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# Comprehensive quality evaluation of Chishao by HPLC

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**Abstract**

***Objective:*** **The purpose of this paper is to comprehensively evaluate the quality of Chishao.**

***Methods:*** **In the experiment of this paper, the fingerprint spectrums of Chishao in all locations are established by RP-HPLC and the model of principle component analysis with the RP-HPLC peak areas is established.**

***Results:*** **The quality of Chishao in the northern part of China or that made of *Paeonia lactiflora* is better than that of these in others or that made of other species. The quality of Chishao comes from *P. veitchii* is in the middle class and is better than those that comes from *P. obovata, P. mairei* and *P. anomala.* The results are consistent with traditional views of the quality of this plant. These results indicates that principal component analysis (PCA) can be used as an effective and economic method to evaluate the quality of Chishao, and may be extended to other Chinese medicinal plants.**

***Conclusions:*** **Due to the complex basis of the efficacy of Traditional Chinese Medicine (TCM), the method such as PCA of several chemical components appears to be a more appropriate method for the quality evaluation of TCM in contrast to the determination of a single or few chemicals.**

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Key words: *Chishao. Paeonia lactiflora. HPLC. Principle component analysis.*

**EVALUACIÓN CUALITATIVA EXTENSA DE CHISHAO MEDIANTE HPLC**

**Resumen**

***Objetivo:*** **El propósito de este artículo es la evaluación cualitativa extensa de Chishao.**

***Métodos:*** **En el experimento de este trabajo, se establecen los espectros de identificación de Chishao en todas las localizaciones mediante RP-HPLC y el método del análisis de componentes principales con las áreas pico de RP-HPLC.**

***Resultados:*** **La calidad de Chishao en el norte de China o del procedente de *Paeonia lactiflora* es mejor que la de otras localizaciones o procedente de *P. obovata, P. mairei* y *P. anomala.* Los resultados son congruentes con la impresión tradicional de la calidad de esta planta. Estos resultados indican que el análisis de los componentes principales (ACP) puede utilizarse como método eficaz y económico para evaluar la calidad de Chishao y podría aplicarse a otras plantas medicinales chinas.**

***Conclusiones:*** **Dada la complicada base de la eficacia de la Medicina tradicional china (MTC), un método como el ACP para diversos componentes químicos parece ser más adecuado para la evaluación de la calidad de la MTC en comparación con la determinación de un único o unos pocos agentes químicos.**

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Palabras clave: *Chishao. Paeonia lactiflora. HPLC. Análisis de los componentes principales.*

## Introduction

Chishao is the dry root of *Paeonia lactiflora* Pall. and *P. veitchii* Lynch in Paeoniaceae.[1,2] It is common in Chinese medicines. Chishao functions are to subside heat and give cooling effect to the blood, remove stagnant blood and reduce swelling. It also can be used in blood stagnation manifested as dysmenorrhea, amenorrhea, acute inflammation with red swelling and pain from external injury. There are many kinds of chemical components of which functions are not clearly known in Chishao. There are a few types of Chishao used for medication. Therefore, the system for evaluating the quality of Chishao should be established and the usage of Chishao should be standardized. The determination of the content of Paeoniflorin is present method for evaluating the quality of Chishao.[1,3] Such method does not fit for the characteristic of medicinal material which emphasize on its function with cooperation of many components.

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*Paeonia* is an only genus in Paeoniaceae. There are three species of *Paeonia*. They are Sect. *Paeonia*, Sect. *Moutan* and Sect. *Onaepia*. There are about 30 species in *Paeonia* in all, and there are 8 species and 6 variations in China.[2] Most roots of these species are used as medication. However, the quality of these Chishao is different.

We investigated the distribution and characteristic of Chishao in China in recent years. The RP-HPLC fingerprint of Chishao is established and the contents of several components in Chishao were determined. We establish the model for comprehensively evaluating the quality of Chishao by principle component analysis firstly and comprehensively evaluated the quality of Chishao in all locations.

## Materials and methods

### *Reagents and materials*

Instruments: HPLC, Agilent 1100 series (degasser: G1379A, JP13206528; quat pump: G1311A, DE239 21359; man. Inj.: G132813, DE11401960; VWD: G1314A, JP24019414; Agilent 1100 workstation). Electronic analytic balance (precision: 0.00001, BP211D, Metler Toledo CO. Switzerland). Rotary evaporator (RE-52, Shanghai Yarong Biochemical Instrument Factory).

Reagents: Methanol (AR, batch: 20080417, Beijing Chemical factory). Ethanol (AR, batch: 20080408, Beijing Chemical factory). Acetonitrile (HPLC grade, Lot: 083485, Fisher scientific).

Materials: The Chishao materials were collected from various locations within the time frame of 2006-2008. All of the lands or mountains where Chishao grew were freely accessed without involving endangered or unprotected species. The information of the material is showed in table I. Medicinal material for control (*P. lactiflora*) was purchased from National Institute for the Control of Pharmaceutical and Biological Products in March 2009.

### *Methods*

Chishao extract preparation: Chishao extract was prepared according to a reported method.[4-6] The Chishao root was crushed and sieved with 80 meshes sieve after being drought at 50º C. 0.2 g material was immersed in 20 ml 50% ethanol solvent for 30 minutes and refluxed for 2 hours at 90º C. The extract was filtered with filter paper, and then the filtrate was evaporated in rotating evaporator under low barometric pressure (75º C, 0.08 MPa). Each sample was extracted twice. The resultant precipitate was dissolved with 10ml methanol and filtered using a 0.45 μm membrane filter. The peak areas of six chemical components in each extract were determined with the HPLC method.

The condition of HPLC: HPLC column: Diamonsil C18, 5 m, 250 mm × 4.6 mm, cat. no.: 99903, Ser. no.: 813184, Dikma Technologies. Mobile phrase consists of acetonitrile (HPLC grade) and 0.2% Phosphoric acid (v/v). The content of acetonitrile in gradient mobile phrase varies as below: from 5 to12% in 0-18 min; 12 to 15% in 18-22 min; 15% in 22-32 min; from 15 to 20% in 32-45 min; 20% in 45-60 min; from 20 to 40% in 60-80 min; 40% in 80-82 min. The flow rate was 1 ml/min with detection wavelength set at 230 nm. The temperature of HPLC column was same as room temperature. Each sample injected was 5 μl. The column plate number was higher than 5,000 when paeoniflorin was determined.

### *Verify the method of determination*

Precision of HPLC: Extraction of Chishao in Zhangxian County in Gansu province was made with the extraction method once and then was determined with the HPLC method for 6 times. The values of the peak areas correspond to the six authentic compounds in each chromatogram were recorded and the RSD of values for each component was computed by SPSS (Statistical Product and Service Solutions). All of the RSD fitted to the demand of HPLC (table II).

Repeatability of experimentation: Extraction of Chishao in Weichang County in Heibei province was made 6 times with the extraction method and then was determined with the HPLC method once for each extract. The values of the peak areas correspond to 6 components in each chromatogram were recorded and the RSD of values for each component was computed by SPSS. All of the RSD fitted to the demand of HPLC (table II).

Stability of sample extracted: Extraction of Chishao in Wuhada of Management Distriction Wulagai in Inner Mongolia Autonomous Region was made with the extraction method once and then was determined with the HPLC method in 2, 8, 14, 20, 32, 44 hours after extraction. The values of the peak areas correspond to 6 components in each chromatogram were recorded and the RSD of values for each component was computed by SPSS. All of the RSD fitted to the demand of HPLC (table II).

Recovery of standard: Chishao material in Emin County in Xinjiang Uygur Autonomous Region was quantified 0.2 g on Electronic balance for 3 shares and then ended standards in each share as table II. Each Extract was made with the extraction method and then was determined with the HPLC method. The values of the peak areas corresponding to 6 components in each chromatogram were recorded and the RSD of values for each component was computed by SPSS. All of the RSD fitted to the demand of HPLC (table II).

**Table I**
*The detail information of germplasm resources*

| *Species* | *Laceyion: province county town* | *Elevetion: n, latitude, longitude* | *Circunstance, distribution* | *Climate: average in year* | | |
|---|---|---|---|---|---|---|
| | | | | *Rain.*: mm* | *Air Tem.*: ºC* | *Acc. Tem*.* |
| *Lactiflora* | Hebei· Qinglong· Anziling | 410, 40°18 20 N, 119°18 41 E | Forest, sunny, cluster | 580 | 9.3 | 3,696 |
| *Lactiflora* | Inner mongolia Duolun· Caimushan | 1370, 42°24 16 N, 116°45 29 E | Brush, cluster | 440 | 0.4 | 1,872 |
| *Lactiflora* | Heilongjiang· Heihe· Xinsheng | 330, 50°30 26 N, 126°47 06 E | Woods and brush, sunny, cluster | 510 | 0 | 2,328 |
| *Lactiflora* | Inner mongolia· Ergun· Enhe | 709, 50°49 03 N, 119°56 15 E | Woods and brush, shady, cluster | 440 | -3.2 | 1,584 |
| *Lactiflora* | Gansu· Zhangxian· Sancha | 2100, 34°50 30 N, 104°19 33 E | Brush, sunny, cluster | 560 | 7.4 | 2,660 |
| *Lactiflora* | Heilongjiang· Muling· Maqiaohe | 409, 44°41 29N, 130°30 45 E | Woods, sunny, cluster | 540 | 3.6 | 2,616 |
| *Intermedia* | Xinjiang· Emin· 168 regiment | 1480, 46°55 30 N, 84°28 28 E | Brush, sunny, cluster | 270 | 6 | 2,904 |
| *Lactiflora* | Heilongjiang· mudanjiang· xinglong | 356, 44°28 02 N, 129°44 44 E | Forest, sunny, cluster | 560 | 4 | 2,680 |
| *Anomala* | Xinjiang· Emin·168 regiment | 1480, 46°55 30 N, 84°28 27 E | Brush, sunny, cluster | 270 | 6 | 2,904 |
| *Lactiflora* | Hebei· Weichang· longtoushan | 720, 41°57 34 N, 117°40 20 E | Forest, sunny, cluster | 420 | 5 | 2,568 |
| *Lactiflora* | Jilin· Lishu· shijiapu | 264, 43°11 46 N, 124°35 53 E | Brush, sunny, cluster | 650 | 6 | 2,970 |
| *Lactiflora* | Hebei· Weichang· Chaoyangwan | 690, 41°57 44 N, 117°55 48 E | Woods, shady, scaterred | 420 | 5.8 | 2,688 |
| *Lactiflora* | Liaoning· Xingcheng· Jianchang | 710, 40°41 02 N, 120°17 28 E | Woods, shady, scaterred | 650 | 9.6 | 3,696 |
| *Lactiflora* | Hebei· Weichang· Yudaokou | 1484, 42°21 28 N, 117°07 50 E | Grassland, sunny, cluster | 460 | 1.4 | 1,944 |
| *Lactiflora* | Shaanxi· Huanglong· Shipu | 1400, 35°39 15 N, 109°51 29 E | Forest, shade, cluster | 580 | 10.2 | 3,264 |
| *Lactiflora* | Inner mongolia· Hailaer | 610, 49°12 27 N, 119°43 51 E | Grassland, sunny, cluster | 340 | -1.3 | 1,992 |
| *Lactiflora* | Shanxi·Jiangxian· Nanfan | 545, 35°36 32 N, 111°35 52 E | Brush, sunny, cluster | 580 | 12.6 | 4,272 |
| *Lactiflora* | Inner mongolia· Wulagai· Hesigewula | 1074, 46°12 43 N, 119°00 29 E | Grassland, cluster | 440 | -0.2 | 1,944 |
| *Lactiflora* | Inner mongolia· Wulagai·Wulahada | 1053, 46°24 20 N, 119°21 04 E | Grassland, cluster | 420 | 0 | 1,944 |
| *Lactiflora* | Liaoning· Fuxin· Daban | 190, 41°55 58 N, 121°52 17 E | Woods, cluster | 580 | 8.6 | 3,528 |
| *Lactiflora* | Shanxi· Fenyang | 760, 37°16 04 N, 111°46 41 E | Woods and brush, cluster | 510 | 10.5 | 3,792 |
| *Lactiflora* | Inner mongolia· Kerqinyouyiqianqi· Huhemachang | 540, 46°18 33 N, 121°23 28 E | Woods, cluster | 490 | 5.6 | 3,072 |
| *Lactiflora* | Henan· Luanchuan· Chengguan | 890, 33°46 05 N, 111°42 30 E | Forest, cluster | 860 | 14.7 | 4,704 |
| *Lactiflora* | Liaoning· Shenyang· Puhe | 110, 41°58 32 N, 123°42 35 E | Woods, cluster | 760 | 7.8 | 3,336 |
| *Lactiflora* | Inner mongolia· Tuquan· Baoshi | 1050, 45°49 55 N, 120°55 12 E | Grassland, sunny, cluster | 420 | 5.8 | 3,048 |
| *Lactiflora* | Inner Mongolia· Erwenkezuqi· Honghuaerji | 740, 48°16 40 N, 119°58 42 E | Woods, cluster | 370 | -1.3 | 1,944 |
| *Lactiflora* | Inner Mongolia· Kerqinyouyizhongqi· Harinula | 730, 45°37 04 N, 120°03 32 E | Grassland, cluster | 420 | 5.4 | 2,976 |
| *Lactiflora* | Ningxia· Longde· Shatang | 1980, 35°35 45 N, 105°58 54 E | Woods, cluster | 460 | 6.4 | 2,400 |
| *Lactiflora* | Inner mongolia· Yakeshi· Wuerqihan | 730, 49°34 15 N, 121°23 16 E | Forest, sunny, cluster | 440 | -1.8 | 1,680 |
| *veitchii·brown root* | Shaanxi· Meixian· Tangyu | 2800, 34°08 15 N, 107°53 42 E | Forest, sunny, cluster | 700 | 11.9 | 3,768 |
| *veitchii·red root* | Shaanxi· Meixian· Tangyu | 2800, 34°08 15 N, 107°53 42 E | Forest, sunny, cluster | 700 | 11.9 | 3,768 |
| *mairei* | Sichuan· Beichuan· Leigu | 1600, 31°50 20 N, 104°21 40 E | Forest, sunny, cluster | 1,110 | 16.1 | 5,184 |
| *veitchii·brown root* | Gansu· Zhangxian· Sancha | 2100, 34°50 50 N, 104°19 58 E | Woods, shade, scattered | 560 | 8.4 | 2664 |
| *willmottiae* | Jilin·Yanji· baihe | 1170, 42°28 50 N, 128°15 38 E | Woods, shade, cluster | 1070 | -1.3 | 1368 |

*Note: Lactiflora.* Means *P. lactiflora. Intermedia means P. anomala var. intermedia. Anomala.* means *P. anomala* subsp. *anomala. Veitchii.* Means *P. anomala* subsp. *Veitchii* (Lynchh) D.Y Hong & K.Y. *Mairei.* means *P. mairei. Willmottiae.* means *P. obovata var.willmottiae* (Stapf) Stern. Rain. means rainfall. Tem. means temperature. Acc means accumulative.

**Table II**
*The result of verify the method and recovery of standard*

| *Item or repeats* | *Gallic acid* | *Catechin* | *Albiflorin* | *Paeoniflorin* | *Benzoic acid* | *Paeonol* |
|---|---|---|---|---|---|---|
| Precision (RSD) | 0.00826 | 0.02843 | 0.01558 | 0.00853 | 0.0612 | 0.0096 |
| Repeatability (RSD) | 0.12005 | 0.033859 | 0.045233 | 0.038667 | 0.039534 | 0.04136 |
| Stability (RSD) | 0.02503 | 0.035290 | 0.023643 | 0.030682 | 0.03235 | 0.02187 |
| Recovered(mg) | 0.06013 | 0.04396 | 0.16305 | 0.86443 | 0.02484 | 0.00504 |
| 1 Added(mg) | 0.061 | 0.045 | 0.17 | 1 | 0.0285 | 0.06 |
| Recovery(mg) | 0.985737705 | 0.976888889 | 0.959117647 | 0.86443 | 0.871578947 | 0.084 |
| Recovered(mg) | 0.07967 | 0.019568 | 0.04579 | 0.82509 | 0.46058 | 0.00453 |
| 2 Added(mg) | 0.08 | 0. 02 | 0.046 | 0.80 | 0.5 | 0.05 |
| Recovery(mg) | 0.995875 | 0.9784 | 0.995434783 | 1.0313625 | 0.92116 | 0.0906 |
| Recovered(mg) | 0.24084 | 0.316083 | 0.04969 | 0.32311 | 0.18756 | 0.06452 |
| 3 Added(mg) | 0.28 | 0.3 | 0.052 | 0.32 | 0.19 | 0.08 |
| Recovery(mg) | 0.860142857 | 1.05361 | 0.955576923 | 1.00971875 | 0.987157895 | 0.8065 |
| RSD | 0.079819 | 0.043736 | 0.022742 | 0.09373 | 0.062574 | 0.059503 |

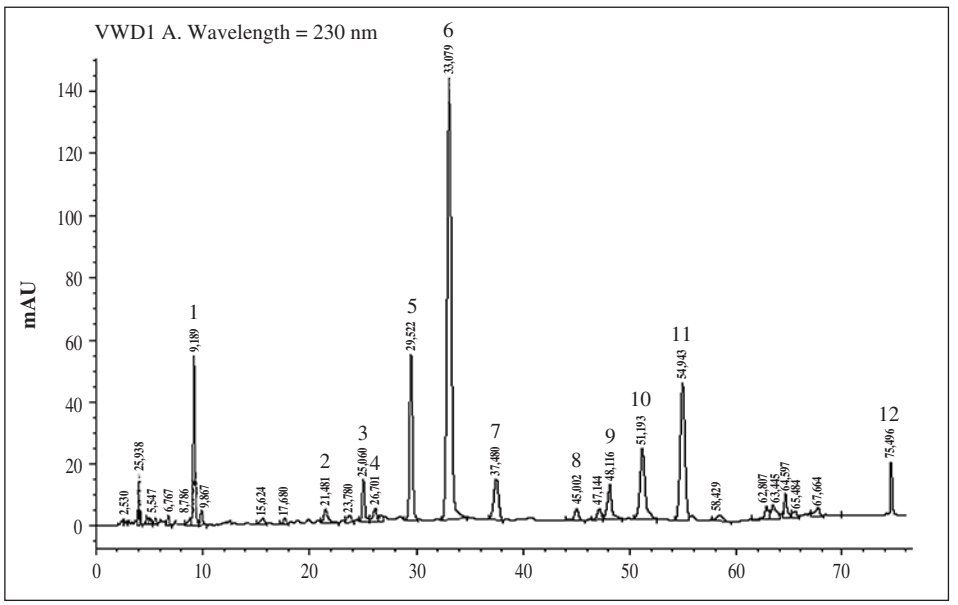


*Fig. 1.—Representative spectum of Chishao. No. 1, 3, 5-6, 9 and 12 peak is respectively gallic acid, catechin, albiflorin, paeoniflorin, benzoic acid and paeonol.*

## Results and analysis

*HPLC fingerprint of Chishao in all of locations*

The ingredients from Chishao extract and a control *(P. lactiflora)* were determined with the extraction method and the HPLC method. There are 12 common Chromatography peaks in the chromatograms of the Chishao extracts. The representative chromatogram is showed in figure 1 *(P. lactiflora* in Kerqinyouyiqianqi of Inner Mongolia). Six components in the chromatograms were identified based on their extract same retention time as that of reference standards. These components recognized in the chromatogram were gallic acid (No. 1 peak); catechin (No. 3 peak); albiflorin (No. 5 peak); paeoniflorin (No. 6 peak); benzoic acid (No. 9 peak) and paeonol (No. 12 peak).

*Comprehensive evaluation of the quality of Chishao in China*

The principal component analysis (PCA) was performed using SPSS. Four principal components are obtained of which the Eigen values are all above 1 and the cumulative load values of these principal components account to 81.095% (table III).

The component matrix (table IV) of 12 peak areas on 4 principal components shows that $F_1$ reflects the information of No. 1-4 peaks, No. 7-8 peaks and No. 11

**Table III**
*Total variance explained in principal component analysis*

| Components | Initial Eigenvalues | | | Estraction sums of squared loadings | | |
|---|---|---|---|---|---|---|
| | Total | % of variance | cumulative % | Total | % of variance | cumulative % |
| 1 | 4.408 | 36.734 | 36.734 | 4.408 | 36.734 | 36.734 |
| 2 | 2.735 | 22.795 | 59.529 | 2.735 | 22.795 | 59.529 |
| 3 | 1.534 | 12.783 | 72.313 | 1.534 | 12.783 | 72.313 |
| 4 | 1.054 | 8.782 | 81.095 | 1.054 | 8.782 | 81.095 |
| 5 | 0.669 | 5.573 | 86.668 | | | |
| 6 | 0.575 | 4.790 | 91.458 | | | |
| 7 | 0.315 | 2.624 | 94.082 | | | |
| 8 | 0.288 | 2.396 | 96.478 | | | |
| 9 | 0.186 | 1.548 | 98.026 | | | |
| 10 | 0.135 | 1.121 | 99.147 | | | |
| 11 | 0.07826 | 0.652 | 99.799 | | | |
| 12 | 0.02408 | 0.201 | 100.000 | | | |

Extraction Method: Principal Component Analysis.

**Table IV**
*Component matrix on 4 principal components*

| Peak area | Component* | | | |
|---|---|---|---|---|
| | $F_1$ | $F_2$ | $F_3$ | $F_4$ |
| No. 1 gallic acid | -0.630 | 0.615 | 0.287 | 0.04318 |
| No. 2 | 0.846 | 0.405 | 0.04342 | -0.01004 |
| No. 3 catechin) | 0.809 | 0.360 | -0.173 | 0.114 |
| No. 4 | 0.691 | 0.483 | -0.06723 | 0.04341 |
| No. 5 (albiflorin) | -0.194 | -0.417 | 0.332 | 0.626 |
| No. 6 (paeoniflorin) | 0.452 | 0.693 | 0.08164 | -0.04297 |
| No.7 | -0.663 | 0.696 | 0.169 | 0.09214 |
| No.8 | 0.838 | 0.04801 | -0.05310 | -0.05685 |
| No.9 (benzoic acid) | 0.456 | -0.09903 | 0.784 | -0.05455 |
| No.10 | 0.208 | -0.300 | 0.721 | -0.488 |
| No.11 | -0.565 | 0.768 | 0.221 | 0.06837 |
| No.12 (paeonol) | 0.457 | -0.02604 | 0.289 | 0.625 |

Extraction method: Principal Component Analysis.
*Note:* 4 components extracted.

peak; $F_2$ reflects the information of No. 1 peak, No. 6-7 peaks and No. 11 peak; $F_3$ reflects the information of No. 9-10 peaks; $F_4$ reflects the information of No. 5 peak and No. 12 peak.

The models of 4 principal components and that of comprehensive principal component (F) are established according to the component matrix and the eigenvalues of 4 principal components. The expression coefficients of these models are showed as tableV.

According to the model of comprehensive principal component and the values of standardized primeval data (peak areas), the values of comprehensive principal component of Chishao in all locations are computed and placed in order (table VI).

It can be seen from the model of comprehensive principal component that the factors affecting the value of comprehensive principal component are mainly No. 2 peak, No. 3 peak (catechin), No. 4 peak and No. 6 peak (paeoniflorin). According to the value of comprehensive principal component, we can think primarily that the quality of Chishao in the northern part of China or that made of *lactiflora* is better than that of these in others or that made of other species. The quality of Chishao comes from *veitchii* is in the middle class and is better than those from *obovata*, *mairei* and *anomala*.

## Discussion

The quality of Chishao is traditionally evaluated by visual characteristic such as appearance, colour, smell and experience or knowledge. There is obvious subjectivity in this method of which the standard is not certain. In recent years, some persons evaluated the quality of Chinese medicine by the content of effective component in it. The content of paeoniflorin in Chishao is not lower than 1.8% regulated in Chinese Pharmacopoeia (2005 edition). However, Chinese traditional medicine takes action by the cooperation of many components in the compound. There is no consistency between some components. Therefore, evaluating the quality of medicinal material by some components is not reasonable, and evaluating the quality of medicinal material should exert the comprehensive index of most components. But, there are too many components in Chishao to determining the contents of all components. Moreover, the effective components are not certain completely and there are some possible components unfound. HPLC fingerprint comparison has been widely used for evaluation and characterization of Chinese traditional medicine.[5-9]

**Table V**
*The expression coefficients of principal components models*

| *Principal components* | $ZX_1$ | $ZX_2$ | $ZX_3$ | $ZX_4$ | $ZX_5$ | $ZX_6$ | $ZX_7$ | $ZX_8$ | $ZX_9$ | $ZX_{10}$ | $ZX_{11}$ | $ZX_{12}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $F_1$ | -0.3001 | 0.4029 | 0.3853 | 0.3291 | -0.0924 | 0.2153 | -0.3158 | 0.3991 | 0.2172 | 0.0991 | -0.26911 | 0.2177 |
| $F_2$ | 0.37191 | 0.2449 | 0.2177 | 0.2921 | -0.2521 | 0.4190 | 0.4209 | 0.0290 | -0.0599 | -0.1814 | 0.4644 | -0.0158 |
| $F_3$ | 0.2317 | 0.0351 | -0.1397 | -0.0543 | 0.2681 | 0.0659 | 0.1365 | -0.0429 | 0.6330 | 0.5821 | 0.1784 | 0.2333 |
| $F_4$ | 0.0421 | -0.0010 | 0.1112 | 0.0423 | 0.6106 | -0.0419 | 0.0899 | -0.0555 | -0.0532 | -0.4760 | 0.0067 | 0.6096 |
| F | 0.0097 | 0.2568 | 0.2258 | 0.22723 | -0.0043 | 0.2211 | 0.00654 | 0.1762 | 0.1756 | 0.0341 | 0.0375 | 0.1970 |

*Note:* F: Comprehensive principal component; ZX: The values of standardized primeval data (peak areas).

**Table VI**
*The values of comprehensive principal component of Chishao in all locations*

| *Species* | *Laceyion: province: county town* | *Comprehensive value* | *Order* |
|---|---|---|---|
| *Lactiflora* | Hebei· Weichang· Yudaokou | 2.023701 | 1 |
| *Lactiflora* | Inner mongolia· Tuquan· Baoshi | 1.833486 | 2 |
| *Lactiflora* | Hebei· Qinglong· Anziling | 1.547112 | 3 |
| *Lactiflora* | Liaoning· Fuxin· Daban | 1.469294 | 4 |
| *Lactiflora* | Henan· Luanchuan· Chengguan | 1.256569 | 5 |
| *Lactiflora* | Heilongjiang· Muling· Maqiaohe | 1.150449 | 6 |
| *Lactiflora* | Inner mongolia· Duolun· Caimushan | 1.118063 | 7 |
| *Lactiflora* | Jilin· Lishu· shijiapu | 0.914743 | 8 |
| *Lactiflora* | Inner mongolia· Wulagai· Hesigewula | 0.869661 | 9 |
| *Lactiflora* | Heilongjiang· Heihe· Xinsheng | 0.853094 | 10 |
| *Lactiflora* | Inner mongolia· Yakeshi· Wuerqihan | 0.635202 | 11 |
| *Lactiflora* | Shanxi·Jiangxian· Nanfan | 0.495933 | 12 |
| *Lactiflora* | Shaanxi· Huanglong· Shipu | 0.431822 | 13 |
| *Lactiflora* | Ningxia· Longde· Shatang | 0.07585 | 14 |
| *Lactiflora* | Hebei· Weichang· longtoushan | 0.063641 | 15 |
| *Lactiflora* | Inner Mongolia· Kerqinyouyizhongqi· Harinula | -0.12265 | 16 |
| *Lactiflora* | Inner mongolia· Hailaer | -0.25253 | 17 |
| *Veitchii (brown root)* | Shaanxi· Meixian· Tangyu (brown root) | -0.30934 | 18 |
| *Lactiflora* | Inner mongolia· Ergun· Enhe | -0.34652 | 19 |
| *Veitchii (brown root)* | Gansu· Zhangxian· Sancha | -0.37925 | 20 |
| *Lactiflora* | Heilongjiang· mudanjiang· xinglong | -0.63519 | 21 |
| *Lactiflora* | Gansu· Zhangxian· Sancha | -0.71584 | 22 |
| *Veitchii (red root)* | Shaanxi· Meixian· Tangyu (red root) | -0.75256 | 23 |
| *Lactiflora* | Liaoning· Shenyang· Puhe | -0.76488 | 24 |
| *Lactiflora* | Inner mongolia· Kerqinyouyiqianqi· Huhemachang | -0.81664 | 25 |
| *Mairei* | Sichuan· Beichuan· Leigu | -0.82892 | 26 |
| *Intermedia* | Xinjiang· Emin· 168 regiment | -0.88992 | 27 |
| *Lactiflora* | Inner Mongolia· Erwenkezuqi· Honghuaerji | -0.94846 | 28 |
| Lactiflora | Hebei· Weichang· Chaoyangwan | -0.99096 | 29 |
| *Lactiflora* | Inner mongolia· Wulagai·Wulahada | -1.02225 | 30 |
| *Anomala* | Xinjiang· Emin·168 regiment | -1.26865 | 31 |
| *Lactiflora* | Liaoning· Xingcheng· Jianchang | -1.29984 | 32 |
| *Willmottiae* | Jilin·Yanji· baihe | -1.43418 | 33 |
| *Lactiflora* | Shanxi· Fenyang | -1.84376 | 34 |

Major component analysis is often used in evaluating the quality of river water, food and so on.[10,11] Hong-Liang Ma thought that simultaneous quantification of multiple components by high-performance liquid chromatography combined with principal component analysis would be a better strategy for the quality evaluation of danshen.[12] We find out that evaluating the quality of Chishao by principal component analysis of HPLC, the peak areas is efficient and practical after comparing and analysis. This method integrates most of the information of components in Chishao. Furthermore, the evaluating index of this method is simple and certain. The result of Chishao quality evaluating with this method is consistent with the practice of Chishao quality recognized in all locations. Therefore, the principal component analysis with HPLC peak areas is an efficient and practical method for evaluating the quality of Chishao.

## Conclusion

The quality of chishao in the north of China or that made of *lactiflora* is better than that of these in others or that made of other species. The quality of chishao come from *veitchii* is middle class and is better than that of these come from *obovata*, *mairei* and *anomala*. PCA can be used as an effective and economic method to evaluate the quality of Chishao, and may be extended to other Chinese medicinal plants.

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