Geographical Origin Discrimination of *Zanthoxylum bungeanum* from Southwest China Using HPLC and PCA: Identification and Determination of Marker Compounds

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Abstract: The geographical origin of 15 samples of *Zanthoxylum bungeanum* fruits obtained from five different regions in Southwest China was differentiated. The pericarps of *Z. bungeanum* were extracted using methanol. Fractions were obtained by column chromatography and analyzed by high-performance liquid chromatography (HPLC) analysis. Principal component analysis (PCA) and partial least-square discriminant analysis (PLS-DA) were performed to develop classification models to differentiate between samples from Hanyuan and those from other regions of Southwest China. The results showed that samples from the Hanyuan region were significantly different from those obtained from the other regions; PLS-DA analysis showed a greater discriminatory effect than did PCA approach. In addition, the chemical compounds with variable influence on the projection (VIP) > 1 from PLS-DA models and $p < 0.05$ for unpaired Student's *t*-test were considered relevant for group discrimination. These included neochlorogenic acid, chlorogenic acid, quercitrin, hydroxy-β-sanshool, and hydroxy-γ-sanshool, which could be potential markers to distinguish Hanyuan *Z. bungeanum* fruits from those obtained from other regions of Southwest China. Hydroxy-γ-sanshool content alone in *Z. bungeanum* fruits from Hanyuan region was higher than that in *Z. bungeanum* fruits from other regions, while contents of other compounds were lower; the differences were significant ($p < 0.05$). The results of this study provide theoretical and experimental basis to some degree for screening and quality evaluation of *Z. bungeanum* fruits from different geographical origins.

Key words: *Zanthoxylum bungeanum*; principal component analysis; partial least square discriminant analysis; marker compound

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基于 HPLC 与主成分分析法区别西南地区不同产地花椒的地域差异:标记化合物的测定和鉴定

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摘要：为研究西南地区不同产地花椒的地域差别，本文选取了 5 个产地花椒的 15 个样品作为实验材料，采用有机溶剂提取并用柱层析方法分离得到不同的组分。通过高效液相色谱法(HPLC)对分离后的组分进行分析。利用主成分分析(PCA)和偏最小二乘判别分析(PLS-DA)，找出汉源花椒与其他产地花椒的主要差异物。结果表明，汉源花椒能够与其他产地花椒明显区分，且 PLS-DA 的分离效果优于 PCA。以 VIP（变量重要性）>1 及 p<0.05 为指标筛选出汉源花椒和其他产地花椒的主要差异物为新绿原酸、绿原酸、榭皮苷、羟基-β-山椒素和羟基-γ-山椒素，其中汉源花椒的羟基-γ-山椒素含量显著高于其他产地花椒，其他差异物含量均低于其他产地花椒，差异有显著性（p<0.05）。本研究为不同产地花椒的筛选及品质评价提供了一定的理论基础和实验数据。

关键词：花椒；PCA；PLS-DA；标记化合物

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Chinese prickly ash (*Zanthoxylum bungeanum* Maxim), commonly called *huajiao* in China, belongs to the genus *Zanthoxylum* of the family *Rutaceae* and is an aromatic tree and shrub. The fruits of *Z. bungeanum* have been widely used as spices in Chinese cuisine owing to their pungent taste, known as *numb* [1]. In addition to culinary applications, many species of *Zanthoxylum* have also been used as traditional Chinese medicine for the treatment of cold, itching, helminth infections, epigastric pain, toothache, and dysentery [2]. The constituents of the fruits include citronellal- and sanshool-type unsaturated alkylamides, which cause a tingling sensation and are pharmacologically active [3]. Antioxidant activity of the fruits and leaves has also been reported, which was attributed to the presence of flavonoids and phenolic acids in the pericarps of *Z. bungeanum* [4]. Recently, distinguishing characteristics of *Z. bungeanum* and *Z. schinifolium* collected from different regions have been identified. Studies indicate that alkylamidess such as hydroxyl-γ-sanshool, volatile oils, and terpenes could be used to differentiate between *Z. bungeanum* and *Z. schinifolium* [5, 6]. Moreover, it has been reported that the content of volatile oil is significantly different in *Z. bungeanum* from various regions, while that of components responsible for the numb taste is not different [7].

The use of *Z. bungeanum* can be traced back two thousand years and it plays a significant role in Sichuanese cuisine, which is famous for a unique taste attribute known as “ma la” (tingling andchili)[1]. *Huajiao* is widely cultivated in the Sichuan province of Southwest China. In particular, *Z. bungeanum* from Hanyuan region is popular for its long history of use and high quality and is well accepted by consumers in China. It is well known that *Z. bungeanum* varies in different regions; however, no information is currently available regarding the characterization of the geographical origin of *Z. bungeanum* from Hanyuan and the other regions of Southwest China.

Metabolomics, the study of low molecular-weight metabolites in a system, is well established and systematic, and has gained importance in many research areas [8]. In food science, it can be used as a tool for evaluating parameters including geographical origin [9], quality [10], and safety [11]. Principal component analysis (PCA) and partial least square (PLS) are widely used to maximize classification and extract valuable information for differentiation of samples [8].

In this study, the chemical compositions of *Z. bungeanum* from Hanyuan region and other regions of Southwest China were measured and compared to differentiate samples on the basis of their geographical origin. In addition, potential marker compounds from *Z. bungeanum* fruits from the Hanyuan region were detected using high-performance liquid chromatography (HPLC) and identified by PCA and PLS-discriminant analysis (DA).

1 Materials and Methods

1.1 Materials and Reagents

Dried pericarps of *Z. bungeanum* were collected from five different regions of Southwest China. In total, 15 samples (three each from Hanyuan, Qingchuan, Maoxian, Wenchuan, and Chongqing) were collected in August 2013 and stored at the Key Laboratory of Food Biochemistry of Sichuan University. The pericarps were divided into two groups, Hanyuan (H) and other origins (Qingchuan, S1; Maoxian, S2; Wenchuan, S3; and Chongqing, S4).

As previously described, hydroxyl-γ-sanshool (purity, HPLC ≥ 90%) was isolated from commercially available *Z. bungeanum* oil [5]. Quercitrin (purity, HPLC ≥ 95%) was obtained from *Z. bungeanum* leaves, as previously described [12]. In addition, neochlorogenic acid (purity, HPLC ≥ 93%) and chlorogenic acid (purity, HPLC ≥ 93%) were isolated from leaves of *Z. bungeanum*. Characteristics of neochlorogenic acid: white powder; ESIMS (negative), *m/z* 353 $[M-H]^-$; ^{1}H-NMR δ (600 MHz, methanol-d_4) ppm (*J* in Hz): 2.06-2.24 (4H, m, H-2, 6), 3.64 (1H, m, H-4), 4.14 (1H, m, H-3), 5.35 (1H, d, *J* = 3.75, H-5), 6.30 (1H, d, *J* = 15.85, H-8’), 6.77 (1H, d, *J* = 8.34, H-5’), 6.93 (1H, dd, *J* = 1.88, 8.34 Hz, H-6’), 7.04 (1H, d, *J* = 1.88, H-2’), 7.58 (1H, d, *J* = 15.85, H-7’). Characteristics of chlorogenic acid: ESIMS (negative), *m/z* 353 $[M-H]^-$; ^{1}H-NMR δ (600 MHz, methanol-d_4), ppm (*J* in Hz): 2.06-2.24 (4H, m, H-2, 6), 3.72 (1H, d, *J* = 6.41, H-4), 4.17 (1H, d, *J* = 5.21, H-3), 5.33 (1H, d, *J* = 4.32, H-5), 6.25 (1H, d, *J* = 15.92, H-8’), 6.77 (1H, d, *J* = 8.18, H-5’), 6.94 (1H, d, *J* = 8.18, H-6’), 7.04 (1H, d, *J* = 1.54, H-2’), 7.55 (1H, d, *J*

= 15.92, H-7'). The NMR data of the two compounds were consistent with those reported in the literature [13].

The solvents for HPLC analysis were of HPLC-grade. All other reagents used were of analytical grade.

1.2 Sample preparation

Before extraction, the dried pericarps of *Z. bungeanum* were crushed into powders (approximately 40 granularity), using a mixer (JYL-350, Jiuyang Co., Ltd., China). The phytochemical compounds (mainly amides and polyphenols) were obtained using an organic solvent. Briefly, 1 g of the powders was extracted in 10 mL of 70% aqueous methanol solution by continuous stirring at room temperature for 12 h. Then, the extract was filtered and condensed using a rotary evaporator (RE-52AA, Yarong Co. Ltd., Shanghai, China) at 45 ℃ under vacuum. Thereafter, the dried extract (0.7 g) was passed through an ODS open column (15 × 130 mm) with a water-methanol gradient to give three fractions: Fr. 1, 15% methanol in water (80 mL); Fr. 2, 50% methanol in water (80 mL); and Fr. 3, 100% methanol (80 mL). Each of the fractions was condensed at 45 ℃ under vacuum, and the dried residue was stored at -20 ℃ prior to further analysis.

1.3 HPLC analysis

HPLC analyses were carried out using an UltiMate 3000 HPLC series instrument. Fr. 1 was run on an Inertsil ODS-3 column (4.6 × 150 mm, 5 μm, GL Sciences, Tokyo, Japan). The mobile phases, consisting of 0.1% formic acid in ultrapure water (A) and methanol (B), were used at a flow rate of 0.8 mL/min. The linear gradient program was as follows: 0 to 5 min, 0% to 20% B; 5 to 10 min, 20% to 30% B; 10 to 15 min, 30% to 40% B; 15 to 20 min, 40% B; 20 to 25 min, 40% to 100% B; 25 to 35 min, 100% B. Separation of Fr. 2 was achieved using a SunFire C18 column (4.6 × 150 mm, 3.5 μm, Waters Corporation, USA). HPLC mobile phases were the same as those for Fr. 1, with the following linear gradient program: 0 to 5 min, 5% to 35% B; 5 to 25 min, 35% B; 25 to 35 min, 35% to 50% B; 35 to 45 min, 50% to 60% B; 45 to 55 min, 60% to 100% B; 55 to 60 min, 100% B. Liquid chromatography run of Fr. 3 was performed on a Nucleodur 100-5 C18 cartridge (4 × 125 mm, 5 μm, Macherey Nagel, Germany) with a gradient of water (A) and acetonitrile (B) (35% to 75% B in 40 min, 75% to 100% B in 10 min, 100% to 100% B in 5 min), flow rate of 0.5 mL/min. Detection was set at ultraviolet (UV) wavelengths of 254, 270, and 320 nm. Each sample was run in triplicate and the average of the results was used for subsequent statistical analysis.

1.4 Statistical analysis

During the optimization of the HPLC analytical procedure, most of the peaks exhibited the largest chromatographic responses at approximately 254 nm for Fr. 1, 254 nm for Fr. 2, and 270 nm for Fr. 3. Thus, the quantitative analyses of chemical components were represented by their relative peak area at the respective wavelength. Statistical analysis of data was performed using SPSS 19.0 software. For multivariate statistical analysis, the HPLC data were imported into SIMACA-P 11.0 (Umetrics AB, Sweden) for data analysis. PCA was used to identify general trends and outliers for an exploratory overview. PLS-DA was used to optimize the differentiation ability and validate the model. R2 and Q2 values were used to assess the amount of variation represented by the principal components and the robustness of the model, respectively. The PLS-DA models were cross-validated by a permutation analysis (200 times) [14].

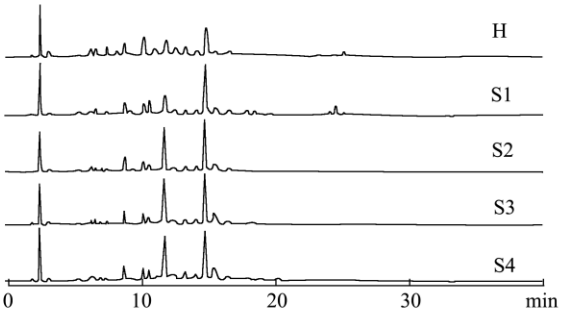
To identify the variables contributing to the differences in spectra between samples from Hanyuan and those from other regions, variable influence on the projection (VIP) values of all peaks from PLS-DA models were calculated, and variables with VIP > 1 were considered relevant for group discrimination. Besides, unpaired Student's *t*-test ($p < 0.05$) was also used to evaluate the significant differences in the peak area for each metabolite. A result of VIP > 1 of multivariate statistical significance and $p < 0.05$ of univariate statistical significance were identified as distinguishing metabolites [15].

2 Results and Discussion

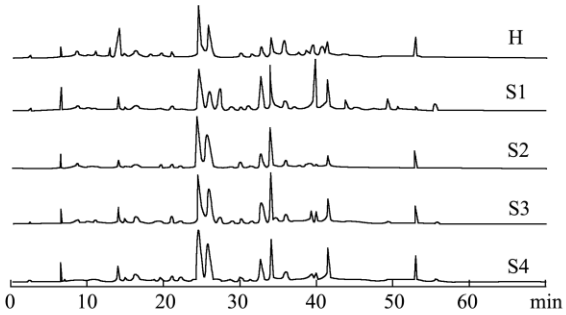
2.1 PCA and PLS-DA models

The 70% methanol extracts from the pericarps of *Z. bungeanum* were subjected to ODS open column to obtain three fractions (Fr. 1-3), using a water-methanol gradient system. Based on the specific nature of the extract, the three fractions were analyzed using different chromatogram columns for HPLC analysis. Representative HPLC chromatograms of Fr. 1-3 are

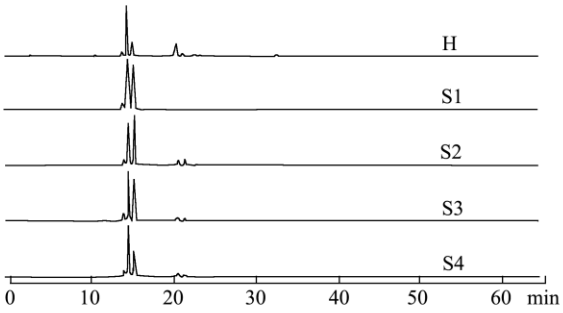
shown in Fig.1. The relative contents are represented by the peak area of each compound (Table 1). The raw data (peak area of each compound and samples) were fed into the SIMCA-P 11.0 software for PCA and PLS-DA analysis; results are shown in Fig.2. The scores t[1] and t[2], one vector for components 1 and 2, are new variables computed as linear combinations of all the original variables to provide a good summary. The results showed a relative separation between Hanyuan region and the other regions on the score plot of the first two principal components (PC) (Fig.2a~c). In this step, samples were identified in a pertinent space of reduced dimension. Unlike supervised methods like PLS-DA, in which both independent and dependent variables are considered, PCA, an unsupervised method, only considers independent variables, which makes it a relatively weaker classification method. Therefore, a visual overview was first acquired using PCA, following which the presence of abnormalities was checked, and then classification was performed using PLS-DA. In addition, the mean-centered scale (ctr) was used in PCA analysis. Thus, low levels of variable information could be suppressed by high content of the signal. The majority of samples lay within the 95% confidence interval; therefore, all samples were used in the subsequent analysis to ensure maximum information. The difference between chemical components was visualized by PLS-DA to optimize the separation between the Hanyuan region and the other regions. As shown in Fig.2D-F, good separation in the score plot of PC1 and PC2 was obtained. This result revealed that the samples were all within the ellipse, meaning that all samples were acceptable by PCA and PLS-DA.



1A



2A



3A

Fig. 1 Representative HPLC chromatograms of Fr. 1-3. (1A) Fr. 1 detected at 254 nm; (2A) Fr. 2 detected at 254 nm; and (3A) Fr. 3 detected at 270 nm

Note: H: Hanyuan; S1: Qingchuan; S2: Maoxian; S3: Wenchuan; S4: Chongqing.

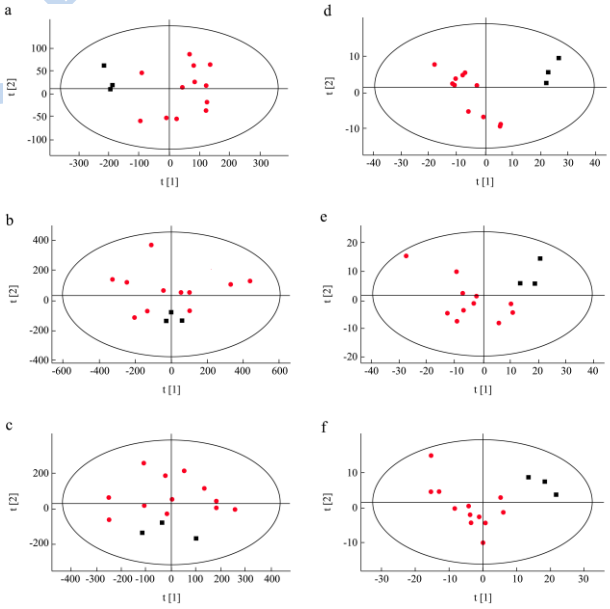


Fig.2 PCA(A-C) and PLS-DA(D-F) scores plot of Z. bungeanum pericarps from Hanyuan (■) and other regions of southwest of China (●). A, D for Fr.1; B, E for Fr.2; C, F for Fr.3

Table 1 Quantitative analysis of chemical components in *Z. bungeanum* pericarps from the Hanyuan region and the other regions of Southwest China

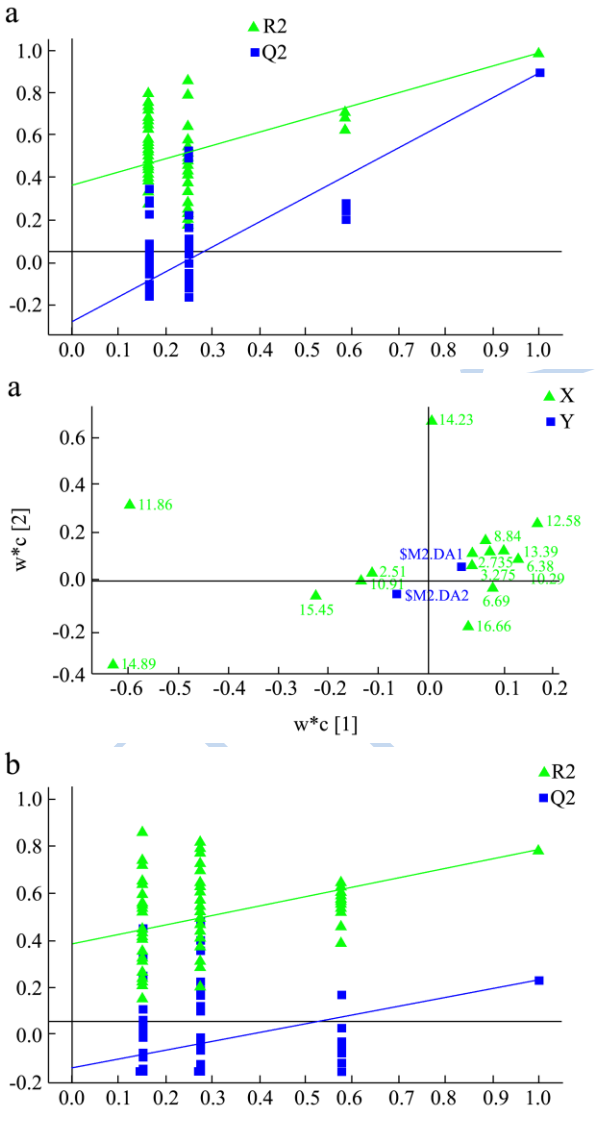
[a]R_t /min	H /(mAU*min)	S1 /(mAU*min)	S2 /(mAU*min)	S3 /(mAU*min)	S4 /(mAU*min)	[b]VIP	[c]*p*-Value
Fr.1							
2.51	34.42± 12.26	44.01±6.20	41.36±10.27	43.60±6.19	47.10±3.61	0.40	0.0706
2.74	11.09± 1.36	6.52±0.52	9.00±1.95	6.03±0.23	6.65±0.45	0.36	0.0010
3.28	11.04±0.84	6.65±0.45	6.99±1.32	7.79±0.48	7.72±2.50	0.32	0.0009
6.38	16.08±4.47	8.37±0.18	9.84±2.11	9.66±0.30	8.18±0.75	0.48	0.0002
6.69	52.66±12.27	50.19±2.20	38.17±6.95	40.56±4.60	35.76±3.95	0.46	0.0453
8.84	47.18±15.98	37.10±3.61	35.16±3.63	39.82±2.84	38.36±2.91	0.48	0.0531
10.29	64.18±6.58	33.69±18.09	34.30±17.47	52.78±6.29	50.74±1.30	0.65	0.0300
10.91	18.81±14.16	42.82±21.27	48.44±27.86	22.57±7.88	31.88±12.77	0.48	0.1663
11.86	81.71±17.37	153.83±61.80	279.31±73.74	324.86±24.00	297.21±10.54	2.23	0.0022
12.58	43.71±2.53	20.40±8.26	22.07±8.20	23.17±10.18	19.10±10.55	0.85	0.0005
13.39	52.40±28.81	33.43±20.82	32.10±10.18	33.01±8.73	37.37±8.67	0.67	0.0868
14.23	52.67±15.04	21.37±7.56	61.77±42.59	62.21±17.92	60.54±23.57	1.10	0.9464
14.89	192.88±13.11	336.86±33.67	390.03±17.99	370.01±21.11	326.27±19.08	2.29	0.0000
15.45	40.03±17.37	72.95±61.89	79.34±65.76	91.40±30.70	98.82±41.60	0.81	0.1204
16.66	30.58±10.00	26.73±8.05	19.43±10.54	25.18±3.24	24.97±3.48	0.44	0.1896
Fr.2							
2.22	7.63 ±5.45	10.56±9.27	11.88±2.61	7.75±6.79	7.12±6.73	0.30	0.7223
6.17	10.13 ±6.20	19.74±12.85	9.26±9.03	11.22±9.16	11.97±15.34	0.42	0.6397
24.44	671.81±66.25	534.65±155.90	689.72±84.25	613.15±239.29	773.83±164.13	0.68	0.8388
25.72	463.75 ±12.90	317.22±106.86	434.37±102.39	484.22±166.97	603.00±130.27	0.38	0.9855
26.97	16.46 ±11.14	277.76±49.86	0.00±0.00	12.09±20.94	42.18±13.00	1.31	0.3343
33.90	178.36±23.31	410.34±66.47	352.36±70.31	349.56±115.36	359.89±39.55	2.21	0.0008
39.09	42.02±72.78	106.02±13.38	33.90±5.49	48.23±33.22	45.30±30.13	0.45	0.5340
41.21	113.71±63.71	192.85±90.45	152.87±125.47	119.65±42.17	266.45±17.87	1.03	0.1992
49.00	20.11±24.44	44.62±28.51	29.96±20.42	7.25±3.93	24.34±6.85	0.50	0.6682
Fr.3							
14.16	25.35±8.30	31.34±7.87	49.79±11.45	57.27±4.93	59.86±8.75	0.67	0.0131
14.73	498.67±108.89	349.07±136.55	508.05±213.63	564.17±114.64	606.99±91.31	0.54	0.9341
15.42	86.00±38.52	165.58±61.75	291.45±129.80	340.36±101.70	279.70±42.20	1.99	0.0106
20.74	130.70±21.62	8.61±5.99	65.42±59.69	49.53±19.01	52.43±15.07	1.55	0.0015
21.59	21.97±6.99	2.27±3.93	31.20±22.23	25.75±7.07	20.15±3.03	0.60	0.8215
22.96	13.42±1.25	2.56±2.22	9.71±6.60	7.77±1.52	8.64±2.04	0.39	0.0294
23.69	3.15±1.19	0.00±0.00	1.04±1.80	1.73±0.92	1.58±1.37	0.21	0.0240
33.02	15.77±0.92	1.58±2.73	9.15±6.00	7.32±2.36	6.32±1.17	0.54	0.0020

Note: H: Hanyuan; S1: Qingchuan; S2: Maoxian; S3: Wenchuan; S4: Chongqing; [a] Retention time; [b] Variable influence on the projection was obtained from PLS-DA model with a threshold of 1.0; [c] *p*-value obtained from unpaired Student's *t*-test. Data are presented as mean ± standard deviation.

The PC1 and PC2 score plots show that all fractions (Fr. 1-3) of samples from the Hanyuan region could be clearly distinguished from samples from the other regions of Southwest China. As shown in Fig.2, the samples can

be divided into two groups based on the position in the score plots of PCA (Fig.2a~c) and PLS-DA (Fig.2d~f), indicating that PCA and PLS-DA methods differentiated between the Hanyuan and other regions.

Meanwhile, individual samples in PLS-DA were more closely grouped into discrete clusters than they were in PCA; the group situated mainly in the first quadrant was from the Hanyuan region, while those in other quadrants were randomly composed of samples from the other four regions evaluated (Fig.2d~f). It was suggested that the three samples collected from the Hanyuan region were obviously different from those collected from the other regions, especially for PLS-DA analysis.



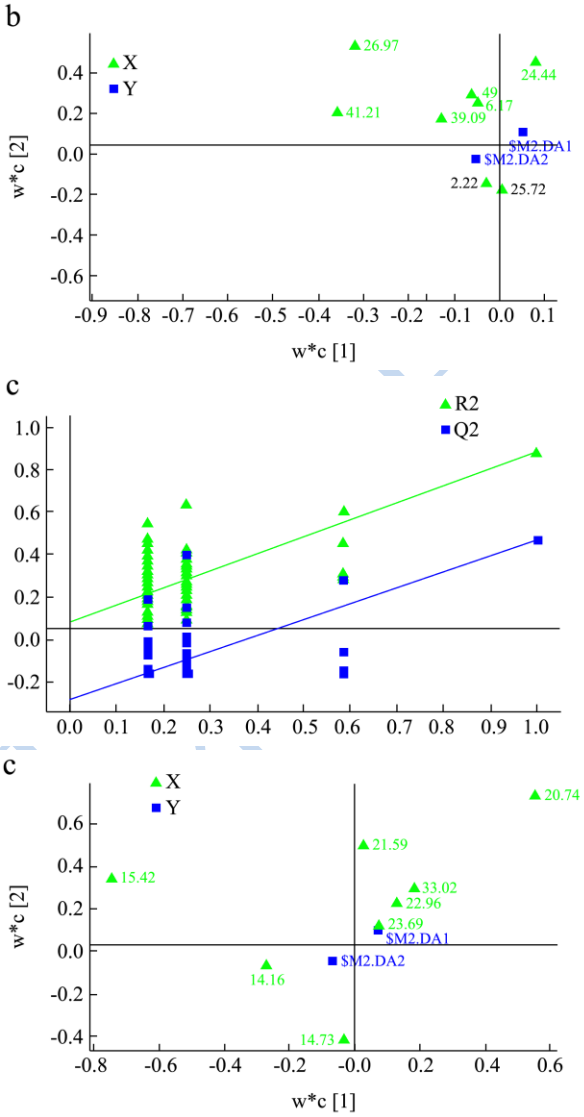


Fig. 3 Validated models and loading plots of PLS-DA analysis. (A) Fr. 1; (B) Fr. 2; and (C) Fr. 3

The total variance of the data explained by the PCA model built for different fractions was as follows: Fr. 1: R^2X_{cum}= 87.5%, with 76.6% from PC1 and 10.9% from PC2; Fr.2: R^2X_{cum}= 82.7%, with 56.2% from PC1 and 26.5% from PC2; and Fr.3: R^2X_{cum}=96.6%, with 59.1% from PC1 and 37.5% from PC2, which was high enough to represent all the variables. Moreover, to prove the validity of the models, PLS regression was applied to connect the information in two sets of variables, by setting *Y* value to 1/2 matrix (the Hanyuan region to 1 and the other regions to 2), with *X* indicating peak area data from the HPLC analysis and *Y* representing samples

from the different regions. Model parameters in the permutation test (n = 200) for the explained variation (Fr. 1: R2 = 0.94; Fr. 2: R2 = 0.96; and Fr. 3: R2 = 0.84) and the predictive capability (Fr. 1: Q2 = 0.84; Fr. 2: Q2 = 0.89; and Fr. 3: Q2 = 0.42) by different fractions were almost significantly high, indicating good quality of the models (Fig.3).

The phytochemical compounds, which contributed to the differences between the two groups, were analyzed by PLS-DA loading plots (Fig.3). Loadings represent the importance of the variables in those components and show the correlation structure between the variables. Therefore, if the score plot can distinguish between the different groups of samples, the loading plot can express the influence of variables on the separation between classes. The influence of the variable has a negative correlation with its distance to the main cluster of variables. In this study, loading plots of PLS-DA and VIP were generated to identify chemical markers for discriminating between the different groups of samples. Eight variables were important for distinguishing the geographical origin of *Z. bungeanum* in the PLS-DA model (Table 1). It was suggested that not all variables with VIP > 1 were significant. Therefore, only variables with the conditions of VIP > 1 and $p < 0.05$ were identified further.

2.2 Targeted identification of maker compounds

The significantly distinguishable chemical compositions were summarized according to VIP > 1 and $p < 0.05$ (Table 1). The main contributing chemical compounds in Fr. 1 (R_t 11.86 and 14.89 min) were identified based on the comparison of their retention time and UV-visible (vis) absorption with those of abundant standard substances by HPLC analysis. The UV spectra of the peak at R_t 11.86 min exhibited two absorption maxima (215.9 and 325.0 nm), similar to that of neochlorogenic acid. The chemical compound at R_t 14.89 min showed UV absorption maxima at 216.5 and 326.4 nm, which is in accordance with that of chlorogenic acid. The significantly distinguishable chemical composition of Fr. 2 was also identified similarly. The results showed that the compound at R_t 33.90 min with UV absorption maxima at 205.3, 257.2, and 350.5 nm showed retention time and UV-vis absorption similar to those of quercitrin. The compounds at 15.42 and 20.74 min from Fr. 3 exhibited three UV absorption maxima at approximately 270 nm, which is characteristic of an aliphatic conjugated double bond system and in accordance with the data previously reported for sanshool derivatives [5,16]. The compound at 15.42 min with UV absorption maxima at 271.7 nm was identified as hydroxy-β-sanshool, while that at 20.74 min with UV absorption maxima at 273.3 nm was identified as hydroxy-γ-sanshool. As shown in Table 2, the results of fold change revealed that levels of neochlorogenic acid, chlorogenic acid, quercitrin, and hydroxy-β-sanshool were significantly lower in the samples of *Z. bungeanum* from the Hanyuan region as compared to those in the samples of *Z. bungeanum* from the other regions. Conversely, the level of hydroxy-γ-sanshool was significantly higher in the samples of *Z. bungeanum* from the Hanyuan region than in the samples of *Z. bungeanum* from the other regions.

Table 2 Identification and quantification of compounds that distinguish between *Z. bungeanum* pericarps of the Hanyuan region and those from the other regions by HPLC

No.	Compounds	[a]R_t	Concentration/(μg/g)					[b]FC
			H	S1	S2	S3	S4	
1	Neochlorogenic Acid	11.86	586.33±160.53[c]	1559.21±710.91	1912.04±540.17	1553.27±121.62	2196.17±82.95	0.32
2	Chlorogenic Acid	14.89	1614.03±121.19[c]	3664.60±387.36	2723.06±131.75	1782.01±106.96	2424.99±150.25	0.61
3	Quercitrin	33.90	1121.18±171.97[c]	3489.07±604.02	2596.63±756.96	2345.79±837.31	2077.12±246.30	0.43
4	Hydroxy-β-sanshol	15.42	334.75±149.93[c]	793.93±296.07	1040.28±463.31	1303.56±389.52	919.19±138.67	0.33
5	Hydroxy-γ-sanshol	20.74	507.09±84.15[c]	41.29±28.73	233.49±213.07	189.68±72.83	172.29±49.52	3.20

Note: [a] Retention time; [b] Fold change (FC) between the samples from the Hanyuan region and those from the other regions. A fold change >1 indicates that a relatively higher concentration is present in the samples from the Hanyuan region than in those from the other regions,

while a fold change <1 indicates that a relatively lower concentration is present in the samples from the Hanyuan region than in those from the other regions; [c] represents statistically significant differences ($p < 0.05$) between the concentrations of the same compound in the samples from the Hanyuan region and those in samples from the other regions. Data are presented as mean ± standard deviation.

The main contributing chemical compounds were quantified using an external standard calibration curve. The linear regression model was $Y = 375.88\ x + 18.284$ ($R^2 = 0.995$), $Y = 482.92\ x + 26.375$ ($R^2 = 0.997$), and $Y = 246039\ x + 524.94$ ($R^2 = 0.998$), where Y was the peak area and x was the concentration of compounds (mg/mL). Quantitative results of compounds that distinguish between *Z. bungeanum* pericarps of the Hanyuan region and those from the other regions of Southwest China are shown in Table 2.

3 Conclusions

In this study, the geographical origin for *Z. bungeanum* samples from five different Southwest China regions was differentiated using HPLC analysis combinated with PCA and PLS-DA. The results showed that *Z. bungeanum* from the Hanyuan region was different from that obtained from the other regions, as determined especially by PLS-DA analysis. Moreover, PLS-DA analysis indicated that neochlorogenic acid, chlorogenic acid, quercitrin, hydroxy-β-sanshool, and hydroxy-γ-sanshool could be used to differentiate between *Z. bungeanum* from the Hanyuan region and those from the other regions of Southwest China. The proposed method in this study can be extended to generate data to trace the geographical origin of samples based on the fingerprinting information of *Z. bungeanum*.

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