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ORIGINAL ARTICLE

Liquid Chromatography-diode Array Detector-electrospray Mass Spectrometry and Principal Components Analyses of Raw and Processed Moutan Cortex

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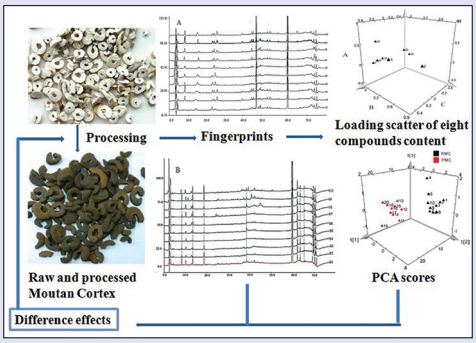
ABSTRACT

Background: Raw Moutan Cortex (RMC), derived from the root bark of *Paeonia suffruticosa*, and Processed Moutan Cortex (PMC) is obtained from RMC by undergoing a stir-frying process. Both of them are indicated for different pharmacodynamic action in traditional Chinese medicine, and they have been used in China and other Asian countries for thousands of years. **Objective:** To establish a method to study the RMC and PMC, revealing their different chemical composition by fingerprint, qualitative, and quantitative ways. **Materials and Methods:** High-performance liquid chromatography coupled with diode array detector and electrospray mass spectrometry (HPLC-DAD-ESIMS) were used for the analysis. Therefore, the analytes were separated on an Ultimate TM XB-C18 analytical column (250 mm × 4.6 mm, 5.0 μm) with a gradient elution program by a mobile phase consisting of acetonitrile and 0.1% (v/v) formic acid water solution. The flow rate, injection volume, detection wavelength, and column temperature were set at 1.0 mL/min, 10 μL, 254 nm, and 30°C, respectively. Besides, principal components analysis and the test of significance were applied in data analysis. **Results:** The results clearly showed a significant difference among RMC and PMC, indicating the significant changes in their chemical compositions before and after the stir-frying process. **Conclusion:** The HPLC-DAD-ESIMS coupled with chemometrics analysis could be used for comprehensive quality evaluation of raw and processed Moutan Cortex.

Key words: Fingerprints, high-performance liquid chromatography coupled with diode array detector and electrospray mass spectrometry, principal components analyses, quantification, raw and processed Moutan Cortex

SUMMARY

- The experiment study the RMC and PMC by HPLC-DAD-ESIMS couple with chemometrics analysis. The results of their fingerprints, qualitative, and quantitative all clearly showed significant changes in their chemical compositions before and after stir-frying processed.



Abbreviation used: HPLC-DAD-ESIMS: High-performance Liquid Chromatography-Diode Array Detector-Electrospray Mass Spectrometry, RMC: Raw moutan cortex, PMC: Processed moutan cortex, TCM: Traditional Chinese medicine, PCA: Principal components analysis, LOD: Limit of detection, LOQ: Limit of quantitation, RSD: Relative standard deviation.

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INTRODUCTION

Processing of herbal materials is a special and important pharmaceutical technique for the preparation of herbal drugs in traditional Chinese medicine (TCM).[1] While some herbs can be used as raw drugs after harvest from the field and undergoing simple postharvest treatments such as garbling, cleaning, and drying; others may require further specific processing procedures such as boiling, steaming, and frying or baking depending on the plant species. The former are often referred to as "raw herbal drugs," whereas the latter are "processed herbal drugs." As a result of the additional treatment processes, which involve prolonged heating, the therapeutic properties of the processed herbal drugs are often altered when compared to the raw drugs, showing reduced toxicity or enhanced efficacy. The processing of herbal materials can also improve the purity and flavor, or facilitate drug administration.[2-5] Formal processing procedures for many TCM herbal drugs are documented in the Chinese Pharmacopoeia.[6]

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Moutan Cortex, the root bark of *Paeonia suffruticosa* Andrews (*Paeoniaceae*), as a common TCM drug has been used in China and other Asian countries for thousands of years. Two kinds of crude drug preparations are available, the Raw Moutan Cortex (RMC) and the Processed Moutan Cortex (PMC). The RMC is prepared by collecting the root bark of *P. suffruticosa*, washed, cleaned, and sun-dried. The dried herbal materials are known as the "raw moutan bark." On the other hand, the PMC is produced after undergoing a further step, that is, stir-frying the dried raw materials until the bark surface turns into dark brown color. The stir-frying procedure involves continual stirring of the bark in a utensil heated to a high temperature. Such processed moutan material is also called "charcoaled moutan bark" in TCM. According to the TCM theory, the raw moutan bark serves to clear excessive heat, cool the blood, promote blood circulation, and remove blood stasis; it is indicated for diseases associated with the "heat syndromes" (typically manifesting in inflammation and related symptoms), stagnated blood conditions, and traumatic injuries. On the other hand, the charcoaled moutan bark not only possesses blood cooling property but more importantly, it is hemostatic. It is indicated for hemoptysis and other kinds of bleeding.[3,6] Pharmacological studies have demonstrated that the RMC possesses anti-inflammatory, analgesia, and blood activating properties while the PMC was able to restore hemostasis and promoting blood clotting.[7,8]

Moutan Cortex has been extensively studied for its chemical composition.[9-15] Qualitative and quantitative analyses using various chemical markers,[16-23] as well as quality control protocols,[24-31] have been reported for this herbal drug. However, little information is available to demonstrate chemical changes, if any that may occur during the stir-frying process. In this study, PMC was prepared from RMC and their chromatographic fingerprints were established and compared; at the same time, qualitative and quantitative analyses of eight marker components were accomplished using the high-performance liquid chromatography coupled with diode array detector and electrospray mass spectrometry (HPLC-DAD-ESIMS) method. Fingerprinting similarity analysis, principal components analysis (PCA), and the test of significance were performed in an attempt to detect the chemical differences between RMC and PMC.

MATERIALS AND METHODS

Chemicals and materials

Samples of Moutan Cortex were collected from different regions of China [Table 1] and authenticated by Dr. Shumei–Wang of the School of Chinese Medicine, Guangdong Pharmaceutical University. Voucher specimens were deposited in the University Herbarium. The RMC samples were prepared according to the procedures specified in the Chinese Pharmacopoeia. From each batch of the RMC samples, a portion was randomly taken to undergo the stir-frying process to produce the PMC in compliance with the standard procedures.[32]

Reference standards (purity >98%) of 5-hydroxymethylfurfural (5-HMF), gallic acid, catechins, oxypaeoniflorin, paeoniflorin, quercetin, paeonol, and benzoylpaeoniflorin were obtained from the National Institute for Food and Drug Control, Beijing, China. All the structures of the references are listed in [Figure 1]. HPLC-grade acetonitrile (Yu-Wang Chemical Factory, Shandong, China), formic acid (Beijing Reagent Co., Beijing, China), and analytical grade methanol (East giant Experimental Instrument Co., Guangzhou, China) were used. Water was purified by the Milli-Q water system (Millipore, Bedford, MA, USA).

Apparatus and chromatographic condition

An Agilent 1100 series HPLC system equipped with a photodiode array detector (Agilent, Palo Alto, CA, USA.), a mass spectrometer with an ESI detector (Applied Biosystems, Foster City, CA, USA), and an Ultimate TM XB-C18 analytical column (250 mm × 4.6 mm, 5 μm, Alltech Associates, Deerfield, IL, USA) were used for all analysis. The mobile phase consisted of acetonitrile and 0.1% (v/v) formic acid in water, with the following gradient program: Acetonitrile was increased from 5% to 15% (first 30 min), then to 50% in the second 30 min, finally increased to 95% in the following 10 min. The flow rate, injection volume, detection wavelength, and column temperature were set at 1.0 mL/min, 10 μL, 254 nm, and 30°C, respectively. Data were processed by using Chemstation for LC3D (Hewlett Packard, Palo Alto, CA, USA). For mass detection, nitrogen was used as dry gas (7 L/min, 400°C). Both positive and negative ESI modes were employed. The values for sprayer voltage, orifice voltage, and focusing ring voltage were set at 5000 V, 101 V, and 380 V, respectively. The scan range was m/z 100–1000. All data acquired were processed by the MaccChrom 1.1 software (Applied Biosystems, Grand Island, NY, USA).

Sample preparation

Each RMC and PMC sample was ground and passed through a 180-mesh sieve. The powder (0.5 g) was accurately weighed and extracted with 50% aqueous methanol (50 mL) for 30 min by sonication at room temperature. The suspension was centrifuged for 10 min at 20,000 rpm by an EBA-20S centrifuge (Hettich, Tuttlingen, Germany). The supernatant was transferred to a volumetric flask, made up to 100 mL with 50% methanol and shaken vigorously. An aliquot (10 μL) was applied to HPLC analysis after filtration through a 0.22-μm membrane filter. Each sample was analyzed in triplicate. The reference solution of a mixture of eight marker compounds was prepared in methanol and serially diluted to appropriate concentrations for the construction of calibration curves.

Data analysis

Similarity analysis was performed using a professional software, the Similarity Evaluation System for Chromatographic Fingerprint of TCM Version 2004A issued by the Chinese Pharmacopoeia Commission, which

Table 1: Sources of RMC and PMC samples, and the results of similarity tests

Sample number		Sample source	Collection date	Similarity		
RMC	PMC			RMC	PMC	RMC-PMC
1	11	Baoding, Hebei province, China	2012-12-04	0.976	0.863	0.018
2	12	Dezhou, Shandong province, China	2012-12-20	0.992	0.988	0.025
3	13	Bozhou, Anhui province, China	2013-03-04	0.985	0.982	0.030
4	14	Haikou, Hainan province, China	2013-03-26	0.933	0.886	0.041
5	15	Luoyang, Henan province, China	2013-04-29	0.930	0.971	0.018
6	16	Heza, Shandong province, China	2013-05-01	0.969	0.975	0.023
7	17	Yaan, Sichuan province, China	2013-05-13	0.976	0.938	0.022
8	18	Tongling, Anhui province, China	2013-05-06	0.972	0.962	0.029
9	19	Shantou, Guangdong province, China	2013-05-17	0.965	0.876	0.018
10	20	Chenzhou, Hunan province, China	2013-05-03	0.982	0.988	0.025

RMC: Raw Moutan Cortex; PMC: Processed Moutan Cortex

was recommended for use by the State Food and Drug Administration of China. The software employs correlative coefficients in the process of evaluating similarities of different chromatograms.[33]

PCA was performed on the contents of eight marker compounds by a software called Soft Independent Modeling of Class Analogy-P + 11.0. And independent-samples *t*-test was performed by the SPSS software (SPSS Statistics 16, SPSS Inc., USA).

Method validation

Linearity of the HPLC method was evaluated by the calibration curves [Table 2]. A range of six concentrations of eight marker compounds was analyzed in triplicates. The limit of detection (LOD) and limit of quantitation (LOQ) for each analyte were determined as the signal-to-noise ratio of 3 and 10, respectively.

The precision of the method was determined by intraday and interday measurements. The standard solution was analyzed in six replicates on the same day to obtain the intraday precision results, and the same replicate samples were analyzed daily for three successive days to obtain the interday results. Stability of the sample solution was evaluated by the same RMC and PMC sample solutions at 0, 2, 4, 8, 12, and 24 h. Meanwhile, recovery tests were performed to determine the accuracy of the method, in which an accurate amount of each standard was added to 0.25 g of Moutan Cortex sample and triplicate experiments were carried out. The results were expressed as % relative standard deviation (RSD) of the measurements [Table 3].

RESULTS AND DISCUSSION

Optimization of the extraction conditions

For sample extraction, different extraction methods (sonication or reflux), extraction solvents (various concentrations of aqueous methanol or aqueous ethanol), and extraction time (30, 40, or 60 min) were attempted [Table 4]. The highest yields of the selected components were obtained after 30 min of sonication in 50% aqueous methanol.

Method validation

The calibration curve of each analyte displayed good linearity ($R^2 > 0.9991$) over a range of concentrations, LOD and LOQ were within the range 2.4–37.5 ng and 0.011–0.2582 μg [Table 2], respectively [Table 2]. The RSD values of the precision test were 0.16-1.97% for intraday assays and 0.39–2.06% for interday assays, while the RSD value for sample solution stability was <3.0% [Table 3]. The recovery of the method was in the range of 91.8–109.5%, with RSD <5.0% [Table 3]. The system was thus considered suitable for the chemical analysis of RMC and PMC.

Optimization of the high-performance liquid chromatography system and peak identification

A mixture of acetonitrile and 0.1% (v/v) formic acid in water was selected as mobile phase after several attempts using different combinations of

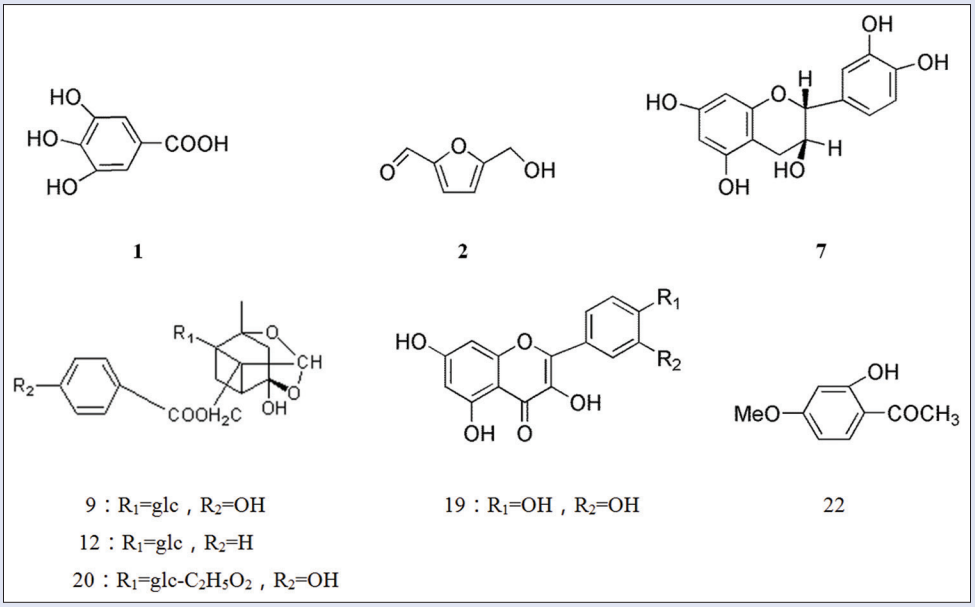

Figure 1: Chemical structures of the identified compounds (1) gallic acid, (2) 5-hydroxymethylfurfural, (7) catechin, (9) oxypaeoniflorin, (12) paeoniflorin, (19) quercetin, (20) benzoylpaeoniflorin, (22) paeonol

Table 2: Calibration curves, linear ranges, LOD and LOQ of eight marker compounds

Compound	Linear regression (*n*=6)	R^2	Linear range (μg)	LOD (μg)	LOQ (μg)
Gallic acid	Y=19538144.17x+1885156.75	0.9992	0.1170-1.1740	0.0375	0.1250
5-HMF	Y=1249408.2x+17268.88	0.9998	0.2500-2.5000	0.0025	0.1050
Catechin	Y=432427465.60x−998095.34	0.9996	0.0480-0.4800	0.0045	0.0180
Oxypaeoniflorin	Y=86252020.16x−1494327.12	0.9991	0.0174-0.3486	0.0083	0.1720
Paeoniflorin	Y=39753512.92x+1370485.22	0.9994	0.0425-0.8496	0.0232	0.0770
Quercetin	Y=1555892.85x−3086.4	0.9994	0.0033-0.0330	0.0024	0.0110
Benzoylpaeoniflorin	Y=2285095.83x+361069.92	0.9992	0.0758-0.7584	0.0077	0.2582
Paeonol	Y=97162921.89x+4493813.70	0.9999	0.0300-0.6000	0.0046	0.1520

LOD: Limit of detection (S/N=3); LOQ: Limit of quantitation (S/N=10); 5-HMF: 5-hydroxymethylfurfural

acetonitrile (or methanol) and water, with or without acid (acetic acid, formic acid, and phosphoric acid). The detection wavelength and column temperature were set at 254 nm and 30°C, respectively. Under the optimized conditions, a good baseline, maximum number of detectable peaks, good resolution, and reasonable analytical time were warranted.

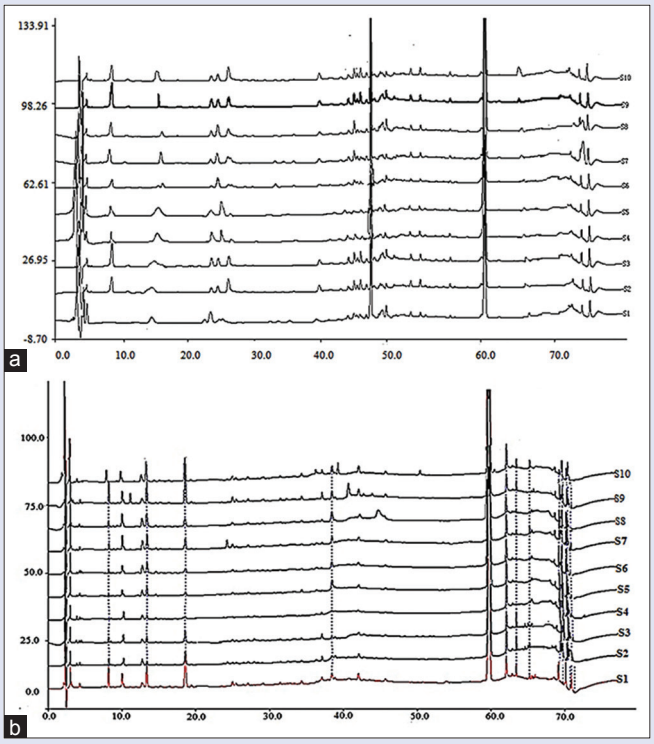

Figure 2: (a) Fingerprints of ten batches of samples detected at ultraviolet 254 nm (A: Raw Moutan Cortex). (b) Fingerprints of 10 batches of samples detected at ultraviolet 254 nm (B: Processed Moutan Cortex)

Table 3: Results of precision, stability, and recovery test

Compound	Precision RSD (%, *n*=6)		Recovery (%, *n*=6)		Stability RSD (%, 24 h)	
	Intraday	Interday	Mean	RSD	RMC	PMC
Gallic acid	1.35	1.97	108.00	4.38	1.23	2.12
5-HMF	0.98	1.27	98.60	4.07	-	2.07
Catechin	1.70	2.06	109.50	4.73	0.93	1.04
Oxypaeoniflorin	1.00	0.39	105.30	3.80	1.41	-
Paeoniflorin	1.81	0.69	92.80	4.85	1.36	1.52
Quercetin	0.16	1.58	98.10	3.68	0.26	-
Benzoylpaeoniflorin	1.97	1.84	93.90	4.98	1.32	1.04
Paeonol	1.29	1.07	91.80	4.23	0.43	0.73

-: Not detected; RSD: Relative standard deviation; RMC: Raw Moutan Cortex; PMC: Processed Moutan Cortex; 5-HMF: 5-hydroxymethylfurfural

Sample analysis

Ten batches each of the RMC and PMC were analyzed under the optimized LC-MS condition [Figure 2]. Eight marker components in the RMC and PMC samples were identified by HPLC-DAD-ESIMS analysis on the basis of retention time, ultraviolet absorption profile, and MS molecular ion in both positive and negative modes, with the respective reference standards [Table 5]. A reference chromatogram, each for RMC and PMC [Figure 2a and b], was synthesized using the software of the Similarity Evaluation System for Chromatographic Fingerprint of TCM,[33] by combining the characteristic features in ten individual LC chromatograms of the samples. These two reference chromatograms (one for RMC and the other for PMC) were subsequently employed in the similarity test for comparison of retentions time and peak area for each sample [Table 1].

Similarity test results showed that within the RMC and PMC groups, most samples displayed high similarity with the reference chromatogram, with similarity indices above 0.93 and 0.86, respectively. The high similarity among the samples indicated high degree of consistency of the samples, on the other hand, when the RMC sample (before processing) was compared to its PMC product (after processing), indices as low as 0.018 were obtained. The low similarity results indicated that significant differences existed before and after the processing procedures. In addition, when the major component signals were compared among the chromatograms, significant variations was detected [Figure 3a and b]. For examples, peaks 15–17 and 19 in the RMC samples disappeared in the PMC samples, with concomitant generation of new peaks (peaks 2–4, 6, 13, and 23) after the stir-frying process.

The contents of eight marker constituents were estimated in the RMC and PMC samples [Table 6]. The independent samples *t*-test results suggested that the average contents of six components, namely, quercetin, oxypaeoniflorin, paeoniflorin, benzoylpaeoniflorin, paeonol, and catechin decreased after the stir-frying processing ($P < 0.05$). On the other hand, the content of gallic acid was increased. 5-HMF, which was not detected in the RMC samples, could be identified in all PMC samples as a newly formed substance. Such chemical changes could be attributed to thermal decomposition during the process of high-temperature treatment.[34-36] It is noteworthy that the four compounds (paeoniflorin, benzoylpaeoniflorin, paeonol, and catechin) that undergo decomposition during the stir-frying procedure have been reported to be able to inhibit platelet aggregation, promote blood circulation, and remove blood stasis,[9,14] while gallic acid had hemostatic and astringent activities.[37] It can, therefore, be rationalized that the RMC is richer in the blood-activating components than the PMC, whereas PMC has higher contents of blood hemostatic components, thus leading to their different clinical properties.

Table 4: Optimization of extraction conditions

Compound	Content (mg/g)						
	A	B	C	D	E	F	G
Gallic acid	6.51±0.49	6.53±0.12	6.50±0.09	6.21±0.59	6.03±0.23	5.78±0.21	6.52±0.05
5-HMF	6.38±0.32	6.36±0.11	6.32±0.31	6.28±0.51	6.21±0.14	6.17±0.41	6.35±0.32
Catechin	0.28±0.51	0.29±0.21	0.26±0.04	0.17±0.30	0.18±0.31	0.17±0.19	0.28±0.31
Oxypaeoniflorin	0.59±0.07	0.68±0.14	0.65±0.07	0.58±0.13	0.49±0.21	0.41±0.21	0.66±0.21
Paeoniflorin	2.71±0.19	2.75±0.13	2.71±0.11	2.49±0.18	2.16±0.36	2.02±0.09	2.71±0.14
Quercetin	0.05±0.31	0.08±0.21	0.07±0.10	0.05±0.07	0.04±0.21	0.04±0.07	0.07±0.11
Benzoylpaeoniflorin	7.98±0.27	8.59±0.20	8.54±0.29	7.31±0.04	7.01±0.11	6.35±0.08	8.55±0.02
Paeonol	4.56±0.19	4.81±0.12	4.79±0.14	3.89±0.02	3.44±0.40	3.04±0.05	4.79±0.03

A-B: Extracted with 50% aqueous methanol for 60 min by reflux and sonication methods, respectively; C-F: Sonicated for 30 min with 50% aqueous methanol, 80% aqueous methanol, methanol, and ethanol, respectively; G: Sonicated with 50% aqueous methanol for 40 min. 5-HMF: 5-hydroxymethylfurfural

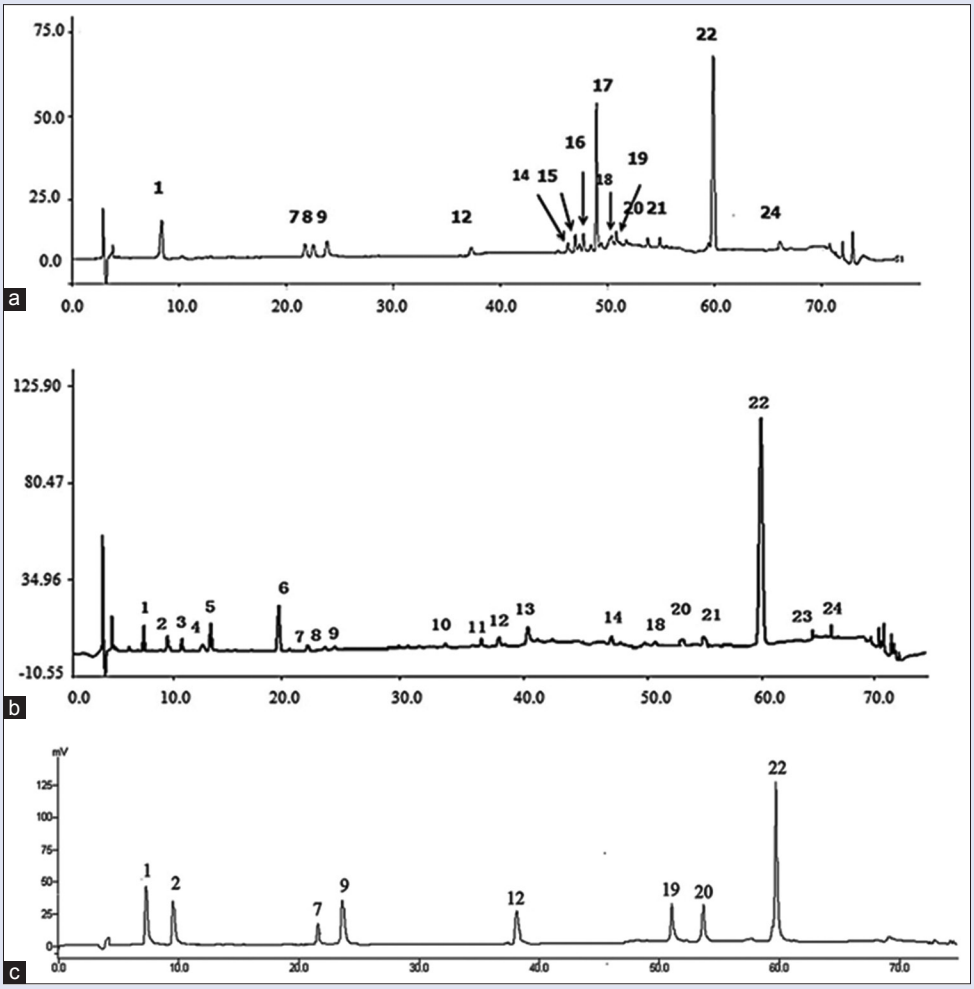

Figure 3: The representative high-performance liquid chromatography chromatograms of Raw Moutan Cortex (a) (b) The representative high-performance liquid chromatography chromatograms of Processed Moutan Cortex (b) (c) The representative high-performance liquid chromatography chromatograms of mixed standards solution (c) (1) gallic acid; (2) 5-hydroxymethylfurfural; (7) catechin; (9) oxypaeoniflorin; (12) paeoniflorin; (19) quercetin; (20) benzoylpaeoniflorin; (22) paeonol

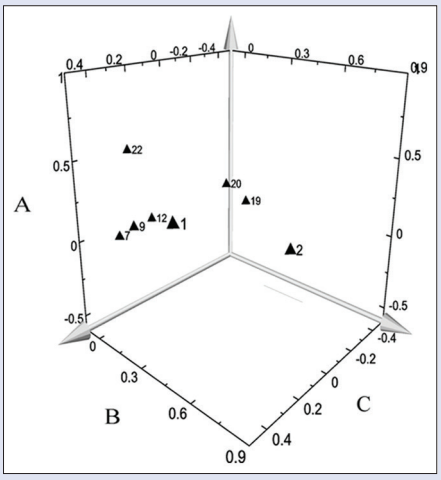

Figure 4: Loading scatter three-dimensional plot of eight variables on three principal components. (a) Component1; (b) component2; (c) component3. (1) gallic acid; (2) 5-hydroxymethylfurfural; (7) catechin; (9) oxypaeoniflorin; (12) paeoniflorin; (19) quercetin; (20) benzoylpaeoniflorin; (22) paeonol

Table 5: Peaks identification by HPLC-DAD-ESIMS

Peak	t_R (min)	$[M-H]^-$ (m/z)	$[M+H]^+$ (m/z)	Other positive ions (m/z)	Other negative ions (m/z)	Identification
1	6.35	169	171	196 315 512	205 283 339	Gallic acid
2	9.45	-	127	106 97 69		5-HMF
7	22.56	289	291	581	579	Catechin
9	24.43	495	514	344 327 179	609 541 991	Oxypaeoniflorin
12	35.78	479	498	463 526	525 593	Paeoniflorin
19	52.43	301	303	344	603	Quercetin
20	53.36	583	602		629 697	Benzoylpaeoniflorin
22	59.34	-	167	208 237		Paeonol

HPLC: High-performance liquid chromatography; DAD: Diode array detector; ESIMS: Electrospray mass spectrometry; 5-HMF: 5-hydroxymethylfurfural

Principal components analysis

PCA was employed to compare the difference between RMC and PMC, using the eight markers as variables and ten batches of samples as the observation object. According to the factor loading matrix calculation, the PCA loading plot [Figure 4] indicated that 5-HMF, gallic acid, and quercetin had great influence on the component 1, component 2, and component 3, respectively. The three-dimensional graphics of PCA scores [Figure 5] showed that RMC

Table 6: Contents of identified components in RMC and PMC samples (mg/g)

Sample	Gallic acid	5-HMF	Catechin	Oxypaeoniflorin	Paeoniflorin	Quercetin	Benzoylpaeoniflorin	Paeonol
1	10.01±0.08	ND	0.41±0.01	1.55±0.01	3.56±0.05	0.13±0.03	8.58±0.02	12.02±0.13
2	14.58±0.34	ND	0.14±0.02	1.25±0.004	5.01±0.03	0.12±0.01	9.48±0.14	11.94±0.15
3	3.63±0.02	ND	0.11±0.13	0.64±0.02	2.85±0.02	0.12±0.24	7.28±0.02	3.92±0.4
4	3.54±0.01	ND	0.15±0.03	0.55±0.001	3.54±0.03	0.11±0.01	7.15±0.03	11.08±0.61
5	6.49±0.02	ND	0.27±0.004	0.64±0.002	2.77±0.09	0.07±0.02	8.59±0.02	4.81±0.13
6	12.31±0.01	ND	0.11±0.01	1.08±0.003	3.95±0.03	0.14±0.01	7.51±0.01	11.07±0.21
7	10.82±0.07	ND	0.11±0.01	0.90±0.01	3.87±0.04	0.12±0.03	7.19±0.10	11.05±0.20
8	2.54±0.02	ND	0.19±0.001	0.98±0.001	6.10±0.03	0.12±0.01	8.62±0.06	11.81±0.72
9	3.56±0.04	ND	0.14±0.02	1.03±0.03	4.23±0.13	0.12±0.32	7.32±0.05	11.32±0.14
10	3.25±0.02	ND	0.24±0.01	0.73±0.23	3.21±0.02	0.12±0.05	8.02±0.06	10.24±0.2
Average	7.07±4.46	ND	0.19±0.09	0.94±0.31	3.91±1.02	0.12±0.02	7.97±0.81	9.92±2.98
11	14.87±0.02	14.15±0.01	ND	0.55±0.35	2.53±0.03	ND	1.65±0.35	2.75±0.14
12	10.61±0.13	5.08±0.03	ND	0.53±0.12	2.13±0.07	ND	2.64±0.23	3.40±0.13
13	4.01±0.23	7.89±0.03	ND	0.53±0.04	ND	ND	2.03±0.16	4.25±0.08
14	13.03±0.04	13.07±0.22	ND	0.54±0.36	ND	ND	2.32±0.36	3.80±0.04
15	8.44±0.14	6.32±0.05	0.083±0.02	ND	2.13±0.07	ND	2.64±0.05	2.95±0.01
16	10.28±0.04	5.52±0.12	0.079±0.023	ND	ND	ND	2.35±0.07	5.14±0.42
17	9.77±0.03	7.71±0.05	0.078±0.08	ND	2.08±0.14	ND	2.16±0.08	3.88±0.15
18	4.90±0.03	11.23±0.34	ND	ND	2.09±0.04	ND	2.75±0.24	4.46±0.02
19	12.25±0.01	14.19±0.04	0.078±0.01	0.64±0.02	2.07±0.05	ND	1.34±0.05	4.40±0.04
20	8.63±0.21	5.82±0.32	ND	ND	ND	ND	3.21±0.05	5.03±0.42
Average	9.68±1.25	9.10±0.48*	0.033±0.04*	0.28±0.29*	1.38±1.10*	ND	2.31±0.55*	4.01±0.81*

Values were expressed as a mean±standard (n=3); ND: Not detectable; *Significant difference was observed when compared to the raw samples (P<0.05). RMC: Raw Moutan Cortex; PMC: Processed Moutan Cortex; 5-HMF: 5-hydroxymethylfurfural

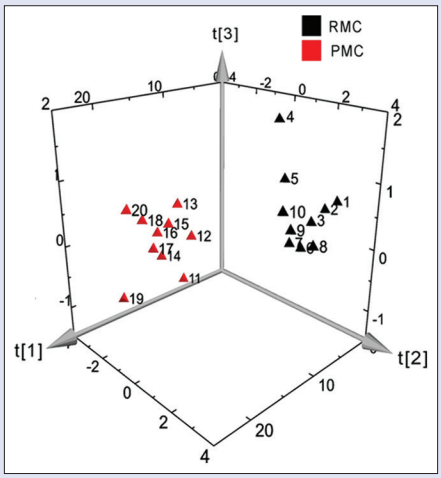

Figure 5: Three-dimensional graphics of principal components analysis scores for Raw Moutan Cortex and Processed Moutan Cortex

and PMC samples formed their own cluster within a small region apart from each other, indicating the two groups are distinctively different. Thus, the PCA analysis clearly supports the observation that the PMC samples had significantly different chemical composition than the RMC samples, which had not gone through the stir-drying process.

During the PCA analysis, it was also noted that a small number of samples distributed outside the core of the clusters. For example, samples RMC-4 and RMC-5 were outliers of the RMC group, and PMC-11, PMC-14, and PMC-19 were outliers of the PMC group. Indeed, these samples displayed lower chromatographic similarity (relative to the reference chromatogram) than other samples in the same group [Table 1]. For samples RMC-4 and RMC-5, the low similarity may have been caused by the relatively low oxypaeoniflorin and quercetin contents, respectively. On the other hand, in PMC-11, PMC-14, and PMC-19 samples, the low similarity could be attributed to the high contents of 5-HMF and gallic acid in these samples. Such variations in the chemical composition may have arisen from environmental and/or growing conditions of the *Paeonia* plants in the field.

CONCLUSION

The raw and processed samples of *Paeonia* bark were compared in the present study on the basis of chemical fingerprinting analysis and simultaneous qualitative and quantitative analysis of the marker components. The results clearly showed significant changes in their chemical compositions before and after the stir-frying process. Chemical variation in the herbal materials may explain, at least partially, the different medicinal properties of RMC and PMC.

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Conflicts of interest

There are no conflicts of interest.

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