Quick comparison of Radix Paeonia Alba, Radix Paeonia Rubra, and Cortex Moutan by high performance liquid chromatography coupled with monolithic columns and their chemical pattern recognition

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ABSTRACT

Background: Radix Paeoniae Alba, Radix Paeoniae Rubra, and Cortex Moutan are important Chinese herbs. Their bioactivities and efficacies are similar. However, they have different superior benefits clinically; so, a comprehensive investigation of the chemical difference is necessary and is of great importance for more reasonable quality assessment and proper clinical application of these three herbal medicines. **Objective:** To establish a high-performance liquid chromatography (HPLC) fingerprint method for the quality control of Radix Paeonia Alba, Radix Paeonia Rubra, and Cortex Moutan, and to compare their main constituents. **Materials and Methods:** The separations of Radix Paeoniae Alba, Radix Paeoniae Rubra, and Cortex Moutan was carried out, respectively, through a gradient elution using a monolithic column and a mobile phase consisting of water (containing 0.1% formic acid) and acetonitrile (containing 0.1% formic acid) at a flow rate of 5.0 ml/min. The detection wavelength was set at 230 nm. The data calculation was performed with CHROMAP v1.51 and Statistical Package for the Social Sciences (SPSS) 18.0 software for principal component analysis. **Results:** A rapid separation method based on high-performance liquid chromatography with diode-array detection (HPLC-DAD) with monolithic columns and a fingerprint analysis method was established. Fifteen Radix Paeonia Alba, 45 Radix Paeonia Rubra, and 21 Cortex Moutan samples were analyzed and 11 chromatographic peaks were identified. Differences of chromatographic peaks among these three herbal medicines in chemical compositions were revealed. **Conclusion:** The separation and analysis method are fast and simple, which can be used for chemical fingerprint comparison of Radix Paeonia Alba, Radix Paeonia Rubra, and Cortex Moutan. The results for the evaluation of the three medicines could provide experimental evidence for chemical affinity.

Key words: Cortex Moutan, fingerprint, high performance liquid chromatography coupled, monolithic column, principal component analysis, Radix Paeonia Alba, Radix Paeonia Rubra

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INTRODUCTION

Radix Paeoniae Alba (Bai-shao in Chinese), Radix Paeoniae Rubra (Chi-shao in Chinese), and Cortex Moutan (Mu- dan-pi in Chinese) are important Chinese herbs used in many herbal preparations.[1] These three herbal medicines all come from *Paeonia*: Bai-shao is the steamed and dried root of cultivated *P. lactiflora*; Chi-shao is the dried root of wild *P. lactiflora* or *P. veitchii*; and Mu-dan-pi is the root bark of *P. suffruticosa*. Some unofficial species from *Paeonia* are also misused as Chi-shao or Mu-dan-pi in some areas of China. They all mainly contain monoterpenoid glucosides, tannins, phenolic acids, triterpenes, and other substances.[2] However, Chi-shao and Mu-dan-pi contain more tannins due to the retained velamen, and Mu-dan-pi possesses a higher level of paeonol compared with the other two herbal

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medicines. Because of the similarity in original plants and characteristic chemical compositions, their bioactivities and efficacies are similar as well.[3] They all play a significant role in treating blood diseases. Moreover, they are uniformly bitter in taste and cold in property and enter the liver meridians. Clinically, they have different superior benefits, namely: Bai-shao is superior in nourishing the blood; Chi-shao is superior in invigorating the blood circulation; and Mu-dan-pi is superior in cooling the blood.[1,4] Thus, a comprehensive investigation of the chemical difference is necessary and is of great importance for more reasonable quality assessment and proper clinical application of these three herbal medicines.

Fingerprinting spectral analysis represented by high-performance liquid chromatography (HPLC) has been used as a major tool for the quality assurance of crude drugs and compound preparations.[5,6] Several chromatographic fingerprint analytical methods have been developed for the qualitative or quantitative evaluation of Bai-shao[7,8] and Mu-dan-pi,[9,10] respectively, or for the comparison of Bai-shao and Chi-shao.[11-15] However, traditional Chinese medicine (TCM) views multi-compound, multi-ingredient preparations as its features representing the activity of the herbal drugs. Selection of individual or a few analytical compounds for evaluating either efficacy or quality is contrary to the principles of TCM.[5] To date, no simultaneous chemical fingerprint analysis of these three herbals has been conducted. In this research, a simple and quick HPLC method for simultaneous comparison of Radix Paeonia Alba, Radix Paeonia Rubra, and Cortex Moutan using HPLC with monolithic columns and their chemical pattern recognition was established.

MATERIALS AND METHODS

Plant materials

A total of 15 Radix Paeonia Alba, 45 Radix Paeonia Rubra, and 21 Cortex Moutan samples were collected either from production areas or markets in China [Table 1]. Most samples were authenticated by Professor Pei-gen Xiao, Yong Peng, and Dr. Chun-nian He. The voucher specimens were deposited in Professor Xiao's laboratory at the Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences, Beijing, P. R. China.

Instrumentation

An Agilent 1200 HPLC system (Agilent Technologies, Palo Alto, CA, USA) equipped with an online vacuum degasser, a quaternary pump, an autosampler, a thermostated column compartment, and a diode array detector (DAD) was used. HPLC separation was performed by a Chromolith performance RP-18e (100×4.6mm, Merck, Germany) using the mobile phase A (purified water containing 0.1% formic acid) and B (acetonitrile containing 0.1% formic acid). The linear gradient elution was optimized as follows: 3→30% B (0→10 min). The flow rate was 5.0 ml/min. The column temperature was set at 30°C. The injection volume was 10 μl and the UV detection wavelength was set at 230 nm. The data were collected and analyzed using Chemstation software (B.02.01).

Chemicals

HPLC grade methanol was purchased from Fisher Scientific (NJ, USA). The purified water was prepared using Millipore purification system (Millipore, Milford, MA, USA) and filtered with 0.45 μm membranes. The 11 chemical reference substances were purified from Cortex Moutan (*Paeonia suffruticosa, Andrews*) in Professor Xiao's laboratory. They were *p*-hydroxybenzoic acid (**1**), methyl gallate (**2**), catechin (**3**), oxypaeonifiorin (**4**), albiflorin (**5**), paeonifiorin (**6**), benzoic acid (**7**), 1,2,3,4,6-penta-*O*-galloyl-β-D-glucose (**8**), quercetin (**9**), paeonol (**10**), and kaempferol (**11**). Their structures were identified by ultraviolet (UV), infra-red (IR), mass spectrometry (MS), and nuclear magnetic resonance (NMR). On the basis of UV, NMR and HPLC analyses, the purities of all the reference compounds were more than 95%.

Plant samples preparation

Each dried sample was ground to fine powders (60 mesh) using a pulverizer. An aliquot of 0.5 g of each sample was accurately weighed into an appropriately sized volumetric flask and macerated with 50 ml of 75% ethanol for 30 min. The sample was placed in an ultrasonic water bath for 30 min and conditioned to room temperature. The extracts were filtered through 0.45 μm filter and 10 μl filtrate was injected for each analysis.

Validation

The reliability of the HPLC method for analysis was validated through its stability, precision, and recovery.

Stability

The same sample (pmg-025, *P. ostii*) was stored in a HPLC vial at 25°C and analyzed at 0, 1, 3, 6, 12, and 24 h. Five chromatographic peaks were chosen to be inspected, of which, paeoniflorin was considered as a reference peak [Figure 1]. The relative standard deviation (RSD) of the relative retention time and of the relative peak area was less than 2%, respectively. Thus, the test solution was stable within 24 h.

Precision

The precision of the method was evaluated by applying the method developed above to analyze the same sample

Table 1: Samples of radix paeonia alba, radix paeonia rubra and cortex moutan for HPLC analyses

No.	Source plant	Collection location	Acquisition time	No.	Source plant	Collection location	Acquisition time
Radix Paeonia Rubra				**Radix Paeonia Alba**			
psg-001	*P. mairei*	Ningshan, Shaanxi	1997.5	psg-007	*P. lactiflora*	Zhongjiang, Sichuan	Unknown
psg-002	*P. lactiflora*	Baotou, Neimeng	Unknown	psg-008	*P. lactiflora*	Anguo, Hebei	Unknown
psg-003	*P. lactiflora*	Bozhou, Anhui	Unknown	psg-009	*P. lactiflora*	Bozhou, Anhui	2007.11
psg-004	*P. lactiflora*	Heze, Shandong	Unknown	psg-012	*P. lactiflora*	Bozhou, Anhui	2007.8
psg-005	*P. lactiflora*	Hailing, Heilongjiang	Unknown	psg-013	*P. lactiflora*	Bozhou, Anhui	2007.8
psg-006	*P. lactiflora*	Duolun, Neimeng	2005	psg-014	*P. lactiflora*	Bozhou, Anhui	2007.8
psg-010	*P. lactiflora*	Beijing	1998.5	psg-015	*P. lactiflora*	Bozhou, Anhui	2007.8
psg-011	*P. lactiflora*	Bozhou, Anhui	2007	psg-016	*P. lactiflora*	Bozhou, Anhui	2007.8
psg-038	*P. lactiflora*	Chifeng, Neimeng	2007.11	psg-047	*P. lactiflora*	Zhongjiang, Sichuan	2008.5
psg-039	*P. lactiflora*	Chifeng, Neimeng	2007.11	psg-057	*P. lactiflora*	Yuncheng, Shanxi	2008
psg-043	*P. lactiflora*	Wuchuan, Guizhou	Unknown	psg-061	*P. lactiflora*	Dongyang, Zhejiang	2008
psg-044	*P. lactiflora*	Sichuan	Unknown	psg-066	*P. lactiflora*	Anhui	2008
psg-045	Not identified	Sichuan	Unknown	psg-067	*P. lactiflora*	Anhui	2008
psg-046	Not identified	Neimeng	Unknown	psg-069	*P. lactiflora*	Anhui	2008
psg-048	*P.lactiflora*	Zhongjiang, Sichuan	2008.5	psg-094	*P. lactiflora*	Zhejiang	2008
psg-049	Not identified	Liaoning	2007				
psg-050	Not identified	Xifeng, Liaoning	2007	**Cortex Moutan**			
psg-051	Not identified	Fenyang, Shanxi	2007	pmg-010	*P. ostii (s. str.)*	Tongling, Anhui	2006
psg-052	Not identified	Meixian, Shaanxi	2007	pmg-011	*P. ostii (s. str.)*	Tongling, Anhui	2005
psg-053	Not identified	Qingling, Shaanxi	2007	pmg-012	*P. suffruticosa*	Kaifeng, Henan	Unknown
psg-054	Not identified	Qiqihaer, Heilongjiang	2007	pmg-013	*P. suffruticosa*	Shandong	Unknown
psg-058	*P. lactiflora*	Yuncheng, Shanxi	2008	pmg-014	*P. suffruticosa*	Shaanxi	1998.6
psg-059	*P. veitchii*	Datong, Qinghai	2008.10	pmg-015	*P. suffruticosa*	Anhui	2008
psg-060	*P. veitchii*	Datong, Qinghai	2008.10	pmg-016	*P. suffruticosa*	Bozhou, Anhui	2007.10
psg-062	*P. lactiflora*	Emei, Sichuan	2008.6	pmg-022	*P. suffruticosa*	Beijing	2007.10
psg-068	*P. lactiflora*	Neimeng	2008	pmg-024	*P. ostii (s. str.)*	Nanling, Anhui	2007.1
psg-070	*P. lactiflora*	Jilin	2008	pmg-025	*P. ostii (s. str.)*	Nanling, Anhui	2007.1
psg-071	*P. lactiflora*	Heilongjiang	2008	pmg-026	*P. ostii (s. str.)*	Nanling, Anhui	2007.1
psg-072	Not identified	Yakeshi, Neimeng	2007	pmg-027	*P. ostii (s. str.)*	Nanling, Anhui	2007.1
psg-073	Not identified	Chenbaerhuqi, Neimeng	2007	pmg-028	*P. ostii (s. str.)*	Nanling, Anhui	2007.1
psg-074	Not identified	Tuquan, Neimeng	2007	pmg-029	*P. ostii (s. str.)*	Nanling, Anhui	2007.1
psg-075	Not identified	Wuchaggou, Neimeng	2007	pmg-030	*P. ostii (s. str.)*	Nanling, Anhui	2007.1
psg-076	Not identified	Duolun, Neimeng	2007	pmg-031	*P. ostii (s. str.)*	Nanling, Anhui	2007.1
psg-077	Not identified	Tuquan, Neimeng	2007	pmg-042	*P. suffruticosa*	Yuncheng, Shanxi	2008
psg-078	Not identified	Alihe, Neimeng	2007	pmg-043	*P. suffruticosa*	Yuncheng, Shanxi	2008
psg-080	Not identified	Hailaer, Neimeng	2007	pmg-044	*P. suffruticosa*	Bozhou, Anhui	2008
psg-081	Not identified	Weichang, Hebei	2007	pmg-045	*P. delavayi*	Lijiang, Yunnan	2008
psg-082	Not identified	Longnan, Gansu	2007	pmg-046	*P. suffruticosa*	Anhui	2008
psg-083	Not identified	Fuxin, Liaoning	2007				
psg-084	*P. sinjiangensis*	Altai, Xinjiang	2007				
psg-085	Not identified	Xingcheng, Liaoning	2007				
psg-090	*P. japonica*	Panshi, Jilin	2009.7				
psg-091	*P. obovata*	Panshi, Jilin	2009.7				
psg-092	*P. lactiflora*	Panshi, Jilin	2009.7				
psg-097	*P. lactiflora*	Luanchuan, Henan	2009.10				

(pmg-025, *P. ostii*) on six consecutive injections. The result showed that the RSD of relative peak area was less than 3%, indicating the method could be considered reliable.

Recovery

Six samples from the same source (pmg-025, *P. ostii*) were extracted and analyzed using the method developed above.

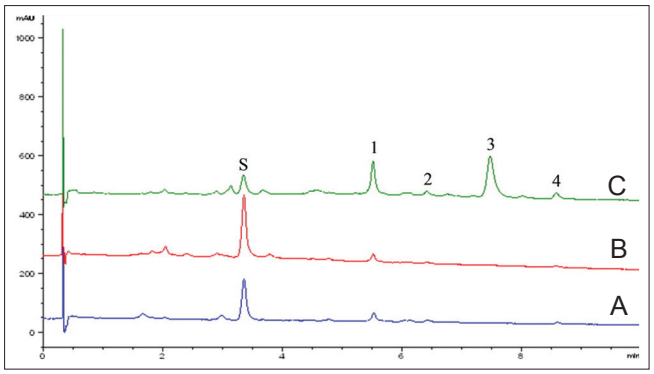

Figure 1: Liquid chromatograms of reference samples (S: paeonifiorin; A: Radix Paeonia Alba, pmg-012; B: Radix Paeonia Rubra, pmg-006; C: Cortex Moutan, pmg-025)

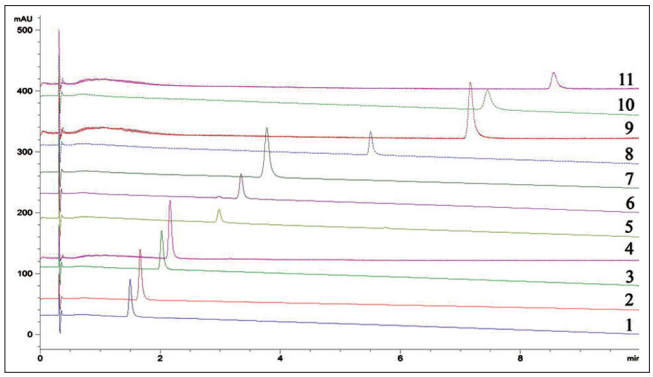

Figure 2: Liquid chromatograms of 11 chemical reference substances chemical reference substances - retention time (min): **1** - 1.497, **2** - 1.665, **3** - 2.023, **4** - 2.16, **5** - 2.980, **6** - 3.348, **7** - 3.774, **8** - 5.506, **9** - 7.164, **10** - 7.450 and **11** - 8.561

The RSD was calculated as the method described above. The result showed that RSD of relative peak area was less than 3%, which showed that the method has excellent repeatability.

Sample analysis and HPLC chromatogram data processing methods

The newly established analytical method was subsequently applied to analyzing 81 samples including 15 Radix Paeonia Alba, 45 Radix Paeonia Rubra, and 21 Cortex Moutan samples collected from different areas in China. Representative chromatograms are shown in Figure 1.

The fingerprint common patterns were generated by inputting the original data suits of all samples acquired from the HPLC workstation to the fingerprint solution software (CHROMAP v1.51, Chromap Institute of Herbal Medicine Research, Zhuhai, China). The following data processing simulated all the HPLC profiles one by one and the results were exhibited on the computer's screen. The major peaks were manually aligned by clicking the peak apexes in order to ensure correct recognition and the common pattern among the species was obtained by averaging all of the computer-simulated profiles. The similarity can be calculated and expressed by the correlative coefficient.

Preparation of chemical reference substance solutions

Q.S. milligrams of each of 11 chemical reference substances were dissolved in 10 ml of methanol for analysis, respectively. Liquid chromatograms of 11 chemical reference substances were obtained [Figure 2].

RESULTS AND DISCUSSION

The common pattern of the three herbal medicines

The common patterns of the HPLC profiles of the 15 Radix Paeonia Alba showed that 34 peaks were detected and 10 out of them were common peaks. Fifty six peaks arose in the common patterns of HPLC profiles of the 46 Radix Paeonia Rubra, of which, 4 peaks were common peaks; 46 peaks arose in the common patterns of HPLC profiles of the 21 Cortex Moutan, of which, 13 peaks were common peaks; and a total of 63 peaks were detected from the three herbal medicines, of which, 4 peaks were common peaks. Some common peaks were identified according to retention time and UV absorption spectra of reference standard compounds [Figure 3].

Similarity analysis of the three herbal medicines

Similarity analysis showed that the similarity coefficient of Radix Paeonia Alba ranged from 0.679 to 0.991 with that of Radix Paeonia Rubra ranging from 0.562 to 0.988 and Cortex Moutan ranging from 0.612 to 0.997. The RSD of the similarity coefficient of the three herbal medicines were more than 5.0%, respectively [Table 2].

Principal component analysis of 81 samples of Paeonia

Principal component analysis (PCA), an unsupervised pattern recognition method, was performed for the analysis of Radix Paeonia Alba, Radix Paeonia Rubra, and Cortex Moutan. PCA uses an N-dimensional vector approach to separate samples on the basis of the cumulative correlation of all metabolite data and then identifies the vector (eigenvector) that yields the greatest separation among samples without requiring prior knowledge of the data sets.[16] Taking the HPLC profile of pmg-025 (*P. ostii*) as reference, the data matrix obtained from the 81 samples above by CHROMAP v1.51 was input into Statistical Package for the Social Sciences (SPSS) 18.0 software for the principal component analysis and the analysis result was visualized through Spotfire 5.0 software.

A three-component PCA model cumulatively accounted for 82.6% of variation. The PCA scores plot in Figure 4 could be readily divided into three relative clusters: Radix Paeonia Alba (I), Radix Paeonia Rubra, and Cortex

Table 2: The correlation coefficient between each chromatogram of the three herbal medicines and the simulative mean chromatogram

Cortex Moutan		Radix Paeonia Alba		Radix Paeonia Rubra			
Chrom ID	Correlation	Chrom ID	Correlation	Chrom ID	Correlation	Chrom ID	Correlation
pmg-010	0.939	psg-007	0.984	psg-001	0.875	psg-062	0.811
pmg-011	0.952	psg-008	0.799	psg-002	0.970	psg-068	0.969
pmg-012	0.785	psg-009	0.820	psg-003	0.971	psg-070	0.978
pmg-013	0.711	psg-012*	0.882	psg-004	0.980	psg-071	0.972
pmg-014	0.860	psg-013	0.839	psg-005	0.952	psg-072	0.971
pmg-015	0.731	psg-014	0.879	psg-006*	0.965	psg-073	0.968
pmg-016	0.914	psg-015	0.762	psg-011	0.899	psg-074	0.962
pmg-022	0.612	psg-016	0.974	psg-038	0.962	psg-075	0.974
pmg-024	0.979	psg-047	0.843	psg-039	0.961	psg-076	0.944
pmg-025*	0.991	psg-057	0.806	psg-043	0.969	psg-077	0.984
Pmg-026	0.987	psg-061	0.791	psg-044	0.959	psg-078	0.980
pmg-027	0.980	psg-066	0.679	psg-045	0.791	psg-080	0.973
pmg-028	0.984	psg-067	0.991	psg-046	0.774	psg-081	0.969
pmg-029	0.991	psg-069	0.751	psg-048	0.965	psg-082	0.925
pmg-030	0.997	psg-094	0.748	psg-049	0.977	psg-083	0.941
pmg-031	0.976			psg-050	0.956	psg-084	0.755
pmg-042	0.937			psg-051	0.975	psg-085	0.981
pmg-043	0.972			psg-052	0.820	psg-086	0.852
pmg-044	0.983			psg-053	0.905	psg-090	0.852
pmg-045	0.957			psg-054	0.847	psg-091	0.831
pmg-046	0.974			psg-058	0.562	psg-092	0.936
				psg-059	0.928	psg-097	0.988
				psg-060	0.924	psg-010	0.803
mean	0.915		0.837			0.918	
RSD%	12.05		11.00			7.55	

*reference sample

Moutan (III) indicating that the content and distribution of secondary metabolites highly varied in the different *Paeonia* herbs. Radix Paeonia Alba was clearly separated from the other herbs by principal component 1 (PC1), while Cortex Moutan was easily discriminated from the others by principal component 3 (PC3). The corresponding PCA loadings plot were utilized to identify the differential metabolic compositions for the discrimination of groups [Figure 5]. For example, the preferential distribution of marker in group A of the loadings plot accounted primarily for the difference of Radix Paeonia Alba. Analogously, the distribution of the loadings plot in group C indicated the variation of cortex moutan. A comparative study on HPLC fingerprinting among these three herbal medicines showed:

(1) Radix Paeonia Alba samples were clustered together closely (I), which showed that its main chemical compositions were stable and could be clearly separated from Radix Paeonia Rubra. According to the load diagram, the contents of chemical compositions of area A in Radix Paeonia Alba samples were higher than those in Radix Paeonia Rubra. It has been identified that area A contains hydroxy acid and albiflorin.

(2) Radix Paeonia Rubra samples appeared to be very scattered, especially psg-001, 045, 046, 052, 054, 084, and 082 (Π area). Psg-084 was *P.sinjiangensis* from the Altai Mountains in Xinjiang, while psg-001 was *P.mairei* from Ningshan city, Shaanxi province. Moreover, the other samples were wild peony from an unidentified source, suggesting that several samples may be different from the *P. lactiflora* or *P. veitchii*. Furthermore, the samples psg-059 and 060 from *P. veitchii* had a certain distance from the main Radix Paeonia Rubra sample, so it was assumed that *P. veitchii* had different chemical compositions from red paeony. This has also been supported by literature.[12,13] *P.obovata* (psg-091) and *P.japonica* (psg-090) were similar to most of the samples; in addition, two cultivated *P. lactiflora* (psg-011 and 058) used as Chishao were much closer to Radix Paeonia Alba, indicating it was inappropriate to apply cultivated *P. lactiflora* as Radix Paeonia Rubra. The contents of the chemical compositions of area B in the Radix Paeonia Rubra samples were higher than the others samples. It has been identified that area B contains paeoniflorin and benzoic acid.

(3) Scattered Cortex Moutan examples can be divided into two groups (III_1 and III_2). Other than pmz-022 being collected from Beijing, the III_2 group samples were collected from the production areas of genuine

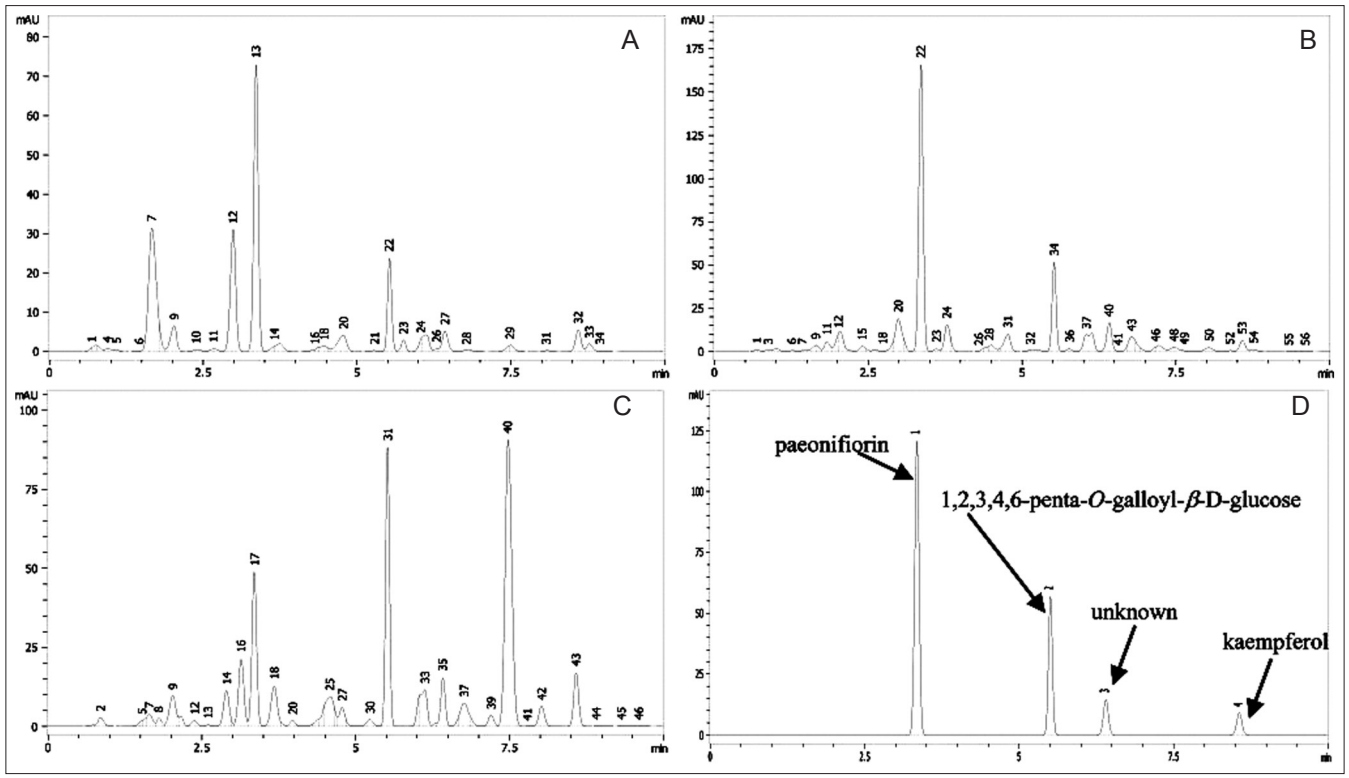

Figure 3: Common Pattern chromatogram of the three herbal medicines A: Radix Paeonia Alba (common peaks: 9-**3**, 12-**5**, 13-**6**, 22-**8**, 32-**11**, 20, 24, 25, 27, 33-unknown); B: Radix Paeonia Rubra (common peaks: 12-**6**, 34-**8**, 40-unknown, 53-**11**); C: Cortex Moutan (common peaks: 9-**3**, 17-**6**, 31-**8**, 39-**9**, 40-**10**, 43-**11**, 14, 18, 25, 32, 33, 35, 37-unknown,); D: common peaks of the three herbal medicines

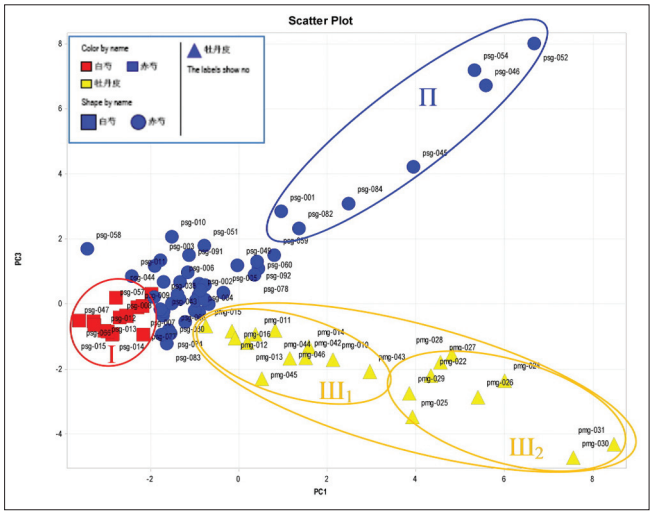

Figure 4: Scores plot of PCA of Paeonia samples on the first three PCs. Accumulated mean square. PC1 = 46.5%; PC2 = 71.6%; PC3 = 82.6%

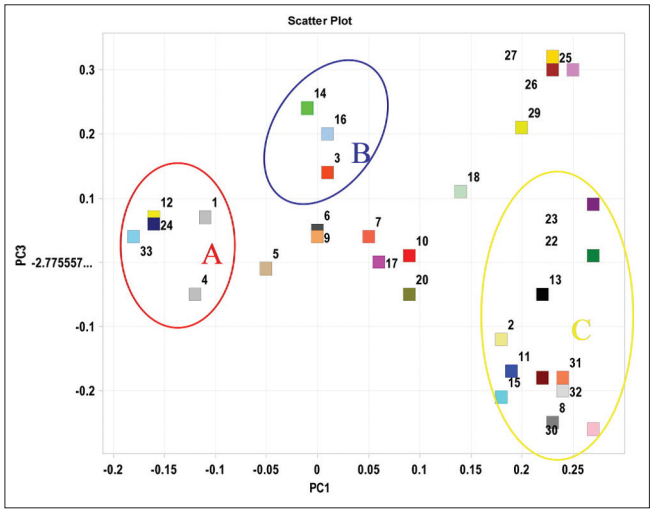

Figure 5: Loadings plot of PCA for the three herbal medicines' components. Identified by reference standard compounds as: 4-**1**, 5-**2**, 7-**3**, 8-**4**, 12-**5**, 14-**6**, 16-**7**, 23-**8**, 29-**9**, 30-**10** and 32-**11**

herbal medicine (Nanling area, Anhui province) in 2007. The III_1 group samples were taken from the drugstore (epidermis of some cortex moutan examples was removed). The PCA analysis showed that the contents of main chemical compositions of cortex moutan were affected by processing methods and storage time, and the latter factor had been confirmed by experiments.[17] According to factor loading plot [Figure 5] derived from the mentation impact factors above, it has been identified that area C contains paeonol, kaempferol, 1,2,3,4,6-penta-*O*-galloyl-*β*-D-glucose, oxypaeonifiorin, etc..

CONCLUSION

A total of 15 Radix Paeonia Alba, 45 Radix Paeonia Rubra,

and 21 Cortex Moutan samples were analyzed by HPLC-DAD with monolithic columns. A rapid separation and fingerprint analysis method has been established which could compare the similarity and PCAs of these three herbal medicines. The results showed that these three herbal medicines could be readily divided into three relative clusters.

HPLC monolithic packing material is a new approach which is increasingly being explored to accelerate the speed of the analysis. Monolithic columns have different structures when compared with conventional silica-based columns. Higher flow rates (up to 9.9 ml/min) can be used while the resolution of the silica rod column is less affected in comparison to particulate materials. After increasing the flow rate, column back pressure is still low (lower than 400 bar). This makes monolithic columns attractive for high throughput applications without a loss of column efficiency.[18-20] Compared with the normal reversed-phase column taking more than 60 minutes for one fingerprint analysis of *Paeonia* herbal medicines, in this research, a very fast method employing monolithic columns has been developed for the analysis of all samples in less than 10 minutes and given the high peak capacity (>30). This method can be applied to the determination of chemical fingerprint chromatogram.

In this investigation, this analytical method has demonstrated its potential for the rapid differentiation and identification of complex TCM extracts that contain similar chemical ingredients as well as the potential for the discrimination of subtle variations in the same plant species with different production areas, cultivation methods, processing methods, and collection time. Through analysis on the sources, Radix Paeonia Alba, Radix Paeonia Rubra, and Cortex Moutan can be clearly distinguished and the main features of their chemical compositions can be revealed by PCA: Cortex Moutan contains high contents of paeonol, kaempferol, 1,2,3,4,6-penta-*O*-galloyl-β-D-glucose and oxypaeonifiorin, etc.; Radix Paeonia Alba contains high contents of *p*-hydroxybenzoic acid and albiflorin; Radix Paeonia Rubra contains high paeoniflorin and benzoic acid. Therefore, speculations about their different bioactivities can be more accurate due to the knowledge of their different chemical compositions.

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