farming System in Southwest China, Ministry of P.R. China, Chengdu 611130, China;

[d] Chengdu Meteorological Bureau, Chendu 610071,China;

**ABSTRACT**

An LC-ESI-Q-TOF MS/MS method coupled with multivariate statistical analysis was developed for chemical fingerprinting of *Cyathula officinalis* Kuan. Ten peaks were selected as the common peaks, and cyasterone was used as a reference. The relative areas of common peaks were used for hierarchical clustering analysis, principal component analysis and similarity calculation. A total of 31 samples collected from different sources were classified into three (principal component analysis) or four groups (hierarchical clustering analysis). The similarities of 31 batches of *C. officinalis* samples were between 0.653 and 0.999. The results obtained suggested

* Corresponding author. Tel.: +86 13980074156.
*E-mail address:* yuanjichao5@163.com

that the chromatographic fingerprint technique could efficiently evaluate *C. officinalis*.

**Keywords:** *Cyathula officinalis* Kuan; quality evaluation; fingerprint; HPLC-ESI-Q-TOF MS/MS

## 1. Introduction

*Cyathula officinalis* Kuan is mainly distributed in Sichuan Province, China. It is a traditional Chinese medicine (TCM), that has been widely used in China for many centuries. Pharmacological and clinical studies indicated that the roots of *C. officinalis* are used for removing blood stasis, restoring menstrual flow and inducing diuresis for treating stranguria[1].

To our knowledge, due to the degradation and hybridisation of varieties, planting limitations, changes in cultivation methods and other factors, led to the huge heteromorphosis in product qualities[2-6]. Exact evaluation of *C. officinalis* plays a significant role in guiding scientific cultivation, breeding purification and rejuvenation. Therefore, development of an evaluation method is necessary and valuable for quality control.

Quality control is now regarded as a core requirement for pharmaceuticals of international trade. In 2000, the State Food and Drug Administration of China promulgated the regulation requiring all injections made from herbal medicine related materials to be standardised by chromatographic fingerprint[7-9]. Fingerprint is a powerful tool for the analysis of multi-ingredient medicines, emphasising on the systemic and comprehensive characterisation of samples. It has been widely accepted as a useful means for the quality evaluation of herbal raw materials combined with stoichiometry[4, 6, 10-19]. The commonly used statistical methods have hierarchical clustering analysis (HCA) and principal component analysis (PCA). HCA is performed to classify samples on the similarities of their chemical properties, and the results often give in the way of a tree diagram which is called a dendrogram[20]. Any valid metric could be used as a measure of similarity between

pairs of observations, and the pairwise distances between observations is the linkage criteria, determines the choice of which clusters to merge of split[20]. PCA is a method for feature extraction and dimensionality reduction, and to provide an overview of class separation, clustering and outliers[20].

Previous studies have reported that the major active ingredients of *C. officinalis* are cyasterone, rhapontisterone, daidzin, sergostesone, isocyasterone, sengosterone, polysaccharides and so on [1, 21, 22]. Therefore, evaluation of raw materials using a few compounds as a quality compound is insufficient. The present study aimed at developing the LC-ESI-Q-TOF MS/MS characteristic fingerprint of *C. officinalis* and then the fingerprint model could accurately reflect the quality and guarantee clinical efficacy.

## 2. Experimental

### 2.1 Material and Chemicals

Thirty-one samples of raw herbs of *C. officinalis* from different areas of China were collected (Table 1). The samples were authenticated by Professor Ji-chao Yuan (College of Agronomy, Sichuan Agricultural University, Chengdu, China). The samples were dried at 60 °C and ground to a fine powder. Cyasterone was obtained from National Institutes for Food and Drug Control, Beijing, China, and used as a standard. Methanol (HPLC grade) was purchased from Fisher Scientific, Inc. Pure water was obtained from a Millipore Milli Q-Plus system (Millipore, Bedford, MA, USA).

**Table 1** Raw material used in this experiment

| No. | Sample code | Sources | growing year | Harvesting time | Similarity [b] |
|---|---|---|---|---|---|
| 1 | s1 | Baoxing, Ya'an City | 2 | 2008.12 | 0.89 |
| 2 | s2 | Baoxing, Ya'an City | 2 | 2007.12 | 0.91 |
| 3 | s3 | Baoxing, Ya'an City | 3 | 2007.12 | 0.87 |

| | | | | | |
|---|---|---|---|---|---|
| 4 | s4 | Baoxing, Ya'an City | 2 | 2007.12 | 0.91 |
| 5 | s5 | Baoxing, Ya'an City | 2 | 2007.12 | 0.89 |
| 6 | s6 | Baoxing, Ya'an City | 3 | 2007.12 | 0.97 |
| 7 | s7 | Baoxing, Ya'an City | 3 | 2007.12 | 0.96 |
| 8 | s8 | Jinkouhe, Leshan City | 2 | 2008.01 | 0.93 |
| 9 | s9 | Jinkouhe, Leshan City | 3 | 2008.01 | 0.94 |
| 10 | s10 | Jinkouhe, Leshan City | 4 | 2008.01 | 0.94 |
| 11 | s11 | Tianquan, Ya'an City | 3 | 2007.12 | 0.87 |
| 12 | s12 | Tianquan, Ya'an City | 3 | 2007.12 | 0.97 |
| 13 | s13 | Tianquan, Ya'an City | 2 | 2007.12 | 0.94 |
| 14 | s14 | Tianquan, Ya'an City | 2 | 2007.12 | 0.90 |
| 15 | s15 | Tianquan, Ya'an City | 2 | 2007.12 | 0.94 |
| 16 | s16 | Tianquan, Ya'an City | 3 | 2007.12 | 0.94 |
| 17 | s17 | Tianquan, Ya'an City | 3 | 2008.12 | 0.84 |
| 18 | s18 | Tianquan, Ya'an City | 3 | 2008.12 | 0.79 |
| 19 | s19 | Tianquan, Ya'an City | 3 | 2008.12 | 0.81 |
| 20 | s20 | Tianquan, Ya'an City | 3 | 2008.12 | 0.80 |
| 21 | s21 | Yingjing, Ya'an City | 3 | 2008.11 | 0.88 |
| 22 | s22 | Yingjing, Ya'an City | 3 | 2008.11 | 0.90 |
| 23 | s23 | Yingjing, Ya'an City | 3 | 2008.11 | 0.82 |
| 24 | s24 | Yingjing, Ya'an City | 3 | 2008.11 | 0.87 |
| 25 | s25 | Yingjing, Ya'an City | 3 | 2008.11 | 0.88 |
| 26 | s26 | Yingjing, Ya'an City | 3 | 2008.11 | 0.88 |
| 27 | s27 | Yingjing, Ya'an City | 3 | 2008.11 | 0.89 |
| 28 | s28[a] | Unknown | Unknown | Unknown | 0.92 |
| 29 | s29[a] | Baoxing, Ya'an City | Unknown | Unknown | 0.90 |
| 30 | s30[a] | Baoxing, Ya'an City | Unknown | Unknown | 0.90 |
| 31 | s31[a] | Baoxing, Ya'an City | Unknown | Unknown | 0.92 |

[a] This batch was bought from medicinal materials market, so the original area, the growing year or harvesting time were not clear.

[b] Similarities of each chromatogram to the corresponding representative standard fingerprint of each areas by Similarity Evaluation System.

2.2 LC-MS conditions

The liquid chromatography equipment was a Shimadzu LC-20A series binary pump and Shimadzu PDA detector. Analysis was carried out on a Diamonsil $C_{18}$ column (250 mm × 4.6 mm, 5 μm). The mobile phase was methanol (A) and pure water (B) with the following gradient program: 0-5 min, linear gradient 5%-46% A; 5-32 min, linear gradient 46%-66% A; 32-40 min, 66%-100% A; 30 °C and flow-rate of 0.9 ml/min. Monitoring was performed at 266 nm and 5 μl

of solution injected.

This LC system was interfaced to a Triple TOF® 4600 mass analyser (AB Sciex, California, USA) operating in a positive ion mode. The following operation parameters were used: ion source gas 1 and ion source gas 2, 55 psi; curtain gas, 25 psi; temperature, 600 °C; ion spray voltage floating, 5500 V; collision energy, 40 V; and collision energy spread, 15 V. For full scan TOF-MS analysis, a scan range of *m/z* 50-600 with an accumulation time of 100 ms was selected, whereas a scan range of *m/z* 50-600 and accumulation time of 50 ms were used in TOF-MS/MS. Instrument control was carried out by Analyst TF 1.6 software (AB Sciex, CA, USA).

2.3 Sample and standard preparations

A SK250LH ultrasonic cleaning instrument (Shanghai Kudos Ultrasonic Instrument Co., Shanghai, China) was used for extraction. 1.0 g of the finely ground powder was accurately weighed and extracted with solvent in an ultrasonic bath for 30 min and then filtered. Extraction was performed as follows the Table 2. Cyasterone linearity of the method was studied by injecting eight known concentrations of the standard in the range of 28.125-450.000 μg/ml in triplicate. The calibration curves were obtained by plotting the peak area (*PA*) versus the amount of the standards.

**Table 2** Factors and levels for optimization of extraction conditions

| Levels | Factors | | |
|---|---|---|---|
| | (A)Methanol concentration | (B)Liquid-to-solid ratio | (C)time(min) |
| 1 | $A_1$:50 | $B_1$:5 | $C_1$:15 |
| 2 | $A_2$:75 | $B_2$:10 | $C_2$:30 |
| 3 | $A_3$:100 | $B_3$:15 | $C_3$:45 |

A, B and C were studied using an orthogonal design $L_9(3^4)$.

2.4 Data analyses of chromatogram

The correlation coefficients of chromatograms of 31 batches of samples were calculated by the

professional software Similarity Evaluation System for Chromatographic Fingerprint of Traditional Chinese Medicine (Version 2004A), which was edited by the Chinese Pharmacopoeia Committee. HCA and PCA of samples were performed using SPSS software (SPSS for Windows 20.0, IBM Inc., USA). In HCA, a method called average linkage between groups was applied, and Euclidean Distance was selected as the measurement standard[23].

## 3. Results and discussion

### 3.1 Optimisation of the extraction condition

Ultrasonic-assisted extraction, an extraction technique used under elevated liquid-to-solid ratio, time, power and temperature, has been used in the extraction of biologically active ingredients from raw medicines for its rapid process and high extraction efficiency[24-26]. The extraction efficiency was evaluated based on the cyasterone yield, and the experiment was performed under the following conditions: methanol concentration, 100%; liquid-to-solid ratio, 5 times; extraction time, 30 min. In this study, methanol concentration, liquid-to-solid ratio and extraction time were studied using an orthogonal design. The experimental factors and corresponding levels are presented in Table 2, and the orthogonal design $L_9$ ($3^4$) and results are presented in Table 3. Intuitionistic analysis of the *R* value in Table 3 indicated that the order of the degree of influence of each variable on the extraction efficiency was A > B > C, and further statistical analysis proved that the experimental factors significantly influenced the results. $A_2$, $B_1$ and $C_2$ were selected based on their high extraction efficiencies.

**Table 3** Experimental and analytical of orthogonal design of cyasterone extraction

| No. | A | B | C | Extraction yield (mg/g) |
|---|---|---|---|---|
| 1 | $A_1$ | $B_1$ | $C_1$ | 91.46 |
| 2 | $A_1$ | $B_2$ | $C_2$ | 79.70 |
| 3 | $A_1$ | $B_3$ | $C_3$ | 58.64 |

| | | | | |
|---|---|---|---|---|
| 4 | $A_2$ | $B_1$ | $C_2$ | 217.47 |
| 5 | $A_2$ | $B_2$ | $C_3$ | 201.13 |
| 6 | $A_2$ | $B_3$ | $C_1$ | 182.65 |
| 7 | $A_3$ | $B_1$ | $C_3$ | 199.26 |
| 8 | $A_3$ | $B_2$ | $C_1$ | 171.48 |
| 9 | $A_3$ | $B_3$ | $C_2$ | 191.68 |
| $K_1$ | 229.80[a] | 508.19 | 445.59 | |
| $K_2$ | 601.25 | 452.31 | 488.85 | |
| $K_3$ | 562.42 | 432.97 | 459.03 | |
| R | 371.45[b] | 75.22 | 43.26 | |

[a] $K_i = i_1 + i_2 + i_3$, (It is means the A, B, or C sum of extraction amount of cyasterone ).

[b] $R_j = \max\{K_i\} - \min\{K_i\}$ .

3.2 Optimisation of LC separation and validation of methodology

Pure water was used as a mobile phase modifier to restrain the peak tailing of cyasterone. The detection wavelength of 266 nm was chosen based on the analysed results. The column temperature was set at 30 °C. The reproducibility method was evaluated by analysing five injections of one sample, and the repeatability method was evaluated by analysing five samples, and prepared independently from *C. officinalis*. The stability of the samples was analysed within 24 h. The relative standard deviations (RSDs) of the retention times ($t_R$) and *PA* of 10 characteristic peaks for reproducibility of the same sample solution were found in the range of 0.05%-0.23% and 0.79%- 0.86%, respectively. In the repeatability experiment, the values were also below 0.12% and 3.07%, respectively. Furthermore, the RSDs of $t_R$ and *PA* in the sample stability test were less than 0.50% and 3.91%, respectively. Calibration plots for cyasterone exhibited the following linear relationship: $Y = 13018X + 43204$, where $Y$ and $X$ are the peak area (mAU) and concentration of the standard solution (μg/ml), respectively. Linear regression showed good linearity in the range of 28.125-450.000 μg/ml with a correlation co-efficient of 0.9994. Thus, cyasterone could be determined over a wide range of concentrations. The results indicated

that HPLC for fingerprint analysis was valid and satisfactory.

3.3 LC fingerprint of *C. officinalis*

A total of 31 batches of samples from different origins (9 from Baoxing, 7 from Yingjing, 4 from Tianquan, 4 from Jinkouhe and 4 were unknown) were analysed, and the representative standard fingerprint of each area was generated from multiple samples using the Similarity Evaluation System software (Fig. 1). *C. officinalis* has been added to the Chinese Pharmacopoeia as an official medicine, and its fingerprint was selected as a standard for comparative evaluation with other samples (species, production area, harvest time and growing year) via HCA, PCA and similarity analysis[27].

Among the five fingerprints, the Baoxing samples had the most complex chromatographic pattern with more than 10 characteristic peaks (Fig. 1), whereas the other four areas had relatively simple patterns. Similarity comparison of the standard fingerprints of the samples from different areas showed a similarity between 0.653 and 0.999. Ten 'common peaks' were observed in the fingerprint chromatograph. In LC-ESI-Q-TOF MS/MS, the accurate molecular mass of the components (common peak) was obtained. One compound (cyasterone, peak 8) was unequivocally identified, and five compounds were tentatively identified based on their MS data. In ESI (+)-TOF MS/MS data, $[M + H]^+$ was 520.3151, whereas $[M + Na]^+$ was 542.2956 ($[M + H]^+$: calculated for $C_{29}H_{44}O_8$ 520.3149; found 520.3151). When the MS results were compared with the cyasterone standard, peak 8 was positively identified as cyasterone. $[M + H]^+$ was 496.6508, whereas $[M + Na]^+$ was 518.6422 ($[M + H]^+$: calculated for $C_{27}H_{44}O_8$ 496.6335; found 496.6508 ), so peak 1 was deduced as rhapontisterone. $[M + H]^+$ was 416.3787, whereas $[M + Na]^+$ was 538.3611 ($[M + H]^+$: calculated for $C_{21}H_{20}O_9$ 416.3781; found 416.3787 ), so peak 5 was

deduced as daidzin. $[M + H]^+$ was 536.6002, whereas $[M + Na]^+$ was 558.5926 ($[M + H]^+$: calculated for $C_{29}H_{44}O_9$ 536.6000, found 536.6002 ), so peak 7 was deduced as sergostesone. $[M + H]^+$ was 520.3151, whereas $[M + Na]^+$ was 542.2956 ($[M + H]^+$: calculated for $C_{29}H_{44}O_8$ 520.3149; found 520.3151 ), so peak 9 was deduced as isocyasterone. $[M + H]^+$ was 536.3078, whereas $[M + Na]^+$ was 558.3018 ($[M + H]^+$, calculated for $C_{29}H_{44}O_9$ 536.3000; found 536.3078 ), so peak 10 was deduced as sengosterone. Peaks 2, 3, 4 and 6 could not be deduced.

Peak 8 was symmetrical and had the highest *PA*, so it was used as a reference to calculate the relative areas of each 'common peak' in the chromatographic patterns (Table 4). Based on this result, we found that the similarity comparison was not always a golden rule for the comparison of samples, and the combination with other methods, such as HCA and PCA, was more efficient. The results of HCA are shown in Fig. 2. The samples could be divided into four clusters. Cluster I was formed by the samples collected from Baoxing and Tianquan. Cluster II consisted of the samples from Jinkouhe. Cluster III comprised the samples of Yingjing. Cluster IV included of the sample from Tianquan.

Fig. 2 shows that the samples from the same source clustered well. The concrete location had a large influence on the clusters. For example, cluster I had a long rescaled distance with cluster II. The rescaled distance between cluster I and II was shorter than that between clusters I, II and III. Compared with cluster III, the quality of cluster I was much similar to the quality of cluster II.

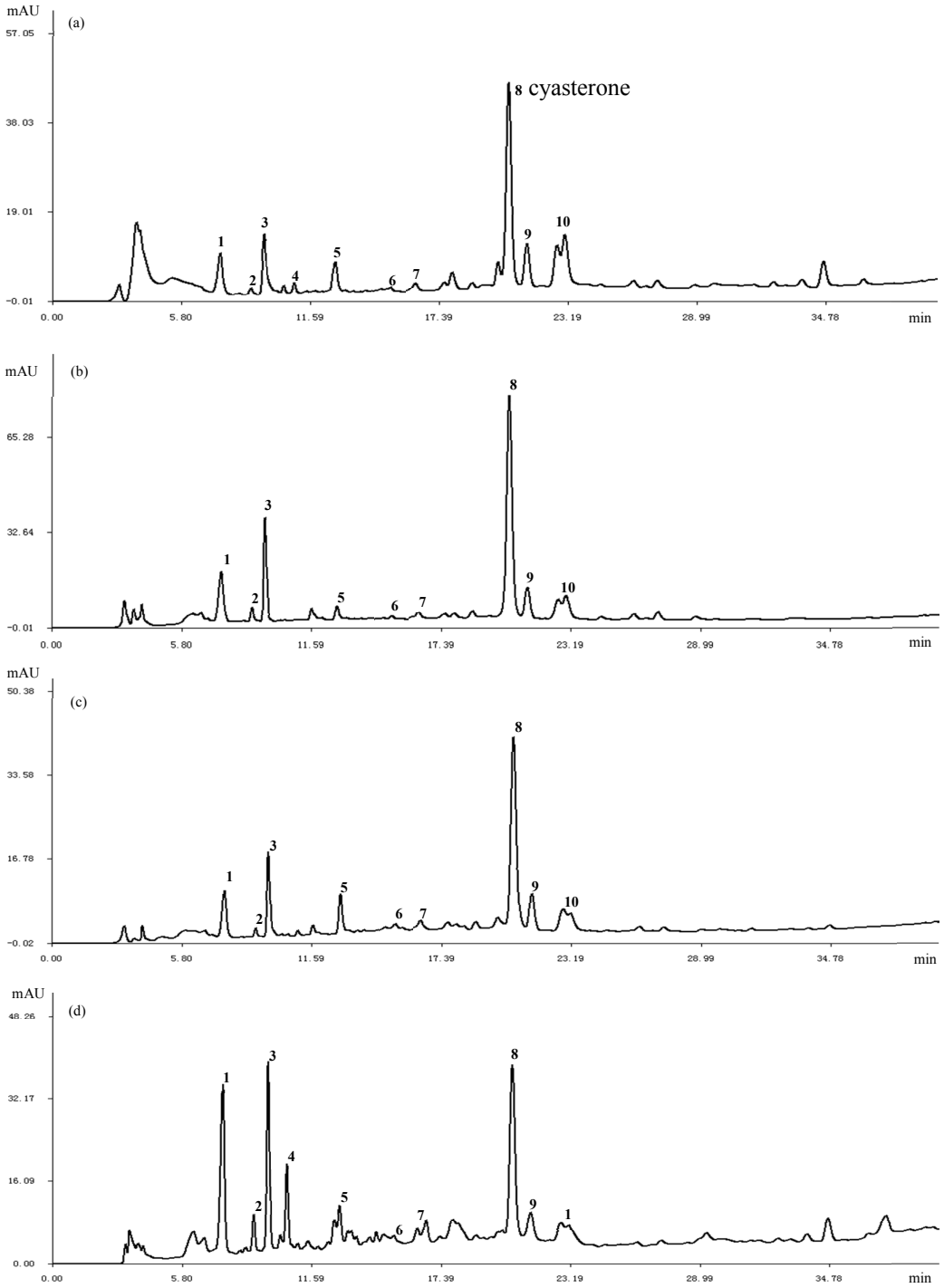


**Fig.1.** The representative fingerprints: (a) Baoxing; (b) Tianquan; (c) Yingjing; (d) Jinkouhe. The numbers of these compounds are common peaks.

**Table 4** Relative areas of ten common peaks in different samples

| No. | The relative areas of common peaks |
|---|---|

| | 7.643 | 9.030 | 9.642 | 10.792 | 12.837 | 15.328 | 16.398 | 20.675 | 21.533 | 23.177min |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 0.3374 | 0.0536 | 0.2790 | 0.1022 | 0.2587 | 0.0604 | 0.2258 | 1 | 0.3598 | 0.3200 |
| 2 | 0.3437 | 0.0584 | 0.3713 | 0.1708 | 0.3069 | 0.0336 | 0.1179 | 1 | 0.3822 | 0.3845 |
| 3 | 0.2607 | 0.0382 | 0.2804 | 0.0604 | 0.2534 | 0.0257 | 0.0866 | 1 | 0.1453 | 0.3046 |
| 4 | 0.3669 | 0.1019 | 0.452 | 0.0905 | 0.1854 | 0.1239 | 0.1805 | 1 | 0.2086 | 0.3906 |
| 5 | 0.3346 | 0.0905 | 0.4721 | 0.0738 | 0.1907 | 0.1035 | 0.1453 | 1 | 0.2015 | 0.3712 |
| 6 | 0.2298 | 0.0582 | 0.3011 | 0.0487 | 0.1720 | 0.0556 | 0.1250 | 1 | 0.2195 | 0.2156 |
| 7 | 0.2220 | 0.0623 | 0.3011 | 0.0431 | 0.1724 | 0.0545 | 0.0928 | 1 | 0.2266 | 0.2145 |
| 8 | 0.1450 | 0.0183 | 0.1648 | 0.0179 | 0.3094 | 0.0564 | 0.0958 | 1 | 0.1480 | 0.1353 |
| 9 | 0.2144 | 0.0438 | 0.2627 | 0.0514 | 0.2216 | 0.0716 | 0.1352 | 1 | 0.1992 | 0.1514 |
| 10 | 0.2187 | 0.0376 | 0.2656 | 0.0339 | 0.1578 | 0.0755 | 0.141 | 1 | 0.1901 | 0.0921 |
| 11 | 0.8264 | 0.1897 | 0.7777 | 0.1454 | 0.3008 | 0.1338 | 0.1932 | 1 | 0.4723 | 0.6973 |
| 12 | 0.1687 | 0.0386 | 0.2704 | 0.0268 | 0.1331 | 0.0580 | 0.1065 | 1 | 0.2248 | 0.1726 |
| 13 | 0.2836 | 0.0223 | 0.3017 | 0.0151 | 0.0909 | 0.0254 | 0.0382 | 1 | 0.1571 | 0.4547 |
| 14 | 0.4755 | 0.1030 | 0.3940 | 0.0333 | 0.2969 | 0.0747 | 0.1449 | 1 | 0.1807 | 0.3197 |
| 15 | 0.1786 | 0.0433 | 0.1936 | 0.0330 | 0.2544 | 0.0477 | 0.0850 | 1 | 0.1497 | 0.2441 |
| 16 | 0.1158 | 0.0253 | 0.2273 | 0.0131 | 0.0844 | 0.0356 | 0.0509 | 1 | 0.1529 | 0.1214 |
| 17 | 0.2371 | 0.0689 | 0.2475 | 0.0901 | 0.1236 | 0.0436 | 0.1038 | 1 | 0.1977 | 0.2240 |
| 18 | 0.2729 | 0.0835 | 0.3539 | 0.0868 | 0.1575 | 0.0537 | 0.1614 | 1 | 0.2181 | 0.2816 |
| 19 | 0.2644 | 0.0803 | 0.3242 | 0.0785 | 0.1484 | 0.0501 | 0.1462 | 1 | 0.2143 | 0.2650 |
| 20 | 0.2622 | 0.0749 | 0.3157 | 0.0752 | 0.1414 | 0.0470 | 0.1363 | 1 | 0.2038 | 0.2443 |
| 21 | 0.6101 | 0.1397 | 0.4812 | 0.2661 | 0.3678 | 0.1189 | 0.2177 | 1 | 0.2436 | 0.1322 |
| 22 | 0.7032 | 0.1413 | 0.6109 | 0.2680 | 0.1220 | 0.1277 | 0.1735 | 1 | 0.2158 | 0.2003 |
| 23 | 0.6509 | 0.1388 | 0.5839 | 0.3262 | 0.2531 | 0.1860 | 0.1295 | 1 | 0.2458 | 0.3433 |
| 24 | 0.8375 | 0.1983 | 0.7106 | 0.4185 | 0.2439 | 0.1658 | 0.2027 | 1 | 0.2688 | 0.3045 |
| 25 | 0.7139 | 0.1564 | 0.6274 | 0.4473 | 0.2302 | 0.1395 | 0.2049 | 1 | 0.3120 | 0.4137 |
| 26 | 0.8354 | 0.1619 | 0.5793 | 0.3079 | 0.1881 | 0.1574 | 0.2071 | 1 | 0.3366 | 0.2133 |
| 27 | 0.6551 | 0.2210 | 0.6470 | 0.2844 | 0.1494 | 0.1118 | 0.2241 | 1 | 0.2485 | 0.202 |
| 28 | 0.2011 | 0.0580 | 0.2102 | 0.0230 | 0.0943 | 0.0848 | 0.1204 | 1 | 0.1597 | 0.2006 |
| 29 | 0.3502 | 0.0633 | 0.4563 | 0.0524 | 0.0993 | 0.1570 | 0.2554 | 1 | 0.3658 | 0.2844 |
| 30 | 0.4281 | 0.1081 | 0.5215 | 0.0848 | 0.1217 | 0.1290 | 0.1650 | 1 | 0.3651 | 0.3377 |
| 31 | 0.5045 | 0.0843 | 0.3950 | 0.0888 | 0.1019 | 0.0718 | 0.1133 | 1 | 0.1629 | 0.2726 |

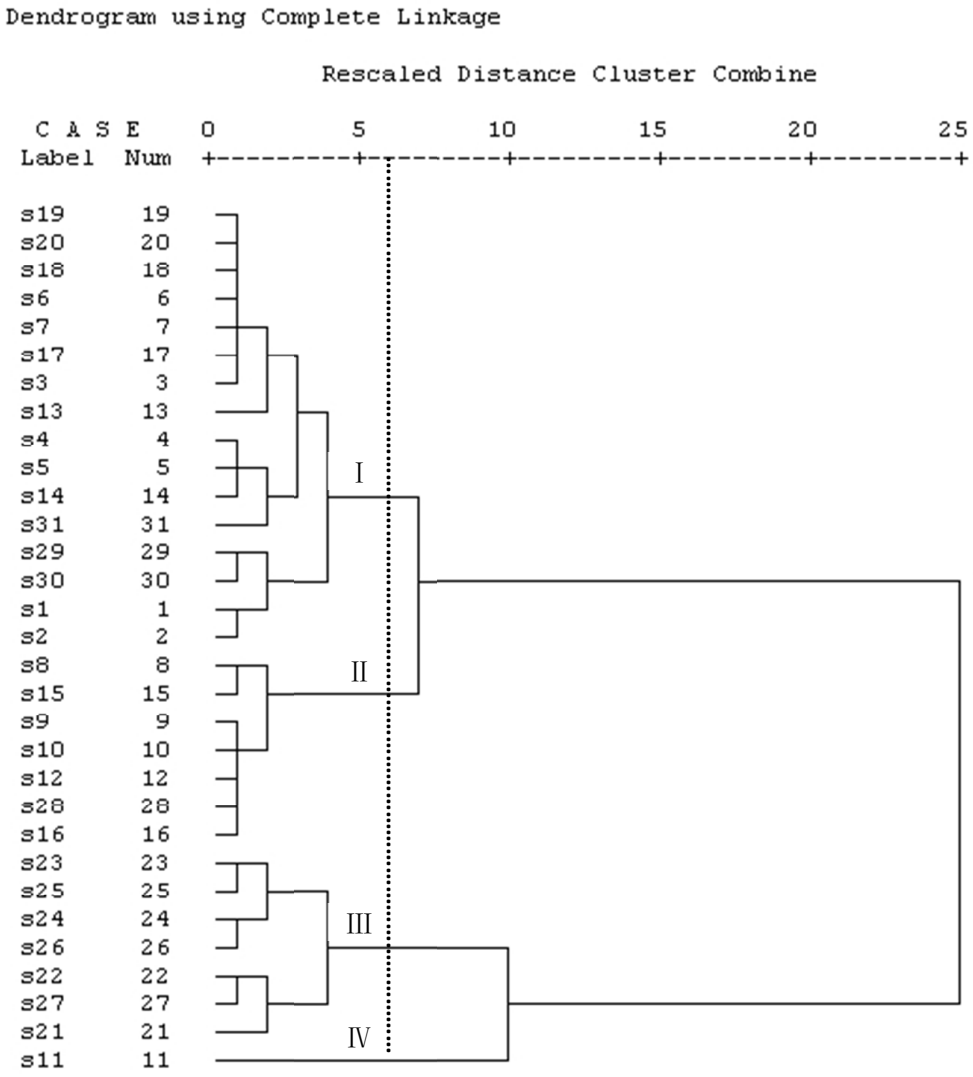


**Fig.2.** Results of hierarchical clustering analysis of 31 batches samples.

To summarise the distribution of the 31 samples, PCA was utilised to classify the LC data. The 3D plot of PCA on the 31 chromatograms shown in Fig. 3 demonstrates interesting results for the identification of the samples of different areas. Three confined clusters were observed in the 3D projection plot; most of these samples were separated with the numbers 21-27 and 11. In this work, numbers 21-27 were named group '1', and the seven samples were from Yingjing, Ya'an City. Numbers 1-10, 12-20 and 28-31 were named group '2'. Numbers 28-31 were purchased from markets with unclear plant habitats, harvest time, planting limitations and cultivation methods.

Number 11 was named group '3', and it was from Tianquan, Ya'an City. This fact indicated that quality problems of *C. officinalis* in herbal markets were very serious.

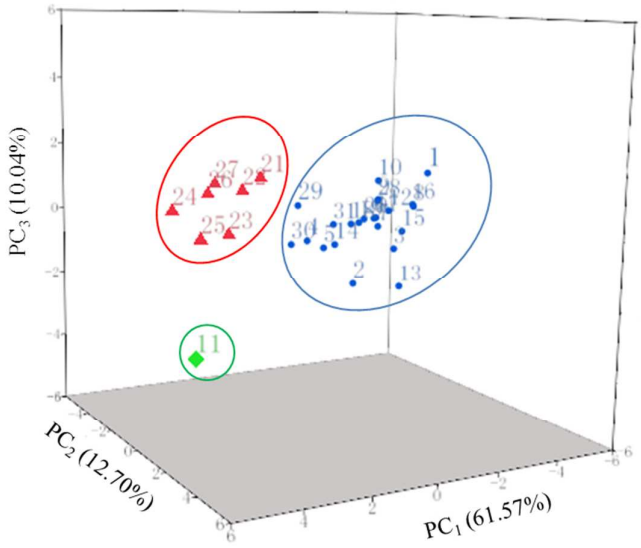


**Fig.3.** Scores plot from principal component analysis (PCA) for the 31 samples.

The quality of *C. officinalis* was not consistent and easily affected by external factors, including the habitats, harvest time, planting limitations and cultivation methods[2-6]. Therefore, more attention should be paid to the quality of materials to ensure their clinical efficacy and safety[28-33].

## 4. Conclusion

The quality control of traditional herbal medicine plays an important role in their safety and effectiveness in clinical applications. In this study, a fingerprint approach for the quality control of processed *C. officinalis* was established. The proposed method was provided high precision, sensitivity, accuracy and reliability. Thirty-one *C. officinalis* samples were investigated for their fingerprints, and the results were compared. This research provides a model for rapid quality control and species differentiation of TCM. The fingerprints of *C. officinalis* greatly varied among different samples. Thus, an effective quality control method and good agriculture practices are necessary to ensure the efficacy and safety of *C. officinalis* in clinical usage.

# Acknowledgement

The authors are grateful for the financial support of the National Key Technology Research and Development Program of the Ministry of Science and Technology of China (2006BAI 06A 12-02).


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