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Full Length Research Paper

Quality assessment for fructus Gardeniae by multi-component quantification, chromatographic fingerprint and related chemometric analysis

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A simple, feasible and effective method of ultra performance liquid chromatography (UPLC) coupled with photo diode array (PDA) were established for fingerprint analysis and simultaneous quantification of three major classes of constitutions including iridoid glycosides, crocins and organic acids of fructus Gardeniae. Extraction method was optimized as 75% methanol ultrasonic extraction for 30 min. Acetonitrile-water (containing 0.2% formic acid) gradient elution on Waters Acquity BEH C18 column (50 × 2.1 mm, 1.7 µm) was used to obtain good chromatographic resolution. 24 characteristic peaks were comprised in the fingerprint common pattern. Among them, seven marked components, geniposide, shanzhiside methyl ester, geinpin, geniposidic acid, crocin I, crocin II, and chlorogenic acid, were quantified. Similarity evaluation, principal component analysis and hierachical cluster analysis were applied to demonstrate the distinction. It was concluded that chemical components of *Gardenia jasminoides* Eills and *Gardenia jasminoides* var*. radicans* Makino from different origins were similar. Other than determination of the content of geniposide and crocin I, comparison of fingerprint atlas could be considered as a suitable quality control method for fructus Gardeniae.

Key words: Fructus Gardeniae, chromatographic fingerprint, multi-component quantification, principal component analysis, hierachical cluster analysis.

INTRODUCTION

Traditional Chinese medicines (TCM) have a long history of use in China and south Asia. Its use in the world was once limited since it was difficult to isolate and quantify the chemical components in herbs. With the transformation of medical model and developments of phytochemistry, the public demand in the world for TCM has increased significantly in recent years. TCM are complex mixtures which contain thousands of chemically constituents. Among them, the content of characteristic constituent may not indicate intensity of the biological activities (Li et al., 2008; Abbasi et al., 2010). It is widely regarded that multiple constituents of TCM work synergistically for the therapeutic effects. Moreover, the chemical constituents in herbs vary obviously with species, origins, harvest seasons, drying processes and other factors (Liang et al., 2004; Hussain et al., 1990). A reliable quality control strategy of TCM is the quantitative determination of characteristic constituents or major ingredients. But major ingredients cannot reflect the overall features comprehensively. Chromatographic fingerprint has gained significant attention in recent years. It has been internationally accepted as a feasible method for the quality control of herbal medicines and other natural products because of its stability and consistency (Liang et al., 2010). But the unavoidable differences in chromatograms of different laboratories and the natural deviation of chemical profiles of individual herbs bring us many difficulties to analyze. Some

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Table 1. Samples of the dried fruit of *G. jasminoides* evaluated in this study.

No.	Code	Species	Origins	Date of collection	Similarity
S1	HB1	*G. jasminoides* Ellis	Hubei, Huanggang	2010.10	0.906
S2	HB2	*G. jasminoides* Ellis var. *grandiflora* Nakai	Hubei	2009.10	0.901
S3	SC1	*G. jasminoides* Ellis	Sichuang, Naxi	2010.10	0.939
S4	SC2	*G. jasminoides* Ellis	Sichuang, Jiangjin	2009	0.864
S5	SC3	*G. jasminoides* Ellis	Sichuang, Luxiang	2009.11	0.896
S6	HN1	*G. jasminoides* Ellis	Hunan, Yiyang	2010.10	0.959
S7	HN2	*G. jasminoides* Ellis var. *grandiflora* Nakai	Hunan, Ningxiang	2009.11	0.769
S8	SX1	*G. jasminoides* Ellis var. *grandiflora* Nakai	Shaanxi, Xian	2010.10	0.849
S9	SX2	*G. jasminoides* Ellis	Shaanxi	2009.11	0.824
S10	JX2	*G. jasminoides* Ellis	Jiangxi,	2008	0.859
S11	JX3	*G. jasminoides* Ellis	Jiangxi, Yichun	2009.10	0.830
S12	JX4	*G. jasminoides* Ellis var. *grandiflora* Nakai	Jiangxi, Fuzhou	2010.11	0.766
S13	JX5	*G. jasminoides* Ellis	Jiangxi, Lichuang	2009.10	0.910
S14	GX1	*G. jasminoides* Ellis	Guangxi	2009.10	0.965
S15	GX2	*G. jasminoides* Ellis	Guangxi	2009.11	0.897
S16	ZJ1	*G. jasminoides* Ellis var. *grandiflora* Nakai	Zhejiang, Pingyang	2010.10	0.865
S17	ZJ2	*G. jasminoides* Ellis	Zhejiang	2009	0.861
S18	JX1	*G. jasminoides* Ellis	Jiangxi, Zhangshu	2009.11	0.926

chemometric methods, such as principal component analysis (PCA) and hierarchical cluster analysis (HCA), can improve applicability and provide more important information (Qin et al., 2009; Xie et al., 2006). The fruit of *Gardenia jasminoides* Ellis (Rubiaceae) is widely used in many Asian countries for its cholagogue, diuretic, antiphlogistic and antipyretic effects (The Pharmacopoeia Commission of PRC, 2010). Its pharmacological activities such as antioxidant (Kim et al., 2010), antihyperlipidemic (Lee et al., 2005; Sheng et al., 2006), antiinflammation (Hwang et al., 2010) and antitumor (Hsu et al., 1997) have also been reported. A number of iridoid glucosides, monoterpenes, flavonoids, and crocetins have been separated from *G. jasminoides* Ellis (Wang et al., 2004; Yu et al., 2009).

The primary origins of fructus Gardeniae are the tropical and subtropical regions of Asian. However, with the growing demand for fructus Gardeniae and the shortage of herbal resources, some fruits of *G. jasminoides* Ellis var. *grandiflora* Nakai were mixed into the genuine fructus Gardeniae in herb market. According to literatures and traditional medicinal customs, the fruits of the same family of plant *G. jasminoides* Ellis var. *grandiflora* Nakai could not be used as herb medicines, which were only used to extract Gardenia yellow pigment (Tsai et al., 2002). The quality of fructus Gardeniae needed the exact specifications. The current method for quality control was mainly implemented by the determination of geniposide, which is the major component in fructus Gardeniae. Though a variety of methods, such as fingerprint analysis, cluster analysis were reported to be employed for quality control, no systematic contrast was carried out until now (He et al., 2006; Yan et al., 2006; Zhou et al., 2010). In the present study, an UPLC–PDA method combined with optimization of ultrasonic extraction were established and optimized for the simultaneous quantitative determination of the seven marker components in the crude extract of samples.

Moreover, a UPLC–based fingerprint was developed for the quality evaluation of fructus Gardeniae. At the same time, chemometric methods including PCA and HCA were applied to analyze the samples.

MATERIALS AND METHODS

Plant materials and reagents

Standard substances of geniposide (abbreviated as GS in tables and figures), crocin I (CI) and chlorogenic acid (CA) were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Reference standards of shanzhiside methyl ester (SME), geniposidic acid (GA), crocin II (CII) and geinpin (GP) were obtained from Zelang Medical Technology Co., Ltd (Nanjing, China). Their structures were identified based on their spectral data (ESI–MS, ^{1}H–NMR, ^{13}C–NMR) by comparing with literature values (El-Naggar and Beal, 1980; Zhou et al., 2010). All standards' purities were demonstrated to be above 98% by HPLC method.

The samples of the dried fruits of *G. jasminoides* Ellis were collected in Jiangxi, Hunan, Hubei, Sichuan, Zhejiang, Guangxi, and Shaanxi Province. The samples of *G. jasminoides* Ellis var. *grandiflora* Nakai were from Hubei, Hunan, Shaanxi, Jiangxi and Zhejiang Province.

All of the plant materials were collected during September and November of the latest years (Table 1). The botanical origins of all the collected samples were identified by Professor Chenggang

Huang. The voucher specimens of all these materials were deposited at Shanghai Institute of Materia Medica, Chinese Academy of Sciences, China. Unless specified otherwise, all chemicals and solvents were of analytical reagent grade and obtained from the Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). Acetonitrile and methanol were of HPLC grade and obtained from Dikma Technologies Inc. (Beijing, China). Deionized water was purified using a Milli–Q purification system (Millipore, MA, USA). All solvents and sample solutions were filtered through 0.45 µm membrane filters before injection into UPLC.

UPLC apparatus

An ultrasonic processor (Kunshan ultrasonic instrument co., Ltd, Jiangsu, China) was employed for sample extraction. An Acquity UPLC-PDA, consisting of a vacuum degasser, binary solvent delivery pump, autosampler, and thermostated column compartment (Waters, Milford, MA, USA) was used for acquiring chromatograms and UV spectrum. This equipment coupled with ion trap mass spectrometer and ESI interface (Agilent, Palo Alto, CA, USA) was used for the identification of components. The chromatographic separation was performed using a Waters Acquity BEH C18 column (50 × 2.1 mm, 1.7 µm) while the column temperature was maintained at 35°C. The separation was carried out with gradient elution procedure and mobile phase A (acetonitrile) and B (0.2% formic acid water) ratios linear changed as follows: 0~2.8 min, 96% B; 2.8~3.6 min, 96~92% B; 3.6~6.2 min, 92% B; 6.2~7.4, 92~86% B; 7.4~12 min, 86~78% B; 12~17 min, 78~60% B; 18~20 min, 96% B. The flow rate was 0.6 ml/min and 2 µl of each sample was injected. The UV spectra by PDA were recorded between 200 and 480 nm.

Preparation of standards

The reference standards of the seven standard compounds (geniposide, genipin, scandoside methyl ester, geniposidic acid, chlorogenic acid, crocin I, crocin II) were accurately weighed and dissolved in methanol and then diluted to appropriate concentration ranges for method validation.

All stock and working standard solutions were stored in brown bottles at -4°C for analysis.

Preparation of samples

The dried and powdered fruits (1.0 g, 40 mesh) of all samples from different origins were extracted with 75:25 methanol/water (V/V, 20 ml) in an ultrasonic bath for 30 min. The extracted solutions were filtered through a 0.22 µm PTFE syringe filter before injection into the UPLC system for analysis.

Data analysis

Similarity analysis was performed by the professional software named similarity evaluation system for chromatographic fingerprint of TCM (Version 2004 A, The Pharmacopoeia Commission of PRC, Beijing, China), which was recommended by the SFDA of China for evaluating similarities of chromatographic profiles of TCMs. In order to explore the variation patterns of the characteristic chemical constituents, the seven marker compounds and the compositions corresponded to peak 8, 14, 16, 21 and 24 were analyzed with chemometric methods. PCA and HCA were performed to analyze the data by using SPSS software (IBM SPSS Statistics 19, Armonk, New York, USA).

RESULTS AND DISCUSSION

Optimization of chromatographic conditions

The major chemical classes in *G. jasminoides* Ellis include iridoid glucosides, organic acid esters, crocetins, triterpenes and essential oils (Wang et al, 2004; Yu et al, 2009). For the comprehensive quality assessment, the characteristic biomarkers should be presented within the chromatographic window.

Firstly, methanol extract of the samples (1.0 g, JX1) were used for optimizing the chromatographic conditions. Several mobile phases, including methanol-water and acetonitrile-water were tested. Due to the variations in chemical properties of the detected compounds, formic acid was applied to restrain the peak tailing. It was found that an acetonitrile-water system containing 0.2% formic acid (v/v) gave the best separation of compounds and the gradient was stated earlier. In order to obtain more information of the samples, UV spectra of the extracted solutions were recorded from 200 to 480 nm.

Optimization of extraction procedure

The same sample (1.0 g, JX1) was extracted in an ultrasonic bath for 1 h with 20 ml water, 25, 50, 75 and 100% methanol respectively to analyze the effects of the solvent. Water could not extract the liposoluble constituents efficiently, and the viscous extract increased the complexity of procedures for post-processing. Comparing the extraction efficiencies of different solvents, it was found that 75:25 methanol/water (V/V) showed best extraction performance. After the solvent was ascertained, the extraction methods were investigated by comparing refluxing for 2 h with ultrasonic processing for 1 h. Ultrasonic extraction for one hour was found to be equivalent to that of refluxing. Ultrasonic extraction was chosen because of its convenience. Then, extraction time was optimized by ultrasonic with 75% methanol for 20, 30, 40 and 60 min, respectively. It was shown that ultrasonic extraction for 30 min was sufficient to extract the analytes.

Identification of the reference materials

In the UPLC-ESI-MS/MS spectra, most of the investigated compounds exhibited their quasi-molecular ions $[M - H]^-$ in negative ion mode. The adduct ions $[M+HCOO]^-$ were also detected due to the addition of 0.2% formic acid in the mobile phase. By comparing their retention times, UV data and MS spectra with those of standards, seven compounds in the extract were unambiguously identified as geniposidic acid, shanzhiside methyl ester, genipin, geniposide, chlorogenic acid, crocin I, crocin II (El-Naggar and Beal, 1980; Zhou et al., 2010). Retention time and spectral data were showed in Table 2, and structures of these key characteristic compounds

Table 2. Structures of the seven reference substances.

t_R (min)	[M–H](*m/z*)	Fragments (*m/z*)	λmax (nm)	Identification
1.26	373	328, 178, 161, 142	238.7	geniposidic acid
3.64	353	191,161	326.226	Chlorogenic acid
4.14	405	373, 243, 225, 185, 167, 119	238.1	Shanzhiside methyl ester
4.90	387	433, 225, 207, 123	240.2	Geniposide
5.41	225	207, 123	241.8	Genipin
9.37	976	651, 533, 687, 510	440.8	Crocin I
10.01	814	533, 510	441.4	Crocin II

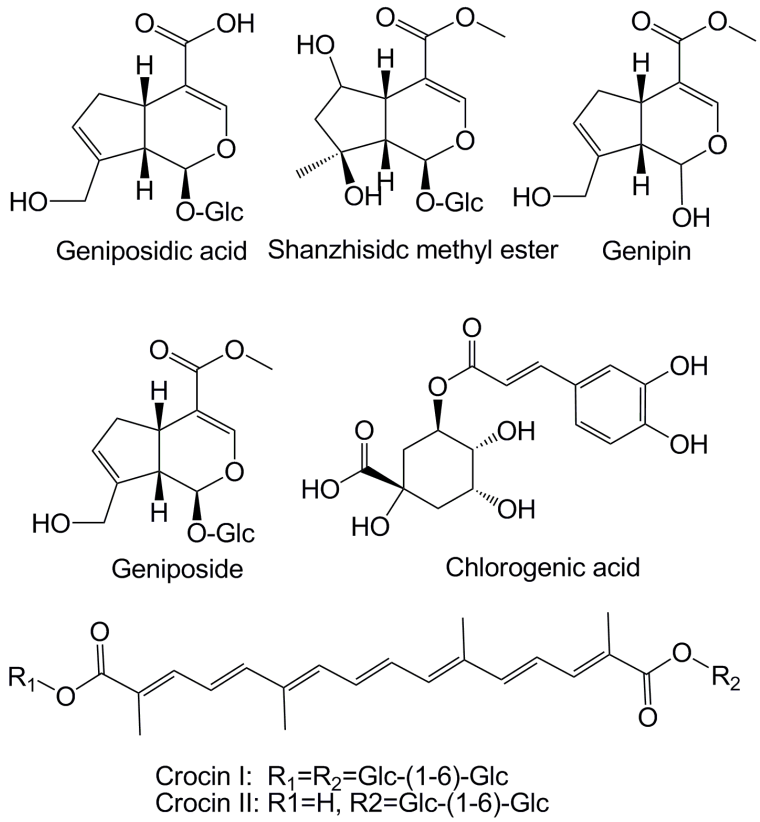

Figure 1. Chemical structures of the reference materials.

were shown in Figure 1.

Method validation

The working solutions containing all the reference compounds were prepared as described previously to construct calibration curves. Triplicate injections were made at seven different concentrations. An aliquot (2 µl) of each standard working solution was subjected to UPLC. The linearity for each compound was established by plotting the peak area (y) versus concentration(x) of each analyte. The standard calibration curves of integrated peak area (n = 3) and linearity (R^2) were shown in Table 3. Calibration curves were linear with correlation coefficients >0.999 for all analytes. The working solutions were further diluted to a series of concentrations to determine the limit of detection (LOD) and limit of quantification (LOQ). The LOD and LOQ were defined as the analyte mass resulting in a signal-to-noise (S/N) ratio of 3 and 10 respectively. The LOD and LOQ for each compound were shown in Table 3. High sensitivity at these chromatographic conditions was confirmed by LOD (0.28 to 0.83 µg/ml) and LOQ (1.48 to 4.37 µg/ml).

Under the optimal conditions, the mixture standard

Table 3. Calibration plots, LOD, and LOQ of the seven analytes.

Compounds	Calibration	R^2	Linear range[a]	LOD[a]	LOQ[a]
GA	Y = 6559.3X-71313	0.9992	21.5–430	0.65	4.37
SME	Y = 6621.2X-171406	0.9993	24.3–486	0.36	2.88
GS	Y = 3454.8X+388958	0.9996	88.4–3536	0.28	1.48
GP	Y = 8348.1X-306034	0.9993	37.5–350	0.37	3.75
CA	Y = 9129.6X-24083	0.9991	22.0–440	0.33	2.31
CI	Y = 7366.2X-21536	0.9996	41.6–832	0.83	4.33
CII	Y = 18153X-73162	0.9994	36.5–365	0.55	2.92

[a] Data are shown in µg/ml.

Table 4. Precision and repeatability test of the method.

Compounds	Precision				Repeatability (n = 6)	
	Intra-day (n = 5)		Inter-day (n = 3)			
	Content[a]	RSD (%)	Content[a]	RSD (%)	Content[a]	RSD (%)
GA	128.7	1.2	128.8	2.4	140.3	1.4
SME	72.9	0.5	73.1	1.1	135.7	2.9
GS	265.1	0.4	264.8	0.8	3153.5	0.7
GP	112.0	1.6	111.7	1.6	54.2	2.7
CA	65.6	1.4	65.4	2.0	44.0	2.3
CI	124.4	1.2	125.0	1.8	373.7	2.4
CII	108.4	0.9	108.4	2.7	46.1	1.8

]a Data are shown in µg/ml.

Table 5. Recovery test determined by the standard addition method (n = 3).

Analyte	Original[a]	Spiked[a]	Determined[a]	Recovery (%)	RSD (%)
GA	70.3	129.0	196.2	97.6	2.6
SME	67.5	72.9	139.4	98.6	2.1
GS	1576.6	265.2	1845.4	101.4	0.9
GP	27.3	112.5	134.7	95.5	3.7
CA	22.3	66.0	89.4	103.2	1.5
CI	186.7	124.8	306.9	96.3	3.3
CII	23.0	109.5	127.2	95.2	2.4

[a]Data are shown in µg/ml.

solution was analyzed five times in one day for intra-day variation and on three successive days for inter-day variation to evaluate the precision. Variations were expressed as relative standard deviations in Table 4. The relative standard deviations (RSDs) of the assays for the intra-day and inter-day precisions were 0.4 to 1.6% and 0.8 to 2.7%, respectively. To confirm the repeatability, six different working solutions prepared from the same sample (1.0 g, JX1) were analyzed. The overall repeatability is excellent for the present experiment with the RSDs in the range of 0.7 to 2.9%. Recoveries were performed by employing the method of standard substances addition. Seven reference standard components were spiked into the samples (0.5 g, JX1), and then extracted, processed and quantified in accordance with the established procedures. Finally, the recovery rates were calculated. As showed in Table 5, the overall recoveries were between 95.2 and 103.2% for all compounds, with RSD less than 3.7%. It was demonstrated that the present method had acceptable precision, accuracy, sensitivity and repeatability from all the previous validation data.

Table 6. Content of the marker compounds in samples (n = 3).

No.	Amount (mg/g, mean ± S, n = 3)						
	GA	CA	SME	GS	GP	CI	CII
S1	5.55±0.31	0.25±0.06	2.17±0.18	85.10±0.51	1.09±0.33	17.7±0.30	1.26±0.04
S2	5.14±0.41	0.43±0.12	2.56±0.20	49.45±0.33	1.23±0.24	10.68±0.25	1.07±0.11
S3	5.86±0.07	0.34±0.06	3.97±0.16	59.60±0.47	1.18±0.19	8.61±0.23	0.68±0.03
S4	2.47±0.21	0.40±0.09	2.76±0.05	54.53±0.34	1.35±0.29	15.83±0.18	1.35±0.09
S5	2.27±0.16	0.32±0.10	2.93±0.20	57.66±0.47	1.97±0.20	13.75±0.26	1.39±0.07
S6	6.17±0.05	0. 07±0.02	2.08±0.12	95.51±0.32	1.46±0.29	9.62±0.32	0.84±0.08
S7	3.37±0.39	1.01±0.13	1.89±0.30	56.70±0.33	1.40±0.47	22.67±0.44	1.43±0.12
S8	3.01±0.17	0.18±0.04	2.05±0.21	61.93±0.40	1.40±0.32	21.08±0.36	1.73±0.06
S9	1.70±0.43	0.27±0.04	1.51±0.21	50.88±0.36	1.12±0.41	15.91±0.51	1.60±0.28
S10	4.28±0.26	1.23±0.08	2.87±0.27	61.27±0.39	1.17±0.23	17.00±0.21	1.09±0.12
S11	2.49±0.18	0.38±0.05	1.93±0.31	45.62±0.41	1.28±0.25	20.88±0.31	1.69±0.09
S12	3.20±0.13	1.18 ±0.13	1.88±0.21	56.10±0.38	1.26±0.25	16.72±0.07	1.21±0.06
S13	2.70±0.32	0.99±0.06	2.69±0.17	81.46±0.54	1.18±0.30	14.64±0.58	1.10±0.08
S14	1.31±0.24	1.44±0.19	2.42±0.06	43.19±0.35	1.34±0.28	3.48±0.39	0.47±0.07
S15	3.24±0.29	1.28±0.12	3.02±0.31	57.17±0.61	1.37±0.31	12.17±0.47	0.90±0.12
S16	2.12±0.31	1.84±0.10	2.18±0.27	46.76±0.25	1.08±0.21	13.04±0.32	1.39±0.11
S17	4.19±0.14	1.09±0.08	3.91±0.10	70.66±0.45	1.29±0.17	15.42±0.42	1.15±0.11
S18	6.13±0.16	0.29±0.07	4.31±0.15	78.95±0.38	1.39±0.16	21.73±0.15	1.42±0.08

Content of seven characteristic compounds in samples

As showed in Table 6, the content of each analyte varied significantly among the different samples. Geniposide, the major oiridoid glucoside in *G. jasminoide* Ellis, was the main active constituent mostly associated with *G. jasminoide* Ellis's pharmacological activities (Tsai et al, 2002). The content of geniposide ranged from 43.19 to 95.51 mg/g. The content of samples from *G. jasminoides* Ellis var. *grandiflora* Nakai was not significantly lower than that of *G. jasminoide* Ellis. In Chinese pharmacopoeia, it is merely needed to determinate the content of geniposide. Thus *G. jasminoides* Ellis var. *grandiflora* Nakai could not be distinguished from *G. jasminoide* Ellis. It was also observed that genipin, the aglycone of geniposide, with the structure of semi-acetal, was the first one to be studied. According to our method, the content of genipin was steadily distributed between 1.08 and 1.97 mg/g. As a marker for the determination, measured concentrations of chlorogenic acid vary considerably, with no significant differences between plant origins and species. The major constituents of crocin I and corcin II shared the structure of carotenoids. It is interesting that crocin I was twelve times more than the content of corcin II, while the total content of crocin I and crocin II from *G. jasminoides* Ellis var. *grandiflora* Nakai was slightly higher than that of *G. jasminoide* Ellis. Although chlorogenic acid was possessed in the samples, the low level of its content was insufficient for the requirements of quality control. As to the content of geniposidic acid, several samples from Hubei, Sichuan, Hunan and Jiangxi province had much more geniposidic acid than samples from other provinces. But no difference was observed between the two species. Finally, we compared the total content of the seven compounds. However, they could not be distinguished.

Fingerprint analysis

The liquid chromatographic fingerprints were shown in Figure 2. To perform the fingerprint analysis, the chromatograms of different samples have to be standardized. The process of standardization included the selection of "common peaks" in chromatograms, and the normalization of retention times of all the common peaks. The RSD of all peaks' relative retention times for all batches less than 0.3% would be assigned as the same substance, and as a 'common peak'. The peak at retention time 5.85 min showed up in all samples, and was clearly recognized. Therefore, it was selected as a reference peak. The similarity indices of all the samples were calculated by the median method and shown in Table 1. The similarity indices of 13 batches of genuine samples were between 0.824 and 0.965, which were not different from those of *G. jasminoides* Ellis var. *grandiflora* Nakai, with similarity indices between 0.766 and 0.901. As shown in Figure 3, the representative standard fingerprints of *G. jasminoides* Ellis were compared with those of *G.*

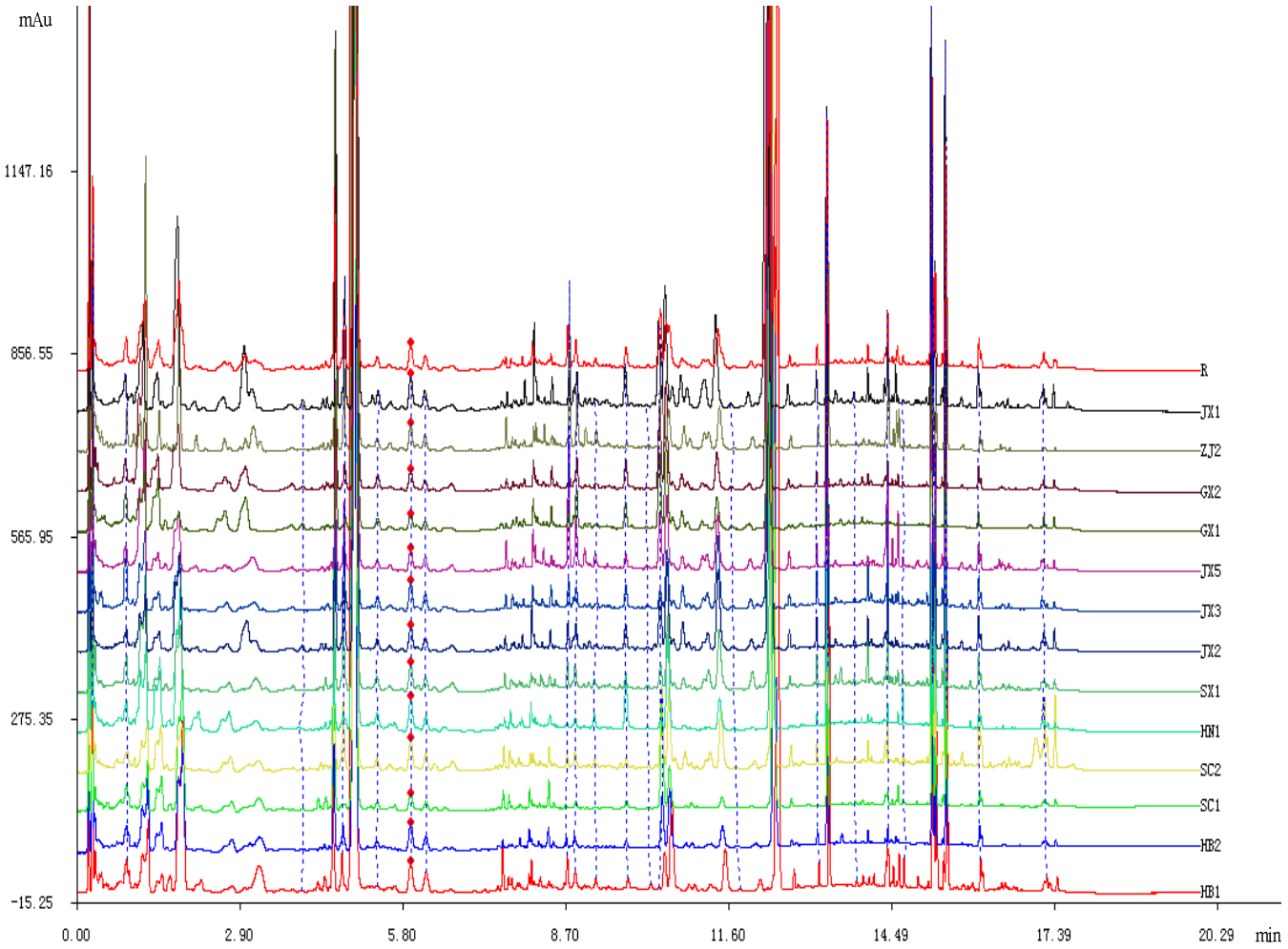

Figure 2. Chromatographic fingerprinting of *G. jasminoides* Ellis.

jasminoides Ellis var. *grandiflora* Nakai. It was shown that there were 24 common peaks for the two species. Based on the comparisons with standard compounds of the UV spectra and their retention time, seven peaks, 3, 4, 5, 7, 8, 18, 20 were unambiguously identified as geniposidic acid, chlorogenic acid, shanzhiside methyl ester, geniposide, genipin, crocin I, crocin II. The two species shared many common aspects in individual peak area and peak height. We magnified two sectional fine regions A and B. Though the close similarity of LC chromatograms explain that some fructus *G. jasminoides* Ellis var. *grandiflora* Nakai were substituted as fructus *G. jasminoides* Ellis, a few tiny differences were shown in fingerprint analysis in compositions between *G. jasminoides* Ellis var. *grandiflora* Nakai and *G. jasminoides* Ellis.

Chemometric study

Medicinal materials of the complexity of the chemical constituents could not be distinguished by simple determinations. Besides similarity analysis of correlation coefficients, chemometric techniques are now being routinely generated in chemical pattern recognition study for its ability to search for interesting structures in a multivariate data set (Alaerts et al., 2010). In the present study, the chemical pattern of the samples was recognized by multivariate statistical tools of PCA and HCA.

PCA is a dimension reduction tool for multivariate statistics (James and Jane, 2005; Kong et al., 2009). We implemented PCA computation by performing singular value decomposition on the data array of the fingerprints, which consisted of a total of 18 × 12 data matrix. Each row represented a medicinal sample. Each column contained the values of 12 characteristic peak areas, including the seven marker compounds, and the compositions corresponded to peak 8, 14, 16, 21 and 24. The data was listed in Table 7, and the visualization of the results was shown as biplot in Figure 4. In the biplot, five crocetin compounds shared a positive correlation.

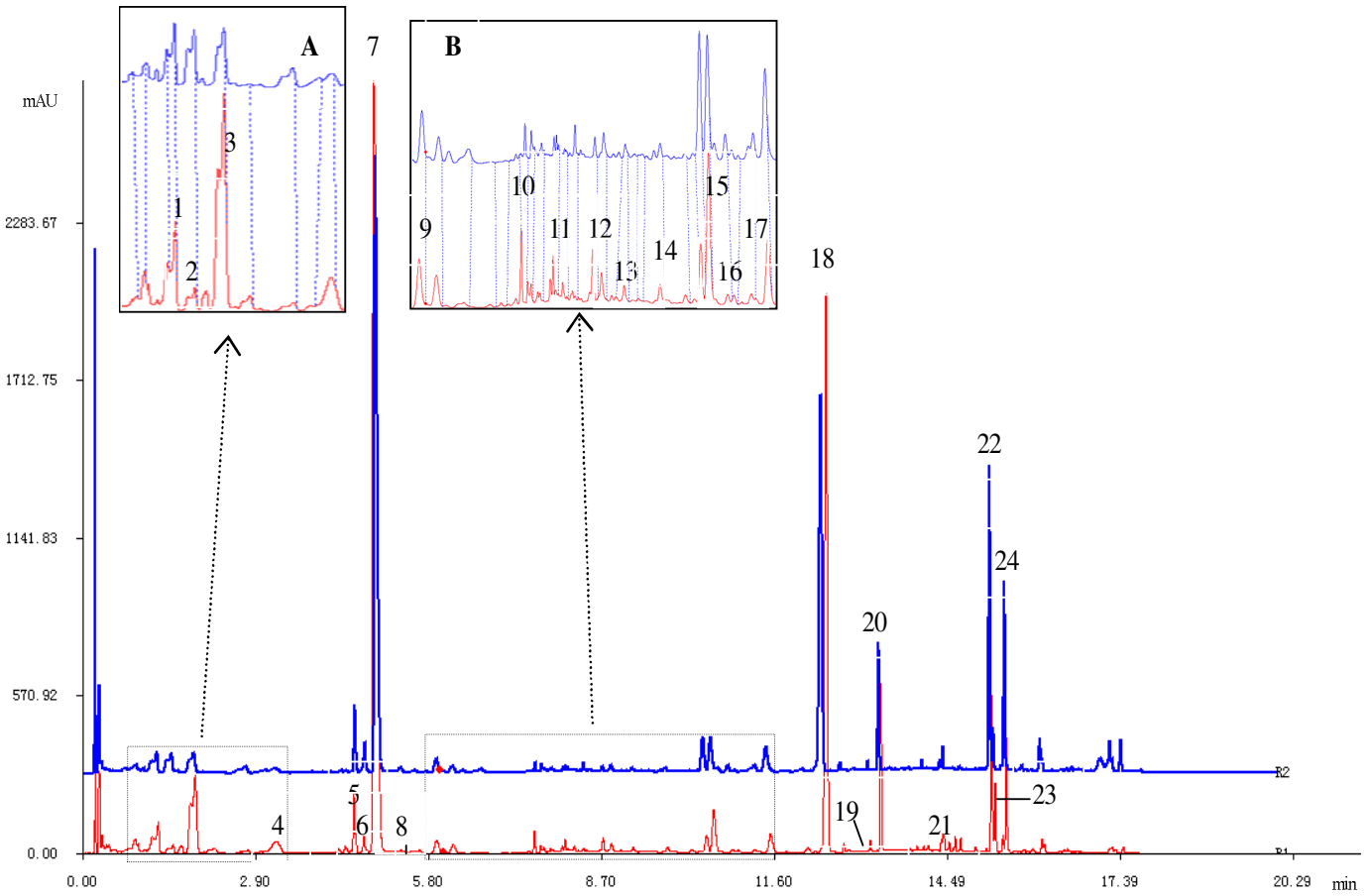

Figure 3. Comparison of the two standard fingerprints of *G. jasminoides* Ellis (R1, the lower red marked fingerprint chromatogram) and *G. jasminoides* Ellis var. *grandiflora* Nakai (R2, the blue marked fingerprint chromatogram). 24 common peaks were marked by numbers and two sectional fine regions of A and B were magnified.

They were crocin I, crocin II, and the other three ingredients corresponded to peak 14, 21, 24, which had the maximum absorption wavelength nearby 440 nm. The samples, which were rich in crocetins, were widely distributed in the Northwest and the Yangtze River Basin. Among them the sample of S11 had the highest content. Furthermore the samples from Yangtze River basin and South China were clustered together in another area. And the points of geniposide, shanzhiside methyl ester, genipin and the other two iridoids compounds corresponded to peaks 8 and 14 (the maximum absorption wavelength was nearby 240 nm) were close to these samples too. It was indicated that there were positive correlations between these substances of iridoids and samples. It was also observed that chlorogenic acid and geniposidic acid shared a weak correlation with the main samples, as the two points were deviated from the main distribution area. The unusual sample of S14, which could be seen intuitively in the figure, contained a very low content of crocetins, which may be related to climatic and geographical conditions. From the biplot, we could draw that compounds of close similar structures shared a positive correlation, which suggested that determination of minority characteristic components, such as geniposide, chlorogenic acid and crocin I could reflect general profile of fructus Gardeniae.

HCA is usually employed to estimate the classification of samples by repeatedly calculating of the distance measures between objects in the field of phytochemical analysis (Alaerts et al., 2010). In this study, the similarity between fingerprints was measured using the squared Euclidean distance, which is often used to detect differences and the correlation. And the between-groups linkage method was applied as the criterion for clustering (James and Jane, 2005). The outcome was represented graphically as a dendrogram in Figure 5. The result visualized that the batches were divided into two clusters. The substitutes of *G. jasminoides* Ellis var. *grandiflora* Nakai were mainly distributed in the first cluster.But the genuine *G. jasminoides* Eills were found distributed in all the two clusters. Reference to the content measured before, it could be observed that the main high-quality

Table 7. The average area of 12 peaks.

No.	GA	CA	SME	GS	GP	P8	P14	P16	CI	CII	P21	P24
S1	1385527	392031	522334	12149962	55299	288141	709119	417726	5193265	839521	1471081	854662
S2	1277643	255707	505132	7222547	105505	240072	418004	198450	3125010	706713	484810	428663
S3	1467025	217429	876145	8625598	89902	142576	292393	104479	2515632	424124	695804	406276
S4	578478	189947	558715	7924359	147983	302902	612474	498089	4643144	903514	2405731	1290777
S5	523065	104328	605203	8356603	93596	285559	472868	367273	4031177	942212	1971108	1081817
S6	1548746	292808	376777	13587611	181693	312141	652998	274441	2811554	538128	1070494	420709
S7	812938	215285	330220	8225111	160189	240339	679831	814344	6660470	971673	1546415	755932
S8	719597	248011	371338	8947446	164476	223875	502125	530578	6189974	1184876	1617476	621989
S9	375903	227140	228287	7420335	98949	205036	422262	341695	4667380	1083994	1584105	349745
S10	1052378	172710	587522	8855728	83266	238333	630051	635479	4986973	721071	1241093	840338
S11	583136	31562	338854	6692170	123911	271093	460658	608550	6129770	1155262	1496564	640029
S12	767552	180411	326145	8141210	113117	238811	557815	473163	4904037	730288	1531563	723503
S13	636346	330128	539877	11645942	88079	176154	537710	473163	4904037	730288	976972	723503
S14	272141	405579	470420	6358037	142035	208962	647714	315225	1003585	265758	580875	400332
S15	778332	441460	628839	8289551	152832	206385	724158	326160	3567123	581884	829295	617147
S16	483110	182138	406733	6851845	58633	218780	200422	459811	3820329	936970	1265972	349087
S17	1028620	340942	866220	10154152	123821	308318	783037	537339	4520518	766927	1047328	545829
S18	1535867	278706	971795	11298582	156079	332466	792647	791033	6381945	959956	1597606	1073243

source of fructus Gardeniae were the five samples of the second cluster, which come from the middle and lower reaches of the Yangtze River. In addition, the quality of sample GX1 was inferior because of the low content of chemical compositions.

Conclusion

This study focused on the simultaneous evaluation of the similarities and differences between *G. jasminoides* Ellis var. *grandiflora* Nakai and *G. jasminoide* Ellis from different regions based on simultaneous determination of seven characteristic constituents, chromatographic fingerprint and related chemometric analysis. The most compounds were observed when the powdered medicinal material was extracted in ultrasonic bath for 30 min with 75:25 methanol/ water (v/v). The chromatographic conditions, including the selection of mobile phases, the gradient of mobile phase, the wavelength for detecting, were optimized. As shown earlier, chromatographic fingerprint had been established based on chromatographic profiles obtained by the UPLC-PDA. It was observed that the finger-print common pattern comprises 24 characteristic peaks. Seven of the 24 characteristic peaks were identified by comparing with the reference com-pounds based on their UV spectra, retention times and the data obtained by LC–MS. The seven com-pounds were quantitatively determined. Though each liquid chromatogram exhibited difference, it was difficult to classify the samples from different origins and species solely relying on comparisons of content of the seven compounds. In the fingerprint, although similarity indices of all samples were very close, a few differences could be observed when some regions were magnified. In this case, chemical pattern recognition methods of PCA and HCA were applied to search the structures in the multivariate data set. More infor-mation could be obtained by dimension reduction and interactive graphical analyses. In the biplot, compounds of close similar structures shared a positive correlation, which means that determina-tion of minority characteristic components, such as geniposide, chlorogenic acid and crocin I, could reflect general profile of fructus Gardeniae. The low content of chlorogenic acid and the widely

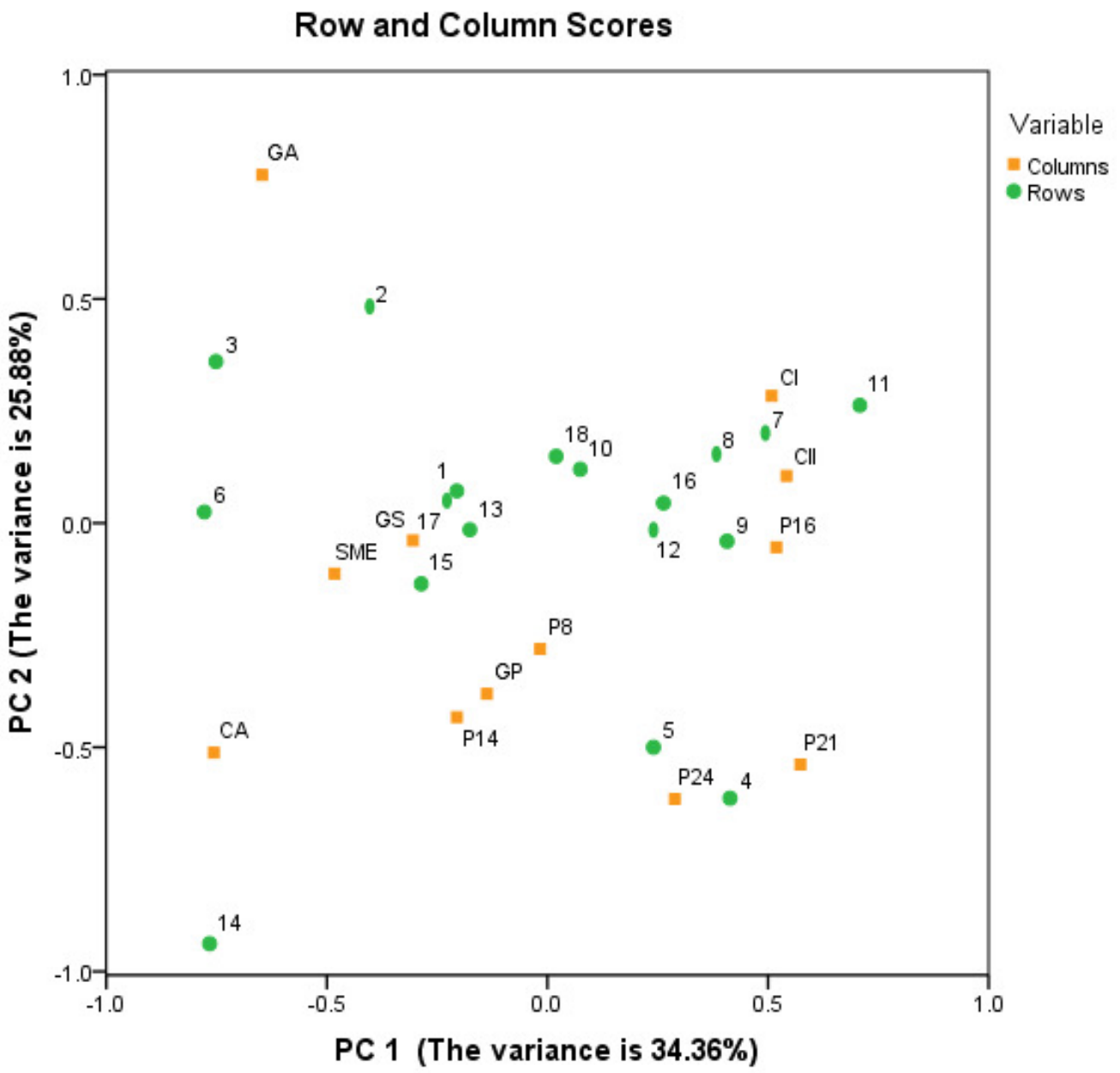

Figure 4. Biplot of the 18 chromatographic responses of samples *G. jasminoides* Ellis (●) and *G. jasminoides* Ellis var. *grandiflora* Nakai (●). The samples of S1-S18 are numbered 1 to 18. The twelve peaks' area was represented as (■). The seven marker compounds were abbreviated earlier and the peaks 8, 14, 16, 21 and 24 were listed as P8, P14, P16, P21 and P24.

distribution of it in phanerogamae prompted that it was not a suitable marked ingredient. For revelation of their differences, HCA was subsequently implicated to explore. From the dendrogram, two clusters were graphi-cally displayed. The substitutes of *G. jasminoides* Ellis var. *grandiflora* Nakai were mainly distributed in the first cluster, while the genuine *G. jasminoides* Eills were found to be distributed in all the two clusters. It was implied that no obvious boundary of chemistry classification could be recommended to differentiate *G. jasminoides* Ellis var. *grandiflora* Nakai and *G. jasminoides* Eills.

In conclusion, for the quality control of fructus Gardeniae, it is simple, feasible and effective to deter-mine the content of geniposide and crocin I, and then make a comparison of fingerprint atlas.

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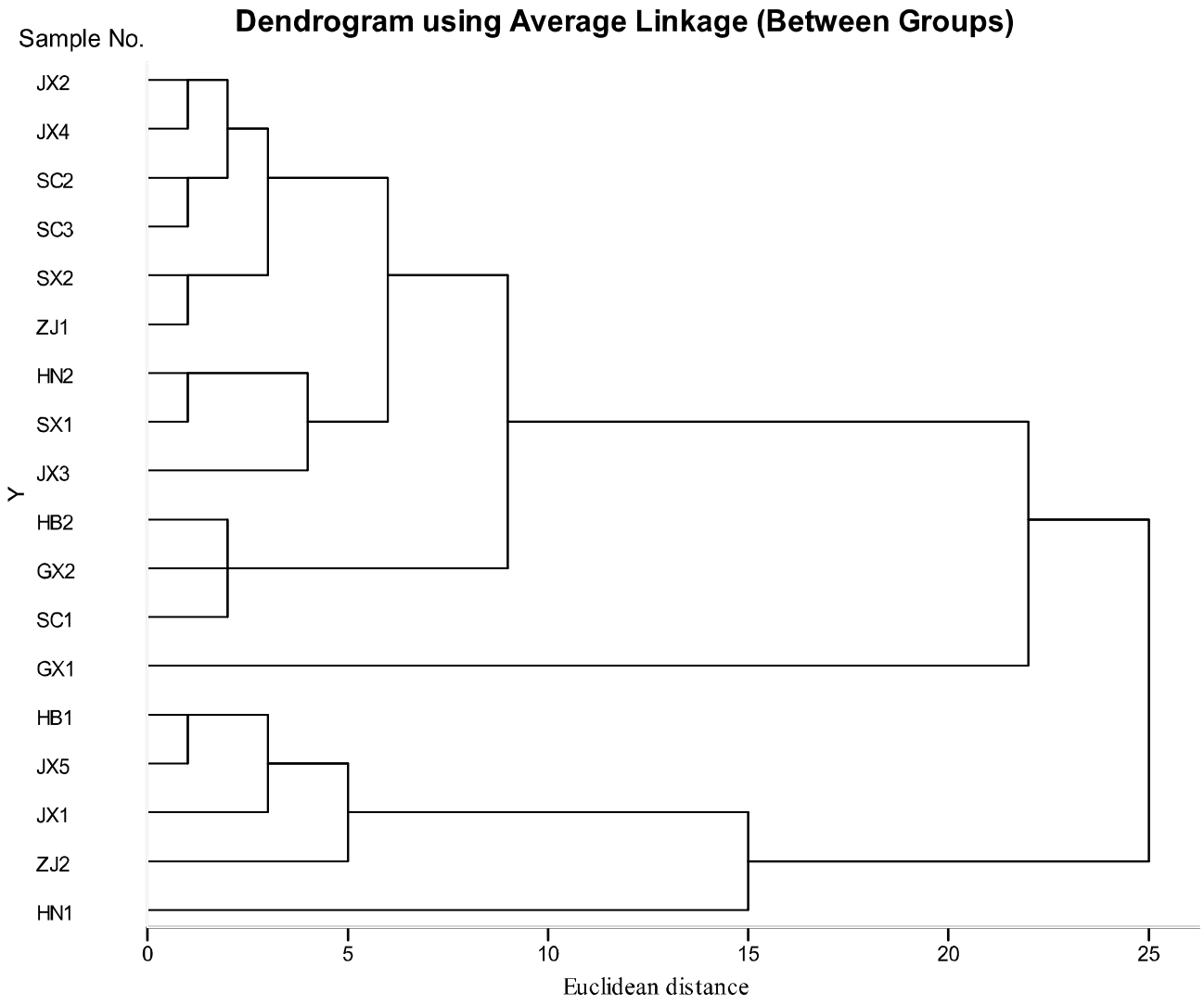

Figure 5. HCA dendrogram for the samples. The abbreviations of samples were the same as listed in Table 1.

support of this work.

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