

Species classification and bioactive ingredients accumulation of *BaiJiangCao* based on characteristic inorganic elements analysis by inductively coupled plasma-mass spectrometry and multivariate analysis

Li Wen-Lan, Zhang Xue¹, Yang Xin-Xin¹, Wang Shuai¹, Zhao Lin¹, Zhao Huan-Jun¹, Bao Yong-Rui¹, Ji Chen-Feng, Chen Ning, Xiang Zheng

Center of Research on Life Science and Environmental Science, Harbin University of Commerce, Harbin 150076, ¹College of Pharmacy, Liaoning University of Traditional Chinese Medicine, Dalian 116600, P.R. China

Submitted: 15-11-2014

Revised: 04-01-2015

Published: 16-09-2015

ABSTRACT

Background: *Patrinia scabiosaefolia* Fisch and *Patrinia villosa* (Thunb.) Juss., two species herbs with the same Chinese name “*BaiJiangCao*”, are important ancient herbal medicines widely used for more than 2000 years. The clinical application of two species herb is confused due to the difficult identification. **Objective:** The objective was to authenticate the species of *BaiJiangCao* and analyze the accumulation of bioactive ingredients based on characteristic inorganic elements analysis. **Materials and Methods:** Content of 32 inorganic elements in *BaiJiangCao* from different habitats were determined by inductively coupled plasma-mass spectrometry (ICP-MS), and the characteristic inorganic elements were picked to distinguish the species of the herb by principal component analysis and cluster analysis. Contents of two bioactive ingredients, luteoloside, and oleanolic acid, in the samples, were also analyzed by high-performance liquid chromatography method. Relationship between accumulation of bioactive ingredients and content of macroelements in *BaiJiangCao* was established by statistics. **Results:** A 4 macroelements (Na, Mg, K, Fe) in 32 determined inorganic elements were picked for characteristic inorganic elements. Content of Na, Mg, K and Fe showed positive correlations with that of luteoloside, content of Na, Mg showed positive correlations with that of oleanolic acid, but content of K and Fe showed negative correlations with that of oleanolic acid. **Conclusion:** It is for the first time to utilize the characteristic inorganic elements as an index to classify the herb species by the method of ICP-MS and multivariate analysis. And it is also the first report to investigate the influence of inorganic elements in herb on the accumulation of bioactive components which could affect the pharmacological efficacy of the herb medicine. And this method could also be utilized in research of corresponding aspects.

Key words: Cluster analysis, inductively coupled plasma-mass spectrometry, *Patrinia scabiosaefolia* Fisch, *Patrinia villosa* (Thunb.) Juss, principal component analysis

Access this article online

Website:

www.phcog.com

DOI:

10.4103/0973-1296.165577

Quick Response Code:



INTRODUCTION

Patrinia, which belongs to family valerianaceae, is a widely distributed plant grown in East Asia and North America. The genus included more than 20 species, 10 of which growing in

China.^[1] Usually, *Patrinia* species are used as leaves vegetables in some areas of China, and research also revealed its leaves with pharmacological properties, especially the species of *Patrinia scabiosaefolia* Fisch and *Patrinia villosa* (Thunb.) Juss.

The *P. scabiosaefolia* Fisch and *P. villosa* (Thunb.) Juss., two species herbs that have been recorded in Chinese Pharmacopoeia (Third edition) with the same name “*BaiJiangCao*” in Chinese, are important ancient herbal medicines widely used for more than 2000 years from *ShenNongBenCaoJing*, a famous ancient Chinese medicinal literary. *BaiJiangCao* is heat-clearing, antipyretic, detoxicant, anti-inflammatory, and is used traditionally

Address for correspondence:

Dr. Bao Yong-Rui, College of Pharmacy, Liaoning University of Traditional Chinese Medicine, Dalian 116600, P.R. China.
E-mail: byr1026@163.com

Dr. Xiang Zheng, Center of Research on Life Science and Environmental Science, Harbin University of Commerce, Harbin 150076, P.R. China.
E-mail: rainbowaftersnow@hotmail.com

in the treatment of wound healing, abdominal pain and intestinal carbuncle, including acute appendicitis, abscess of the liver, dysentery in enteritis, carbuncle and deep-rooted ulcer.^[2,3]

Previous research on the chemical constituents of *BaiJiangCao* has revealed that it contains several compound classes. Triterpenoid saponins, iridoids, flavonoids, flavonones, and polysaccharides are the dominant bioactive constituents in the leaves of *P. villosa* Juss and *P. scabiosaefolia* Fisch, which displayed potential ability of anti-tumor and anti-inflammatory.^[4-7] Other components, such as sterols and fatty acids were also confirmed.^[8,9]

Inorganic elements play a key role in Chinese medicine. The medicinal efficacy of herb medicine is largely affected by the soil and the climate of the habitats, so, organic ingredients and inorganic elements in the herbs of different areas showed variances in content.^[10] Most bioactive ingredients in herbs are organic ingredients, and the efficacy of herb medicine is evaluated by the “fingerprint” based on the bioactive compounds in it. Inorganic elements in the herb could also affect the medicinal efficacy.^[11] Previous reports indicated that the inorganic elements could be coordinated with organic ingredients in the herb to construct the coordination compounds, which were also considered to be the bioactive compounds of the herb,^[10] the contents and structure characteristics of organic ingredients might be important factors influencing the distribution of inorganic elements.^[12] However, there is no research to reveal the relationship between inorganic elements and organic ingredients. As such, this study is designed to analyze the inorganic elements in two species of *BaiJiangCao* of different habitats by inductively coupled plasma-mass spectrometry (ICP-MS) firstly, principal component analysis (PCA) and cluster analysis were used to identify the characteristic inorganic elements in two species of *BaiJiangCao* and its adulterants, then correlation analysis were achieved to explain the relationship between content of macroelements and content of luteoloside and oleanolic acid, two main bioactive components in *BaiJiangCao*.

MATERIALS AND METHODS

Plant materials

The *P. scabiosaefolia* Fisch and *P. villosa* (Thunb.) Juss were collected from different provinces in China [Table 1]. All the materials were identified by Dr. Jian Wu, Harbin University of Commerce. And voucher specimens (NO. PVJ2013-1 to PVJ2013-9, PSF2013-1 to PSF2013-4, PSB-1, and SAL-1) were deposited at the Pharmacognosy Laboratory, Harbin University of Commerce.

Table 1: Samples collected from different habitats

Number	Species	Origin
1	<i>P. villosa</i> (Thunb.) Juss	Hebei
2	<i>P. villosa</i> (Thunb.) Juss	Liaoning
3	<i>P. villosa</i> (Thunb.) Juss	Hebei
4	<i>P. villosa</i> (Thunb.) Juss	Heilongjiang
5	<i>P. scabiosaefolia</i> Fisch	Jilin
6	<i>P. scabiosaefolia</i> Fisch	Jilin
7	<i>P. scabiosaefolia</i> Fisch	Liaoning
8	<i>P. scabiosaefolia</i> Fisch	Heilongjiang
9	<i>P. scabiosaefolia</i> Fisch	Heilongjiang
10	<i>P. scabiosaefolia</i> Fisch	Heilongjiang
11	<i>P. villosa</i> (Thunb.) Juss	Hebei
12	<i>P. scabiosaefolia</i> Fisch	Liaoning
13	<i>P. scabiosaefolia</i> Fisch	Hubei
14	<i>P. scabiosaefolia</i> Fisch	Hubei
15	<i>P. scabiosaefolia</i> Fisch	Heilongjiang
16*	<i>P. scabra</i> Bunge	Hebei
17	<i>P. scabiosaefolia</i> Fisch	Hebei
18	<i>P. villosa</i> (Thunb.) Juss	Hebei
19	<i>P. scabiosaefolia</i> Fisch	Heilongjiang
20*	<i>Sonchus arvensis</i> L	Liaoning
21	<i>P. villosa</i> (Thunb.) Juss	Hebei
22	<i>P. scabiosaefolia</i> Fisch	Heilongjiang
23	<i>P. villosa</i> (Thunb.) Juss	Hebei
24	<i>P. scabiosaefolia</i> Fisch	Heilongjiang
25	<i>P. villosa</i> (Thunb.) Juss	Hebei

*Sample 16 (*P. scabra* Bunge) and 20 (*Sonchus arvensis* L) were the adulterants of *BaiJiangCao* which used as folk medicine in some places of China. *P. villosa*: *Patrinia villosa*; *P. scabiosaefolia*: *Patrinia scabiosaefolia*; *P. scabra*: *Patrinia scabra*

General

An Agilent 7500a ICP-MS (Agilent Technologies Co., Ltd, USA) was used for the determination of microelement with a quantitative analysis. Agilent 7500 ICP-MS ChemStation software was used for data acquisition. A MDS-6 digester/extractor including a microwaver and polytetrafluoroethylene (PTFE) vessels was from Shanghai Xinyi Microwave Chemical scientific and technology Co., Ltd., (Shanghai, China).

The instrument was optimized daily in terms of sensitivity (Li, Y, and Tl), level of oxide (CeO/Ce) and doubly charged ion (Ce⁺²/Ce) using a tuning solution containing 10⁻⁹ g/mL of Li, Y, Tl, Ce and Co in 2% HNO₃ to meet the demands of the trace element determination. The operating conditions of ICP-MS instrument are summarized in Table 2.

Chemicals

Ultrapure water was prepared with a Milli-Q deionization unit (Millipore, Bedford, MA, USA). Luteoloside and oleanolic acid were purchased from Weikeqi Biological Technology CO., LTD (Sichuan, China). Nitric acid used for sample digestion was of high-purity grade and purchased from Kermel Chemical Reagent Co., Ltd. (Tianjin, China).

Table 2: Operating conditions for ICP-MS

ICP system	
Carrier gas	1.14 L/min
RF power	1250W
Atomizer chamber	2°C
Sample uptake rate	0.1 rps
Points per spectral peak	6
Number of replicate	3
Sampling depth	7.8 mm
Mass spectrometer	
Sampling cone	Nickel, -96.2 V
Skimmer cone	Nickel, -22 V
Vacuum	5×10 ⁻⁷ Mba
Mass range	2–260 amu
Total acquisition	181 s

ICP-MS: Inductively coupled plasma-mass spectrometry; RF: Radio frequency

Wash-nitric acid blank: Part #G1820-60258 (5% HNO₃), wash-water blank: Part# G1820-60259, tuning solution of MS optimization: 10⁻⁸ g/mL of Li, Y, Ce, Tl, and Co (2% HNO₃) (Part# G5184-3566) was used to perform external calibration, mixed standard solution including 10⁻³ g/mL of Fe, K, Ca, Na, Mg and 10⁻⁵ g/mL of Ag, Al, As, Ba, Be, Cd, Co, Cr, Cu, Mn, Mo, Ni, Pb, Sb, Se, Tl, V, Zn, Th, U. (Part # G5183-4688) and the internal standard solutions including six elements of 10⁻⁵ g/mL of Li, Sc, Ge, Y, In, and Bi (5% HNO₃) (Part# G5183-4680) used to reduce matrix effect and compensate for instrument drift during the analysis were purchased from Agilent (NJ, USA) and diluted to approximately 8 ng/g by 5% HNO₃ before the experiment. All the chemical reagents were from Kermel Chemical Co. (Tianjin, China).

Sample preparation for inductively coupled plasma-mass spectrometry and analysis procedure

The herbal samples were washed thoroughly with tap water, followed by distilled water, dried at 105°C, grounded using a mortar and stored in the bags.

To the 50 mL closed PTFE vessel, about 200 mg (accurately weighed) of leaves, together with 3 mL of concentration HNO₃, was added and then digested by the MDS-6 microwave digestion system. The optimized digestion conditions were a four-step procedure for 6 vessels including step 1–3 (Pressures were 0.3, 0.6 and 1.0 MPa for 4 min, respectively) and step 4 (Pressure was set at a maximum pressure of 1.5 MPa for 10 min), the microwave power was 600 W for every step. After cooling, the decomposed sample solutions were heated almost to dryness to remove any excess HNO₃. Finally, the residue dissolved in deionized water, and made up to 10 mL in a volumetric flask with deionized water, the solution was stored at 4°C and subjected to analysis within 48 h. Blank experiments were carried out, in the same way.

Content of inorganic elements in *BaiJiangCao* from different places were determined using ICP-MS as the method of reported with minor modification.^[13] Before the analysis, wash-nitric acid blank and wash-water blank were used to rinse the system and flow path, respectively. Then, tuning solution (10⁻⁸ g/mL of Li, Y, Ce, Tl, and Co) was utilized to modulate the conditions of the system. Samples were directly introduced by a peristaltic pump at the flow rate of 0.3 mL/min following the sample diluted with 5% HNO₃. The internal standard (8 ng/g of Li, Sc, Ge, Y, In, and Bi) was added on-line as the reference solution to alleviate matrix effect and compensate for signal drift in each individual run on the ICP-MS.

Luteoloside and oleanolic analysis by high-performance liquid chromatography

Preparation of standard solutions for high-performance liquid chromatography analysis

Luteoloside and oleanolic acid were dissolved in methanol to produce stock solutions containing 40.0 and 50.0 µg/mL, respectively.

Preparation of samples for luteoloside analysis

Dry, powdered herbal leaves (approximate 2 g, accurately weighed) were extracted by refluxing with 80% aqueous methanol (50 mL) for 1 h, twice. After filtration, the remaining solvent was evaporated to dryness using a rotary evaporator; the residue dissolved in methanol, and made up to 10 mL in a volumetric flask with methanol. The sample solution was filtered through 0.45 µm organic membranes prior to use.^[14]

Preparation of samples for oleanolic acid analysis

Dry, powdered herbal leaves (approximate 2 g, accurately weighed) were extracted by refluxing with EtOH (25 mL) for 2 h, twice. After filtration, the remaining solvent was evaporated to dryness using a rotary evaporator; the residue dissolved in methanol, and made up to 10 mL in a volumetric flask with methanol. The sample solution was filtered through 0.45 µm organic membranes prior to use.^[15]

High-performance liquid chromatography analysis

Content analysis was achieved using analytical high-performance liquid chromatography (HPLC) with an Agilent 1100 series HPLC system with pump (Agilent model G1314A VWD), and an Agilent reverse-phase TC-C₁₈ column (4.6 × 250 mm, 5 µm particle size) protected by a pre-column from the same company, eluted with water-phosphoric acid (100:0.5) (A) and acetonitrile (B) as mobile phase at the flow rate of 1 mL/min. The isocratic elution for determining luteoloside and oleanolic acid consisted of 17% B and 73% B, respectively. And

ultraviolet absorption of the luteoloside and oleanolic acid were determined at 350 nm and 210 nm, respectively. The injection volume was 20 μL for each fraction. Peak identification was performed by comparison of retention times and thermostated at 30°C.

Development and validation of high-performance liquid chromatography method

Calibration

Curves were constructed using standard solutions of luteoloside in the concentration range 2–40 $\mu\text{g/mL}$,

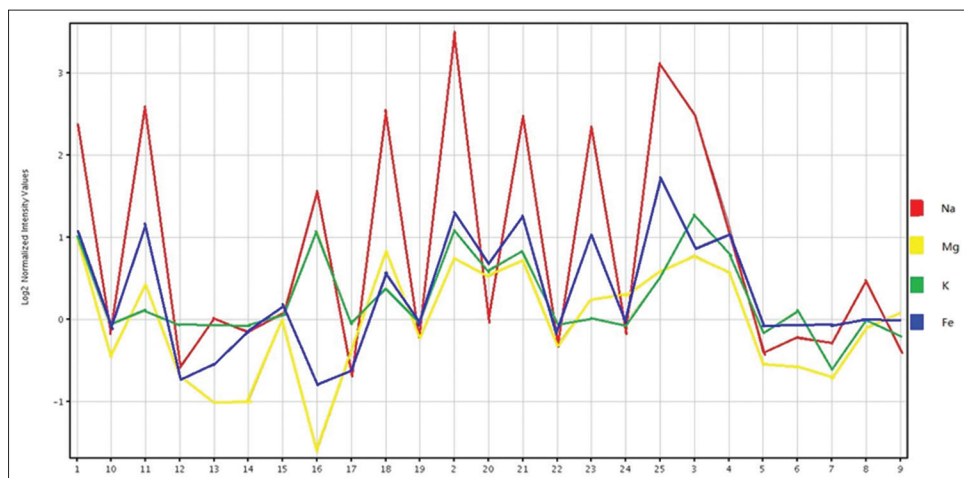


Figure 1: Content of characteristic inorganic elements in *BaiJiangCao* from different habitats

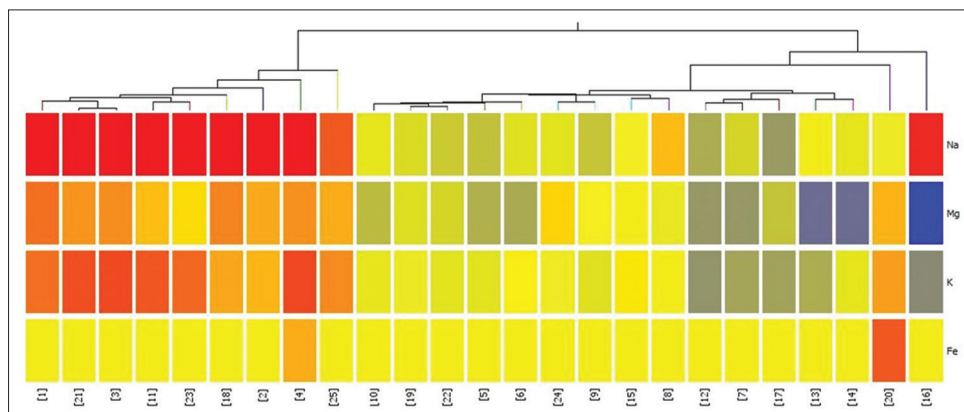


Figure 2: Clustering analysis of characteristic inorganic elements in *BaiJiangCao* from different habitats

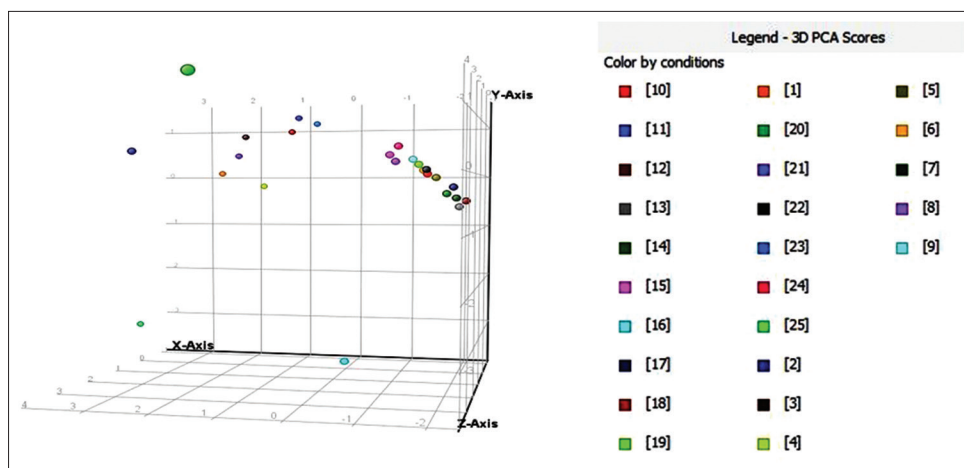


Figure 3: Principal component analysis of characteristic inorganic elements in *BaiJiangCao* from different habitats

oleanolic acid 0.3–6.0 mg/mL. Each sample (20 μ L) was determined in triplicate, and the average detector responses were used by the software to construct the curves.

Linearity

Aliquots (20 μ L) of six solutions of luteoloside and oleanolic acid were analyzed in triplicate, and the average

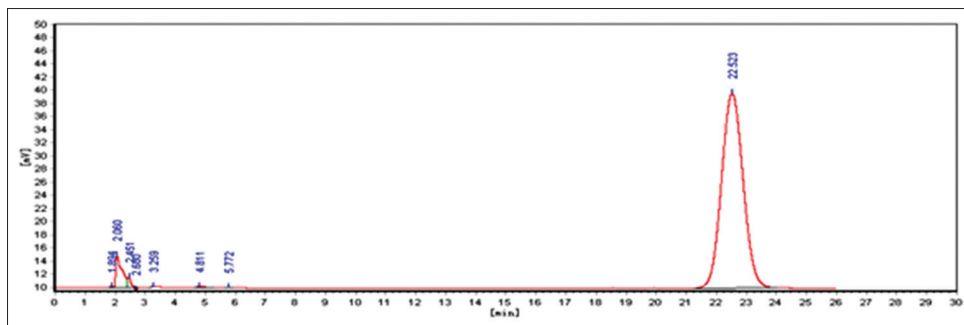


Figure 4: High-performance liquid chromatography of Luteoloside

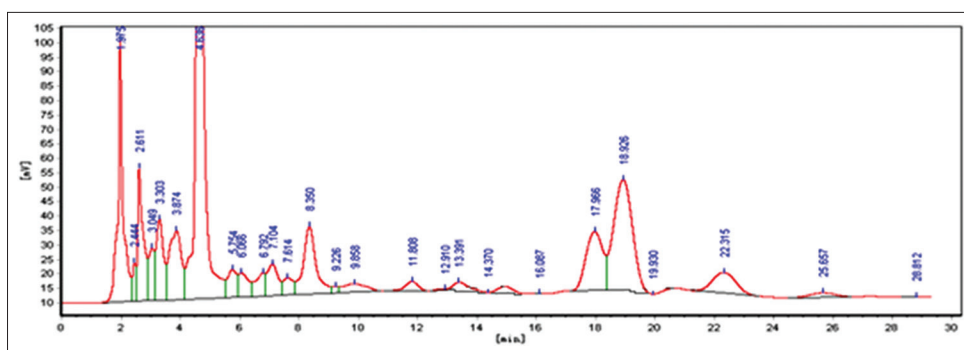


Figure 5: High performance liquid chromatography of luteoloside in sample 6

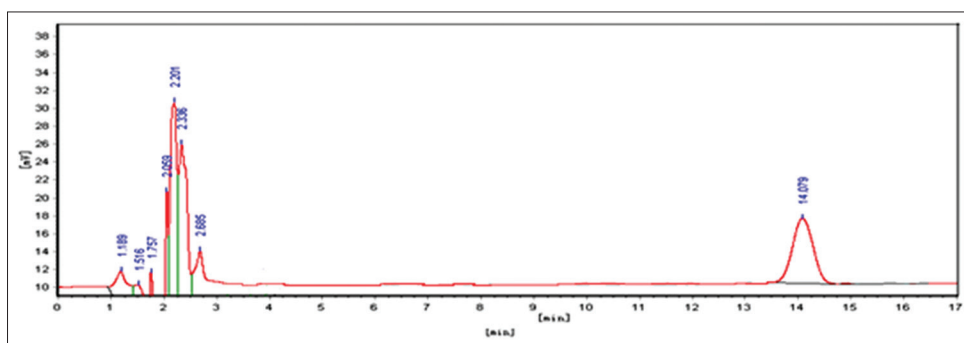


Figure 6: High-performance liquid chromatography of oleanolic acid

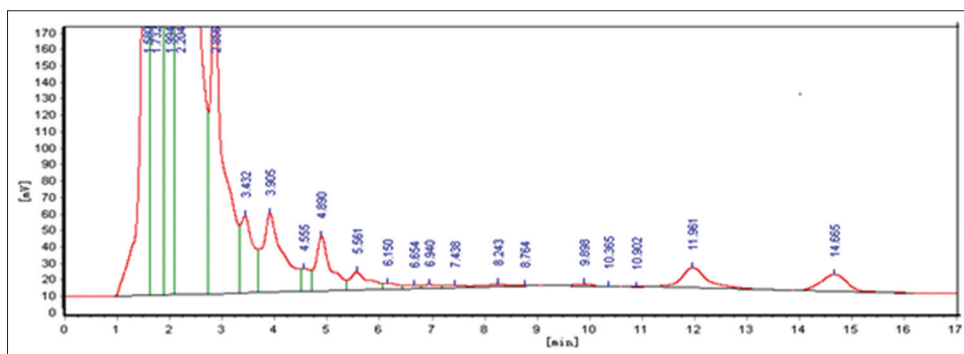


Figure 7: High-performance liquid chromatography of oleanolic acid in sample 15

detector responses were used by the software to construct the curve.

Precision

Standard solutions of luteoloside (40 µg/mL) and oleanolic acid (6.0 mg/mL) were injected in sextuplicate, respectively, in order to determine the standard deviation of the method on the same day.

Accuracy

The accuracy and recovery of the method were determined by application of the standard addition method. Standards (80, 100, 120% of the content in 1.0 g herbal leaves, respectively) of luteoloside and oleanolic acid were added to 1.0 g of herbal leaves and then extracted and analyzed in triplicate using the method described above. The total amount of each compound was calculated from the corresponding calibration curve, and the recovery of each compound was determined.

RESULTS AND DISCUSSION

Content of inorganic elements in *BaiJiangCao*

Before quantitative analysis, semiquantitative analysis of inorganic elements in samples was accomplished by ICP-MS. The semiquantitative method enables us to automatically determine the concentrations of up to 32 inorganic elements. And the result of statistical analysis (One-way ANOVA) indicated that four macroelements (Na, Mg, K, Fe) were picked for characteristic inorganic elements to evaluate the variances of inorganic elements in *P. scabiosaefolia* Fisch and *P. villosa* (Thunb.) Juss collected from different places. Contents of the four characteristic elements were quantitated subsequently. The calibration curves (correlation coefficient) and limits of detection (LOD) were obtained [Table 3]. The variation coefficients of elements were <10%, which showed good precision. And the accuracy of the method was examined by performing recovery experiments. The recoveries of elements were in the range of 91.04 ~ 107.38%, which presented good accuracy for the analysis. And the four characteristic inorganic elements content in the sample were analyzed [Figure 1].

Statistical analysis

Because of the feature identification more difficult and the non-standard folk medication name, several species of herb, such as *P. scabra* Bunge and *Sonchus arvensis* L., confused with *BaiJiangCao* frequently. Hence, in this experiment, *P. scabra* Bunge and *S. arvensis* L were also collected to make a comparison of the characteristic inorganic elements with *P. scabiosaefolia* Fisch and *P. villosa* (Thunb.) Juss. Clustering analysis (CA) and PCA between species and characteristic elements content were confirmed by the software of GeneSpring 12.1 subsequently [Figures 2 and 3].

All the herb samples could be divided into two classes clearly as the CA result [Figure 2]. Samples 1, 2, 3, 4, 11, 18, 21, 23, and 25 classified as one group, which were identified as *P. villosa* (Thunb.) Juss. The contents of Na, Mg, K and Fe in *P. villosa* (Thunb.) Juss showed a little difference, especially that of Na. The remaining samples were classified as another group, which containing *P. scabiosaefolia* Fisch, *P. scabra* Bunge and *S. arvensis* L. Although there are three species of herb sample in this group, it is obviously to distinguish samples 20 (*S. arvensis* L) and 16 (*P. scabra* Bunge) from other samples according to the content of macroelements which can be reflected by the color depth.

Principal component analysis result could exhibit this tendency in a three-dimensional model [Figure 3]. Plants with the same species could be accumulated as one group, which is far from the other groups in space in the chart. And samples in the same group were not overlapped in space because of the variances of production area and climate.

The above statistics result indicated that the characteristic inorganic elements in samples could be used to distinguish the herb of different species, such as *P. villosa* (Thunb.) Juss and *P. scabiosaefolia* Fisch.

Content of luteoloside and oleanolic acid in *BaiJiangCao* Validation of high-performance liquid chromatography method

The HPLC method was validated as follows. The calibration

Table 3: Regression equations and LOD of determined elements

Trace elements	Regression equations	<i>r</i>	LOD (ng/mL)	Linear range (µg/L)
Na	$y=0.5394x+97.14$	0.9997	1.0826	0-500
Mg	$y=0.3404x+7.555$	0.9999	0.0341	0-500
K	$y=0.5938x+82.38$	0.9997	0.5048	0-500
Fe	$y=0.7672x+70.90$	0.9999	0.1118	0-100

x: Concentration of elements (ppb); y: The ratio of response of tested elements to internal standard elements. LOD: Limit of detection

curves for luteoloside $y = 2968353.2x - 1302.6$ ($r = 0.9999$, $n = 6$, $0.04\text{--}0.8\text{ }\mu\text{g}$) and oleanolic acid $y = 136618.4\text{--}1481.7$ ($r = 0.9998$, $n = 6$, $0.6\text{--}12.0\text{ }\mu\text{g}$), respectively. The variation coefficients of luteoloside and oleanolic acid were 0.16% and 0.18% for the intra-day assays, respectively. The accuracy of the method was examined by performing recovery experiments according to the method of standard additions. Luteoloside and oleanolic acid stock solutions were added before the extraction at different concentration levels around half of the analyzed amounts in sample, respectively. Samples were prepared in triplicate at each level. Mean recovery of luteoloside and oleanolic acid were 102.47% (RSD% = 1.71) and 101.05% (RSD% = 1.62), which presented good accuracy for the analysis. Contents of luteoloside and oleanolic acid in the herb samples from different habitats were determined by HPLC subsequently [Table 4] and [Figure 4-7].

Statistical analysis

Content of macroelements (Na, Mg, K, and Fe) in *P. villosa* (Thunb.) Juss and *P. scabiosaefolia* Fisch from different places of production were determined by ICP-MS, and the contents of luteoloside and oleanolic acid in the same herb samples were also confirmed by HPLC. Hence, the relationship between content of inorganic macroelements and bioactive chemical ingredients was built up by statistics. The experiment data were analyzed by SPSS 17.0 International Business Machines Corporation (Armonk, State of New York, USA), and the results of correlation analysis [Table 5] indicated that the content of luteoloside showed high and significant positive correlations with that of Na, Mg, K, and showed weak positive correlation with that of Fe. Content of oleanolic acid showed weak positive correlations with that of Na and Mg, and showed weak negative correlations with that of K and Fe.

CONCLUSION

Content of 32 inorganic elements in *P. villosa* (Thunb.) Juss and *P. scabiosaefolia* Fisch from different places of production were determined by ICP-MS, and four macroelements were used as the characteristic inorganic elements to distinguish the species of the herb by PCA and cluster analysis. It is for the first time to use the characteristic inorganic elements as an index to classify the herb species by the method of ICP-MS and multivariate analysis. And it is also for the first time to investigate the influence of inorganic elements in herb on the accumulation of bioactive components which could affect the pharmacological efficacy of the herb medicine. In *BaiJiangCao*, content of Na, Mg, K, and Fe showed positive correlations with that of luteoloside, content of Na, Mg showed positive correlations with that of oleanolic acid, but content of K, and Fe showed the

Table 4: Content of luteoloside and oleanolic acid in *BaiJiangCao* from different habitats ($\mu\text{g/g}$, mean \pm SD, $n=3$)

Sample*	Luteoloside	Oleanolic acid
1	97.02 \pm 1.21	1628.35 \pm 19.21
2	130.13 \pm 1.84	1982.71 \pm 25.36
3	160.68 \pm 1.74	739.46 \pm 12.47
4	85.04 \pm 1.33	305.82 \pm 5.35
5	37.66 \pm 0.69	1401.67 \pm 16.23
6	68.14 \pm 1.03	1590.91 \pm 18.07
7	27.77 \pm 0.57	1976.23 \pm 17.82
8	30.71 \pm 0.50	2101.37 \pm 29.5
9	71.01 \pm 1.02	1577.74 \pm 20.42
10	3.82 \pm 0.06	1207.87 \pm 21.36
11	13.72 \pm 0.29	134.04 \pm 2.14
12	11.33 \pm 0.2	485.09 \pm 8.74
13	4.30 \pm 0.07	1160.49 \pm 13.53
14	16.29 \pm 0.23	706.3 \pm 11.48
15	2.62 \pm 0.05	922.61 \pm 12.09
17	14.22 \pm 0.19	256.7 \pm 2.36
18	4.87 \pm 0.08	2511.03 \pm 21.75
19	7.24 \pm 0.10	1804.26 \pm 23.22
20	3.43 \pm 0.07	548.36 \pm 9.04
21	28.64 \pm 0.47	759.48 \pm 10.83
22	13.53 \pm 0.18	787.41 \pm 14.94
23	5.59 \pm 0.07	459.73 \pm 8.26
24	100.20 \pm 0.89	1236.06 \pm 22.02

*Sample information was the same as that of table 1. SD: Standard deviation

Table 5: The correlation coefficients (r) of contents of macroelements and chemical ingredients

	Mg	Na	K	Fe
Luteoloside	0.539	0.554	0.579	0.275
Oleanolic acid	0.134	0.159	-0.035	-0.032

Correlation coefficients: $|r| < 0.3$, weak correlation; $0.3 \leq |r| \leq 0.5$, moderate correlation; $0.5 \leq |r|$, high correlation^[10]

opposite tendency. And this method could also be utilized in research of corresponding aspects.

ACKNOWLEDGMENTS

Thanks for the financial support of this work by the National Natural Science Foundation of China (81303211) and China Postdoctoral Science Foundation (2013M540301).

REFERENCES

- Xie Y, Peng J, Fan G, Wu Y. Chemical composition and antioxidant activity of volatiles from *Patrinia villosa* Juss obtained by optimized supercritical fluid extraction. J Pharm Biomed Anal 2008;48:796-801.
- Hsu HY, Chen YP, Shen SJ, Hsu CS, Chen CC, Chang HC. Oriental Materia Medica. Long Beach, California: Oriental Healing Arts Institute; 1986. p. 238-9.

3. Chang HM, But PP. Pharmacology and Applications of Chinese Materia Medica. Teaneck, New Jersey: World Scientific; 1987. p. 790-2.
4. Gao L, Zhang L, Li N, Liu JY, Cai PL, Yang SL. New triterpenoid saponins from *Patrinia scabiosaeifolia*. Carbohydr Res 2011;346:2881-5.
5. Peng J, Fan G, Wu Y. Studies on chemical constituents of *Patrinia villosa* Juss. Zhong Yao Cai 2005;28:883-4.
6. Xu JC, Zeng XY, Yu DQ. Studies on the chemical constituents of *Patrinia villosa* Juss. Acta Pharm Sin 1985;20:652-6.
7. Zhang T, Li Q, Li K, Li Y, Li J, Wang G, *et al.* Antitumor effects of saponin extract from *Patrinia villosa* (Thunb.) Juss on mice bearing U14 cervical cancer. Phytother Res 2008;22:640-5.
8. Taguchi H, Yokokawa Y, Endo T. Studies on the constituents of *Patrinia villosa* Juss. Yakugaku Zasshi 1973;93:607-11.
9. Peng JY, Fan GR, Wu YT. Studies on chemical constituents of *Patrinia villosa*. Zhongguo Zhong Yao Za Zhi 2006;31:128-30.
10. Hu D, Fang Y, Cui Y, Sun Z. Scavenging effects of daphnetin and its Cu, Zn complexes on superoxide radical. Zhongguo Zhong Yao Za Zhi 1995;20:749-50, 764.
11. Zhu WZ. A review on the factors affecting trace elements in Chinese medicine. Wei Liang Yuan Su Yu Jian Kang Yan Jiu 2002;19:66-8.
12. Li SL, Gu X, Ma D, Hu YZ. Correlation studies of contents of copper and organic components in Astragalus roots. Zhongguo Zhong Yao Za Zhi 2006;31:1249-53.
13. Yong-Rui B, Xin-Xin Y, Shuai W, Xian-Sheng M, Rui-Qing Z, Yue-Ming X, *et al.* Study on the *in vivo* toxic mechanism of xixin based on trace elements determination by inductively coupled plasma-mass spectrometry. Pharmacogn Mag 2014;10:141-6.
14. Ma LY, Yao LW, Ma SC, Lin RC. Optimization of an HPLC method for determination of luteolin-7-O-glucoside in *Flos Ionicerae Japonicae*. Chin J Pharm Anal 2009;29:1496-50.
15. Mao HM, Ping YH, Zong XX, Liu B. Simultaneous determination of hederagenina, oleanolic acid and ursolic acid in herba *Patriniae* by HPLC. Zhongguo Shi Yan Fang Ji Xue 2012;18:89-92.

Cite this article as: Wen-Lan L, Xue Z, Xin-Xin Y, Shuai W, Lin Z, Huan-Jun Z, *et al.* Species classification and bioactive ingredients accumulation of *BaiJiangCao* based on characteristic inorganic elements analysis by inductively coupled plasma-mass spectrometry and multivariate analysis. Phcog Mag 2015;11:756-63.

Source of Support: Thanks for the financial support of this work by the National Natural Science Foundation of China (81303211) and China Postdoctoral Science Foundation (2013M540301), **Conflict of Interest:** None declared.