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Original article

Distribution of Metabolites in Root Barks of Seven Tree Peony Cultivars for Quality Assessment Using NMR-based Metabolomics

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ABSTRACT

Objective To determine the distribution of metabolites in the root barks of different tree peony cultivars for quality assessment. **Methods** Seven tree peony phenotypic cultivars with different colors were systematically analyzed using NMR-based metabolomics. **Results** A total of 16 metabolites from their methanol extracts were simultaneously identified and quantified, including one primary metabolite (sucrose) and 15 secondary ones (acetophenones, phenolics, monoterpene glycosides, flavonoids, and unsaturated fatty acids). The quantitative data indicated that sucrose (90-180 mg/g) and acetophenones (15-100 mg/g), and non-phenolics, monoterpene glycosides, flavonoids, and unsaturated fatty acids (2-15 mg/g) were the major metabolites in these tree peony cultivars. The significantly increasing levels of paeonoside with bioactivity were observed in "Xiangyu", "Wujinyaohui", "Roufurong", "Yaohuang", "Zhaofen", "Doulǘ", and "Yingrihong" in order. Opposite trends in the levels of paeonoside and paeonol were observed in "Xiangyu" and "Yingrihong", suggesting that the changes of the secondary metabolites in plants were influenced by primary metabolites, such as sucrose/glucose, and the different physiological processes occurred in different tree peony cultivars. **Conclusion** "Yingrihong" with red flower has the highest medicine quality whereas "Xiangyu" with white flower has the worst one based on the content of paeonoside.

Key words

metabolomics; NMR; quality assessment; tree peony

1. Introduction

Tree peony (*Paeonia suffruticosa* Andrews, "Mudan" in Chinese), a woody deciduous shrub, belongs to the section Moutan DC in genus *Paeonia* L. of *Paeoniaceae* family. The tree peony flower is a symbol of good fortune and happiness

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in China, and its root barks are widely used in East Asia as a remedy for cardiovascular and female genital diseases (Picerno and Meucherini, 2011). At present, approximately 600 tree peony cultivars with different-colored flowers (Li et al, 1999) have been produced by conventional breeding in China, and several other unique cultivars (Haw, 2011) have been bred in Western countries for their ornamental and medicinal values. Flower color, one of phenotypic characteristics for tree peony, was used as the taxonomic evidence of different phenotypic cultivars. These phenotypic cultivars contain a wide variety of constituents, many of which may have close relationships with biological activities (Wang et al, 2004a; He and Peng, 2014). Therefore, adequate understanding of the metabolite distribution is helpful for classifying many cultivars and assessing their qualities.

Detailed qualitative and quantitative analyses of the chemical constituents in tree peony have been performed since 1887, and more than 130 highly diverse compounds (He and Peng, 2010) in this plant have been isolated and identified, including monoterpenoid glucosides, flavonoids, tannins, stilbenes, triterpenoids, steroids, paeonols, and phenols. Most of these compounds have been found to be related to the quality of the herbal drug because different functions are associated with different active constituents. Moreover, primary metabolites, such as sucrose, glucose, and fructose, are widely present in plants. The amounts of primary metabolites have been reported to most likely affect changes in the secondary metabolites associated with the quality of certain herbal drugs (Drew and Demain, 1977). Considering the complicated chemical composition in tree peony, the separation and determination of individual constituents will be challenging using conventional phytochemistry methods.

In recent years, increasing number of bioactive constituents in tree peony have been simultaneously determined using chromatographic methods (He et al, 2013) and the hyphened chromatography-mass spectrometry techniques (Liang and Wen, 2005; Xu and Yang, 2006; Li and Chen, 2012; Li and Yuan, 2015). In particular, HPLC and profiling analysis have been employed for the determination of chemical variation in the seeds (He and Peng, 2013a; 2013b) and root barks (Ding et al, 2009; He and Peng, 2014) of different tree peony species. However, these studies focused on the profiling analysis of certain secondary metabolites and lacked sufficient information about the relationships between the primary and secondary metabolites of tree peony. Therefore, it is essential to use simple and accurate methods for the simultaneous determination and comparison of the bioactive substances and other co-existing components in tree peony.

High-field ^{1}H-NMR is particularly well suited for the detection and quantification of both abundant primary metabolites and various groups of secondary metabolites in complex mixtures, such as plant extracts, and in a single measurement without chemical separation. In addition, quantitative NMR data are obtained relative to a single internal standard without the need for calibration curves, and thus, all measured concentrations are estimated. Combining NMR with metabolic profiling has been increasingly applied in metabolomics-related fields to analyze phytomedicines (Wang et al, 2004b; Holmes et al, 2006; Xiao et al, 2008), foods (Duarte et al, 2002; Wishart, 2008), and biological samples (Zhao et al, 2011; Shi et al, 2013).

In this work, seven phenotypic cultivars with different flower colors were selected as the representative examples among enormous amounts of tree peony cultivars. Their metabolomic profiles were systematically investigated using NMR-based metabolomics. The main aims were (1) to elucidate the metabolite distribution in different tree peony cultivars and (2) to assess their potential values as medicines.

2. Materials and methods

2.1 Chemicals

Methanol of HPLC-grade from Fisher Scientific Products (USA) was used for the analyses. Deuterated methanol (CD_3OD, 99.8% D) and sodium 3-trimethlysilyl [2,2,3,3-$^{2}H_4$] propionate (TSP) were purchased from Sigma-Aldrich, Inc. (USA).

2.2 Sample collection and extraction

Seven representative cultivars of tree peony (*Paeonia suffruticosa* Andrews) with different-colored flowers of three years old were collected from Heze Xinjun Peony Garden in Shandong province, China, in October 2012. The seven cultivars were identified by Prof. Min-feng Fang such as “Xiangyu” with white flowers, “Wujinyaohui” with blackish-purple flowers, “Yaohuang” with light-yellow flowers, “Yingrihong” with red flowers, “Roufurong” with deep-pink flowers, “Zhaofen” with pink flowers, and “Doulǔ” with light-green flowers. Five plants of each cultivar were gathered, and their main roots (diameter of 8−9 mm) were carefully separated. The roots were peeled and the internal cores eliminated according to *Chinese Pharmacopeia* 2015. The remaining materials (root barks), called “Mudanpi” in Chinese, were immediately dried at 40 °C for 24 h.

The dried root barks were ground and strained through a 2-mm sieve. In all cases, the raw materials (1.0 g) were extracted for three times sequentially in a flask with methanol (25 mL) by vortexing for 30 s followed by continuous ultrasonication in ice water bath for 30 min. The three resulting stock solutions were combined and centrifuged at 8000 r/min for 10 min. The supernatants were concentrated at 40 °C with a rotary evaporator to remove most of the solvent before vacuum drying.

2.3 NMR measurements

The extracts (10 mg) in the form of dried powder were dissolved in 600 μL of CD_3OD containing TSP (0.05%). The supernatants (500 μL) were transferred into 5-mm NMR tubes after agitation and centrifugation (10 000 r/min, 5 min). The ^{1}H-NMR spectra of 35 samples were recorded at 298 K on a

Varian VNMRS 600 MHz NMR Spectrometer (599.904 MHz for proton frequency) with a cold probe. A standard one-dimensional NOESYPR pulse sequence (RD-90º-t_1-90º-t_m-90º-acquisition; t_1 = 6 μs) was employed with irradiation at the water frequency during the recycling delay (RD, 2 s) and the mixing period (t_m, 100 ms) to suppress the water signal. A 90 pulse length was adjusted to approximately 10 μs for each sample. Totally 64 transients were collected into 32K data points for each spectrum with a spectral width of 20 ppm. All free induction decays (FID) were multiplied by an exponential function with a 1-Hz line-broadening factor prior to Fourier transformation (FT). For NMR signal assignment, a set of two-dimensional (2D) NMR spectra including ^{1}H-^{1}H J-Resolved, ^{1}H-^{1}H COSY and TOCSY, ^{1}H-^{13}C HSQC and HMBC spectra were acquired and processed using the 600-MHz spectrometer for selected samples.

2.4 Data analysis

All ^{1}H-NMR spectra were manually phase- and baseline-corrected and referenced to TSP (δ 0.00). These spectra were then integrated into regions with a bucket width of 0.002 ppm using the AMIX package (V3.8, Bruker Biospin, Germany) in the spectral region (δ 0.50−9.50). To eliminate the effects of imperfect water suppression and exogenous compounds, the regions of δ 4.80–4.90 for H_2O signal and δ 3.35−3.33 and δ 3.33−3.29 for CH_3OH and CD_3OD signals were carefully discarded from the ^{1}H-NMR spectra. Multivariate data analysis was conducted using the SIMCA-P$^+$ package (V.11, Umetrics, Sweden). Principal component analysis (PCA) was performed on mean-centered data to obtain an overview of the data distribution.

For analysis of variance (ANOVA), the metabolite concentration was calculated from the integrals of selected metabolite NMR signals (least overlapping ones) relative to an internal reference (TSP) of known concentration. Although the relaxation time (*T*1) for the metabolites and references was different and the concentration measured here was semi-quantitative, our treatments were still valid when the concentration changes between samples were compared because the inter-sample *T*1 variations were small for the same metabolite (or reference). The obtained metabolite concentration was subjected to classical statistical analysis (one-way ANOVA) using SPSS 13.0 software with a Tukey post-test.

3. Results and discussion

3.1 Metabolites in tree peony assigned by NMR

Methanol is generally accepted as a solvent for extraction owing to its purging enzymic activity by precipitating protein and its extracting efficiency for the overwhelming majority of metabolites in phytomedicines. However, the weak polar components in phytomedicines are hardly extracted from methanol with its poor solubility. Figure 1 shows a typical ^{1}H-NMR spectroscopy for the methanol extract of the root bark from one of the seven tested tree peony cultivars. The peaks were assigned to individual metabolites based on 1D and 2D NMR data. A total of 16 metabolites (Table 1) were characterized in this study, including one primary metabolite and 15 secondary ones. The ^{1}H-NMR spectroscopy was clearly dominated by sucrose (Catherine et al, 1991; Kwon and Song, 1996), four acetophenones including paeonol, paeonolide, paeonoside (Kuwajima and Shibano, 1996) and apiopaeonoside (Yu and Lang, 1986; Ha et al, 2010), six phenolics including benzoic acid, 4-hydroxybenzoic acid (Peungvicha and Temsirirkkul, 1998), gallic acid, methyl gallate (Lee and Kwon, 2005), glucogallin (Puppala and Ponder, 2012) and 1,2,3,4,6-pentagalloyl glucose (Piao and Piao, 2008; Beretta et al, 2010), two monoterpene glycosides including paeoniflorin and oxypaeoniflorin (Lee and Kwon, 2005), one flavonoid such as catechin (Qi and Wu, 2013) and two unsaturated fatty acids (unassigned).

To compare the metabolite composition of different tree peony cultivars, ^{1}H-NMR spectra were acquired for the methanol extracts from root barks of seven tree peony cultivars: "Zhaofen", "Roufurong", "Yingrihong", "Yaohuang", "Wujinyaohui", "Xiangyu", and "Doulű" (Figure 2). The extracts from the different cultivars exhibited marked concentration differences in certain metabolites (i.e., sucrose and unsaturated fatty acid), although the metabolite types were broadly similar. The complexity of tree peony extracts and multiple spectral data make it prohibitively difficult for the spectra to be analyzed with the naked eye; Therefore, multivariate data analysis is more appropriate forming such complex data.

3.2 Principal component analysis (PCA) for different tree peony cultivars

Figure 3 displays a 3D PCA score plot of the extracts from seven tree peony cultivars. Samples from the same cultivars were closely clustered, indicating their similar metabolite compositions and the excellent reproducibility of extraction procedures and NMR measurements. Moreover, a clear classification can be observed for "Zhaofen", "Roufurong", "Yingrihong", "Yaohuang", "Wujinyaohui", "Xiangyu", and "Doulű" samples, suggesting large differences in metabolite composition among the different cultivars. To understand the significance of the contribution of metabolites to classification and elucidate the distributions of the metabolites, one-way ANOVA analysis was employed to explore the differential metabolites from these seven cultivars.

3.3 Distribution of metabolites and assessment of medicine values for different tree peony cultivars

The concentrations of most metabolites were calculated by integrating the areas of selected NMR proton signals (least overlapping ones) relative to the internal standard TSP (Dai et al, 2010). All data were expressed as $\bar{x} \pm s$ (mg/g of the dried root bark material) from five plant samples (Table 2). The metabolic profiles of these tree peony cultivars show that sucrose exhibits the highest concentration ranging from 90 to

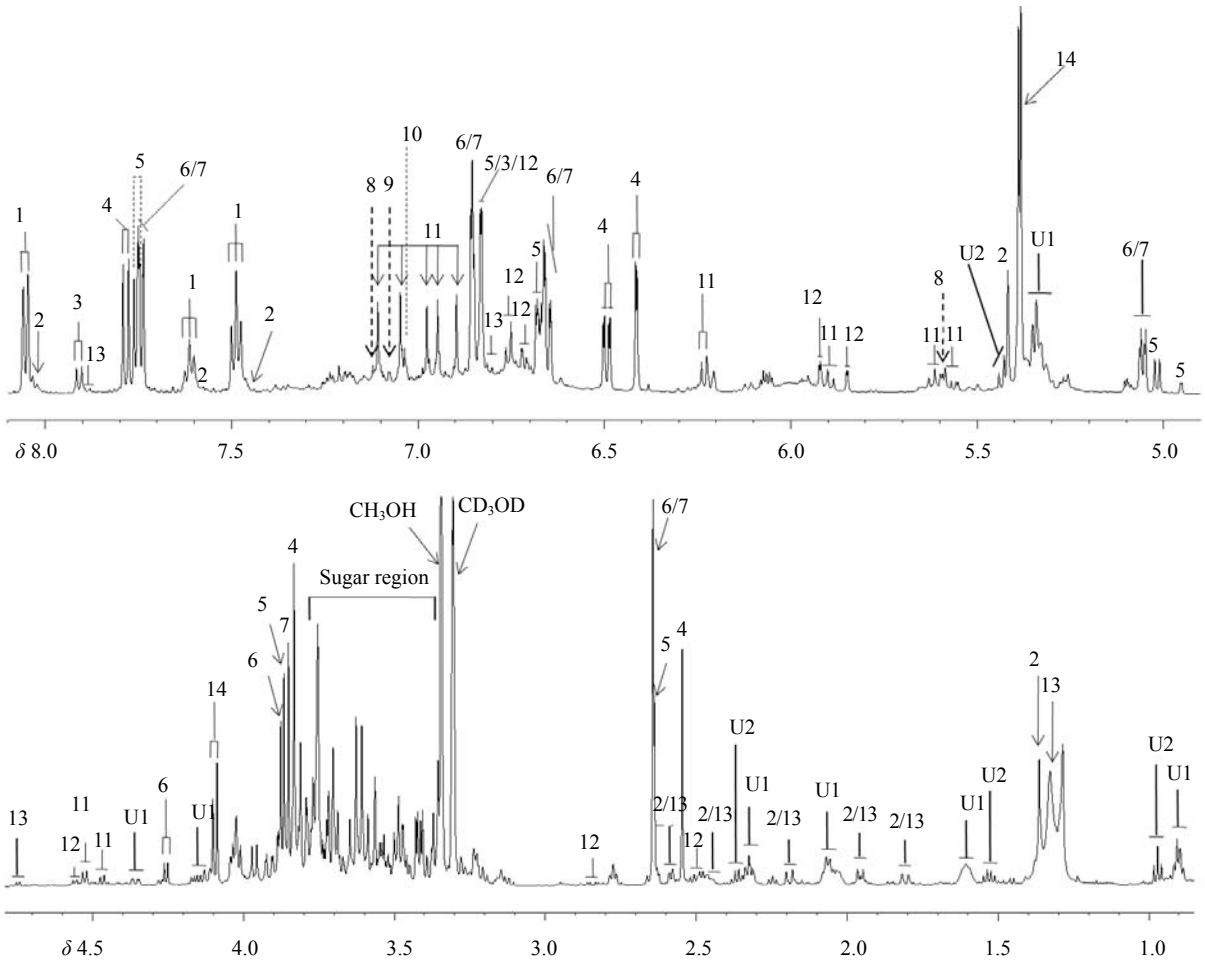
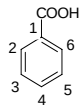
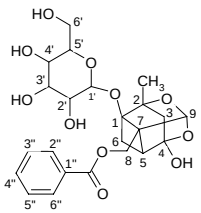


Figure 1 600-MHz ^{1}H-NMR spectroscopy of root bark extract from one of seven typical tree peony cultivars

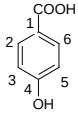
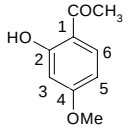
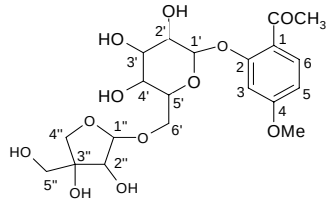
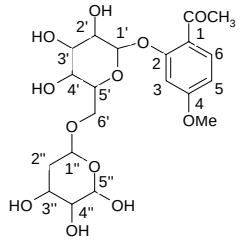
Region δ4.90−8.10 is magnified two times relative to the region δ0.8−4.8 (see Table 1 for metabolite identification key)

Table 1 Assignment of metabolites in root bark of tree peony by NMR

NM	Metabolites	Groups	δ ^{1}H (J/Hz)	δ ^{13}C
1	Benzoic acid	1-C		#
		2,6-CH*	8.05(m)	130.6
		3,5-CH	7.49(m)	129.8
		4-CH	7.61(m)	134.5
		COOH		168.1
2	Paeoniflorin	1-C		89.3
		2-C		87.3
		3-CH_2	1.81(d,12.0), 2.19(d,12.0)	44.6
		4-C		106.5
		5-CH	1.96(m)	23.7
		6-CH_2	2.49(m), 2.58(m)	44.3
		7-C		72.3
		8-CH_2	4.75(d,12.0), 4.72(d,12.0)	61.3
		9-CH	5.42(s)	102.4
		CH_3	1.36(s)	19.8
		C=O		168.2
		1′-CH	4.52(d,7.8)	100.2
		2′-CH	3.22(#)	#
		3′-CH	3.84(#)	#
		4′-CH	3.31(#)	#

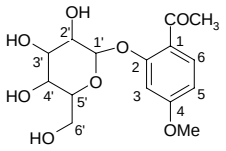
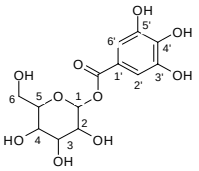
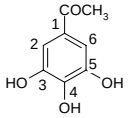
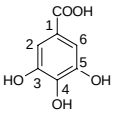
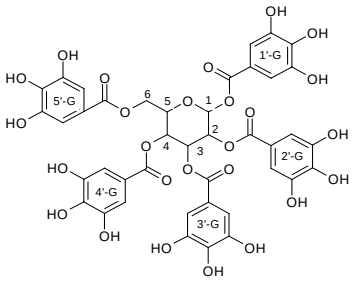
To be continued

Continued Table 1

NM	Metabolites	Groups	δ ^{1}H (J/Hz)	δ ^{13}C
2	Paeoniflorin	5′ -CH	3.61(#)	#
		6′ -CH_2	4.46(#)	#
		1″ -C		111.4
		2″, 6″-CH*	8.03(m)	130.6
		3″, 5″-CH	7.47(m)	129.8
		4″ -CH	7.60(m)	134.5
3	4-Hydroxybenzoic acid	1-C		122.8
		2,6-CH*	7.91(d,8.8)	133.0
		3,5 -CH	6.83(d,8.8)	115.7
		4-C		163.9
		COOH		168.1
4	Paeonol	1-C		115.0
		2-C		166.4
		3-CH	6.41(d,1.8)	101.9
		4-C		167.7
		5-CH	6.49(dd,1.8,7.8)	108.4
		6-CH*	7.79(d,7.8)	134.0
		-OCH_3	3.83(s)	56.2
		CH_3-C=O	2.55(s)	26.3
		C=O		204.8
5	Apiopaeonoside	1-C		122.7
		2-C		160.7
		3-CH	6.83(d,2.0)	103.5
		4-C		166.4
		5-CH	6.68(dd,2.0,7.8)	109.1
		6-CH	7.75(d,7.8)	133.3
		1′CH*	5.02(d,7.8)	102.5
		2′CH	3.54(m)	74.8
		3′CH	3.48(m)	78.3
		4′CH	3.36(m)	71.4
		5′CH	3.63(m)	77.2
		6′CH	4.03(m)	74.5
		-OCH_3	3.87(s)	56.4
		O=C-CH_3	2.64(s)	32.2
		O=C		200.6
		1″CH	4.95(d,2.5)	111.0
		2″CH	3.88(d,2.5)	78.1
		3″C		80.0
		4″CH_2	4.05(m), 4.03(m)	74.9
		5″CH_2	3.56(d,12.0), 3.96(d,12.0)	65.5
6	Paeonolide	1-C		#
		2-C		#
		3-CH	6.85(d,2.0)	102.5
		4-C		106.5
		5-CH	6.67(dd, 2.0,8.5)	109.3
		6-CH	7.75(d,8.5)	133.0
		-OCH_3*	3.88(s)	56.1
		CH_3-CO	2.64(s)	32.2
		C=O		200.6
		1′CH	5.06(d,7.8)	100.2
		2′CH	3.55(#)	#
		3′CH	3.49(#)	#
		4′CH	3.36(#)	#
		5′CH	3.70(#)	#

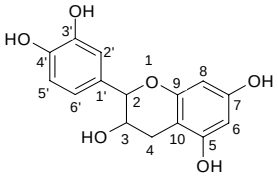
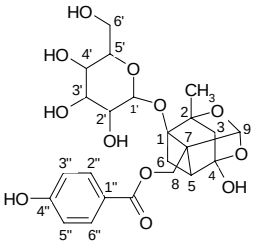
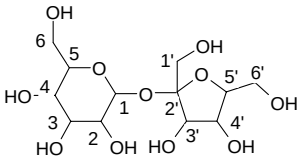
To be continued

Continued Table 1

NM	Metabolites	Groups	δ ^{1}H (J/Hz)	δ ^{13}C
6	Paeonolide	6′CH_2	3.79(#)	#
		1″CH	4.26(d,7.5)	#
		2″CH_2	3.56(#)	#
		3″CH	3.37(#)	#
		4″CH	3.48(#)	#
		5″CH	3.76(#)	#
7	Paeonoside	1-C		#
		2-C		#
		3-CH	6.86(d,2.0)	102.5
		4-C		106.5
		5-CH	6.65(dd,2.0,8.7)	109.3
		6-CH	7.74(d,8.7)	133.0
		-OCH_3*	3.85(s)	56.1
		CH_3-CO	2.60(s)	32.2
		C=O		200.6
		1′CH	5.05(d, 7.8)	100.2
		2′CH	3.55(#)	#
		3′CH	3.49(#)	#
		4′CH	3.36(#)	#
		5′CH	3.70(#)	#
		6′CH_2	#	#
8	Glucogallin	1-CH	5.65(d)	96.2
		2,3,4,5-CH	3.41-3.48	#
		6-CH_2	3.85-3.70	#
		1′-C		#
		2′,6′-CH*	7.12(s)	110.3
		3′,5′-C		139.9
		4′-C		146.6
		C=O		167.7
9	Methyl gallate	1-C		121.4
		2,6-CH*	7.08(s)	110.1
		3,5-C		140.2
		4-C		146.4
		C=O		168.1
		CH_3	3.81(s)	52.6
10	Gallic acid	1-C		122.8
		2,6-CH*	7.03(s)	110.1
		3,5-C		139.8
		4-C		146.4
		COOH		169.0
11	1,2,3,4,6-Pentagalloylglucose	1-CH	6.24(d,9.5)	93.9
		2-CH	5.58(t,11.4)	69.9
		3-CH	5.91(t,11.4)	74.3
		4-CH	5.61(t,11.4)	72.2
		5-CH	4.51(d,12.6)	63.9
		6-CH_2	4.40(m), 4.42(m)	63.4
		1′- G-CH	7.05(s)	110.4
		2′- G-CH	6.95(s)	110.4
		3′- G-CH*	6.90(s)	110.4
		4′- G-CH	6.98(s)	110.4
		6′- G-CH	7.11(s)	110.4
		1′- G-C=O		166.4
		2′- G-C=O		167.0
		3′- G-C=O		167.4

To be continued

Continued Table 1

NM	Metabolites	Groups	δ ^{1}H (J/Hz)	δ ^{13}C
11	1,2,3,4,6-Pentagalloylglucose	4′- G-C=O		167.0
		6′- G-C=O		168.1
12	Catechin	2-CH	4.56(d,7.8)	83.0
		3-CH	3.97(ddd,5.4,8.2,7.8)	68.7
		4-CH_2	2.84(dd,16.0,5.4), 2.50(dd,16.0,5.4)	28.5
		5-C		157.1
		6-CH*	5.84(d,2.0)	95.6
		7-C		156.7
		8-CH	5.91(d,2.0)	96.3
		9-C		156.7
		10-C		100.2
		1′-C		132.1
		2′-CH	6.83(d,2.0)	115.5
		3′ -C		145.1
		4′ -C		146.3
		5′ -CH	6.75(d,8.0)	117.8
		6′ -CH	6.71(dd,2.0,8.0)	120.1
13	Oxypaeoniflorin	1-C		89.3
		2-C		87.3
		3-CH_2	1.80(d,12.0), 2.18(d,12.0)	44.6
		4-C		106.5
		5-CH	1.96 (m)	23.7
		6-CH_2	2.48(m), 2.57(m)	44.3
		7-C		72.3
		8-CH_2	4.76(d,12.0), 4.73(d,12.0)	61.3
		9-CH	5.39(s)	102.4
		CH_3	1.33(s)	19.8
		C=O		168.2
		1′-CH	4.64(d,7.8)	100.2
		2′-CH	3.26(#)	#
		3′-CH	#	#
		4′-CH	3.36(#)	#
		5′-CH	3.59(#)	#
		6′ CH_2	4.50(#)	#
		1″-C		111.4
		2″, 6″-CH*	7.88(d,7.8)	132.9
		3″, 5″-CH	6.82(d,7.8)	115.8
		4″-C		163.6
14	Sucrose	1-CH*	5.39(d,3.8)	93.6
		2-CH	3.43(m)	73.3
		3-CH	3.71(m)	74.7
		4-CH	3.36(#)	71.3
		5-CH	3.82(#)	74.5
		6-CH_2	3.62(#)	64.1
		1′ CH_2	#	63.4
		2′-C		105.3
		3′-CH	4.10(d,8.5)	79.2
		4′-CH	4.04(#)	75.7
		5′-CH	4.02(#)	75.6
		6′-CH_2	3.78(#), 3.76(#)	63.9
U1	Unsaturated fatty acid	CH_3	0.90(t,7.8)	14.5
		$(CH_2)_n$	1.32(m)	23.7
		CH_2-*CH_2-CO*	1.61(b)	26.5
		CH_2-*CH=CH*	2.03(m), 2.06(m)	28.1

To be continued

Continued Table 1

NM	Metabolites	Groups	δ ^{1}H (J/Hz)	δ ^{13}C
U1	Unsaturated fatty acid	CH_2-*COOH*	2.32(m)	35.0
		CH_2-*OCOR*	4.15(m), 4.36(m)	63.4
		CH-*OCOR*	5.35(m)	#
U2	Unsaturated fatty acid	COOH		174.5
		CH_3	0.97(t,7.8)	14.2
		CH_2	1.53(ddd,7.8, 7.8, 7.8)	23.3
		CH_2	2.36(q,7.8)	29.2
		CH=CH	5.43(t,7.8)	114.7
		COOH		150.0

* The protons used for NMR quantitation. s: singlet; d: doublet; t: triplet; q: quartet; dd: doublet of doublets; ddd: doublet of doublets of doublets; m: multiplet; b: broad peak; U1 and U2: unidentified signal; #: signals or multiplicities not determined.

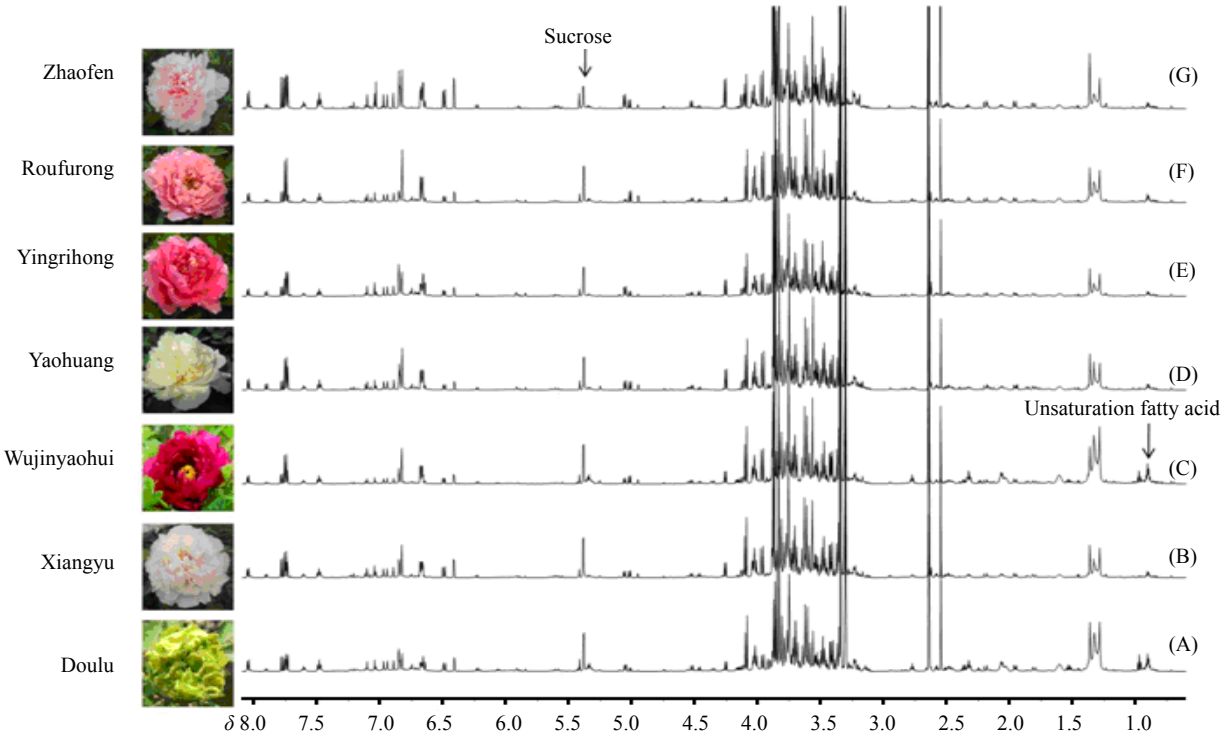


Figure 2 Metabolomic profiling of different tree peony cultivars including "Doulǔ"(A), "Xiangyu"(B), "Wujinyaohui"(C), "Yaohuang"(D), "Yingrihong"(E), "Roufurong"(F), and "Zhaofen"(G)

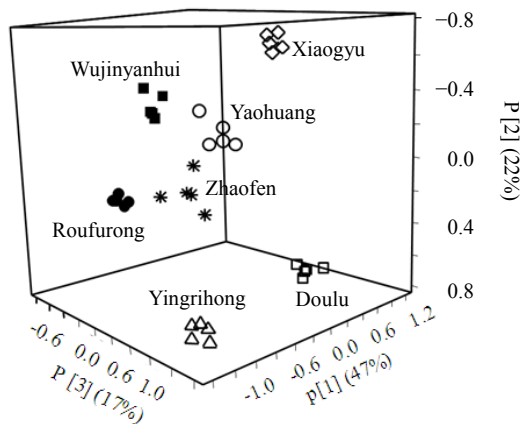


Figure 3 3D PCA score plots of root bark extracts from seven tree peony cultivars

180 mg/g; Acetophenones (paeonol, apiopaeonoside, paeonolide, and paeonoside), and 1,2,3,4,6-pentagalloyl glucose are present at intermediate levels ranging from 15 to 100 mg/g; and the phenolics (benzoic acid, 4-hydroxy-benzoic acid, gallic acid, methyl gallate, and glucogallin), flavonoids (catechin), and monoterpenes (paeoniflorin and oxypaeoniflorin) exist at very low or trace levels (2−15 mg/g). These results indicate that acetophenones are the major secondary metabolites in the methanol extract of tree peony, which is consistent with the results of previous studies (Do et al, 2010). However, flavonoids are minimal compounds in the root bark of tree peony in that abundant flavonoids occur in its petal (Wang and Cheng, 2005; Li and Du, 2009). Additionally, the relatively low contents of phenolics and monoterpenes might be attributable to their poor solubility in methanol solvent.

The statistical significance of these differences in

metabolites among tree peony cultivars was analyzed by one-way ANOVA analysis (Table 2). The concentration of paeonoside was found to be significantly changed within seven phenotypic cultivars. The increasing levels of paeonoside were observed in "Xiangyu", "Wujinyaohui", "Roufurong", "Yaohuang", "Zhaofen", "Doulǔ", and "Yingrihong" in order. It was reported that acetophenones such as paeonoside and paeonol were the bioactive members of *Paeonia* L. species and are generally the key metabolites for quality assessment when tree peony is used as a medicinal plant (Do et al, 2010; Hu et al, 2010). Based on this, it can be inferred that "Yingrihong" has the highest medicine quality whereas "Xiangyu" has the worst one.

For clarity, changes in the content of metabolites between different cultivars were described in Figure 4. Compared with other cultivars, extract obtained from "Xiangyu" showed significantly lower concentration of paeonoside and higher concentration of paeonol, paeoniflorin, and sucrose. Meanwhile, the samples collected from "Yingrihong" had lower level of paeonol and higher quantity of paeonoside. Paeonol and glucose/sucrose are known to be involved in the biosynthesis of acetophenone glycosides, such as paeonoside and paeonolide. The opposite trends for the levels of paeonol and paeonoside in "Xiangyu" and "Yingrihong" suggested that two different plant physiology processes exist in the two cultivars. In contrast, the samples collected from "Zhaofen" had lower amounts of sucrose and higher quantities of paeonolide, apiopaeonoside and oxypaeoniflorin than those harvested from other cultivars. The decreased sucrose and increased paeonolide and apiopaeonoside in "Zhaofen" suggested that the biosynthesis of acetophenone glycosides was activated in this cultivar. Opposite trends of paeonolide and apiopaeonoside were observed in the samples from "Doulǔ" and "Roufurong", suggesting that different physiological mechanisms were active in these two cultivars. These results demonstrated that the variations in secondary metabolites were influenced by primary metabolites, such as sucrose/glucose, and that different physiological processes occur in different tree peony cultivars.

Table 2 Metabolite contents in root bark of seven tree peony cultivars ($\bar{x} \pm s$, $n = 5$)

Metabolites	Doulǔ	Xiangyu	Wujinyaohui	Yaohuang	Yingrihong	Roufurong	Zhaofen
methyl gallate	1.64 ± 0.05	2.28 ± 0.21	1.15 ± 0.11	1.96 ± 0.24	1.52 ± 0.21	2.39 ± 0.26	1.41 ± 0.10
4-hydroxybenzoic acid	2.35 ± 0.11	3.06 ± 0.25	1.57 ± 0.15	4.93 ± 0.66	4.01 ± 0.28	2.50 ± 0.28	3.62 ± 0.14
gallic acid	3.34 ± 0.09	3.73 ± 0.68	1.93 ± 0.27$^{\#}$	2.56 ± 0.43	2.05 ± 0.53	2.29 ± 0.17	4.74 ± 0.19
glucogallin	2.23 ± 0.20	3.31 ± 0.27	2.07 ± 0.20	3.48 ± 0.50	2.28 ± 0.20	3.39 ± 0.45	2.58 ± 0.09
benzoic acid	12.27 ± 0.72	11.88 ± 0.98	9.39 ± 0.91	11.59 ± 1.54	8.26 ± 0.60	9.80 ± 1.03	13.12 ± 0.59
catechin	7.39 ± 0.26	5.15 ± 0.47	2.7 ± 0.22$^{\#}$	7.84 ± 0.84	10.17 ± 0.39§	6.73 ± 0.54	3.73 ± 0.13
oxypaeoniflorin	2.88 ± 0.10	5.58 ± 0.47	4.28 ± 0.38	6.53 ± 0.86	5.81 ± 0.36	5.70 ± 0.60	8.44 ± 0.66*
paeoniflorin	8.81 ± 0.84	14.36 ± 1.07$^{\&}$	9.20 ± 0.82	8.92 ± 1.28	8.65 ± 0.63	9.14 ± 0.97	11.13 ± 0.47
pentagalloyl glucose	30.73 ± 1.23	31.29 ± 3.61	17.91 ± 1.71$^{\#}$	29.33 ± 3.03	29.18 ± 2.03	28.30 ± 2.47	31.02 ± 1.19
paeonol	27.64 ± 3.94	35.40 ± 2.21$^{\&}$	18.33 ± 1.98	17.98 ± 2.05	13.60 ± 1.16§	18.72 ± 1.94	28.63 ± 1.67
apiopaeonoside	20.72 ± 0.81^{+}	46.76 ± 3.77	46.48 ± 4.07	47.43 ± 5.32	28.39 ± 1.61	60.71 ± 5.11	61.44 ± 2.39*
paeonoside	57.95 ± 2.14^{+}	15.62 ± 1.85$^{\&}$	24.66 ± 2.47$^{\#}$	36.24 ± 4.99$^{\Psi}$	80.72 ± 5.52§	30.03 ± 3.53^	40.03 ± 1.26*
paeonolide	63.24 ± 3.24	107.25 ± 9.48	72.75 ± 7.50	110.36 ± 17.44	88.71 ± 9.01	38.08 ± 5.94^	112.16 ± 10.43*
sucrose	152.26 ± 9.11	172.92 ± 15.63$^{\&}$	163.00 ± 17.33	129.97 ± 16.44	125.31 ± 10.65	138.52 ± 16.26	94.57 ± 4.72*

$P < 0.05$ $^{+}$ in "Doulǔ", $^{\&}$ in "Xiangyu", $^{\#}$ in "Wujinyaohui", $^{\Psi}$ in "Yaohuang", § in "Yingrihong", $^{\wedge}$ in "Roufurong", and * in "Zhaofen" compared with other cultivars

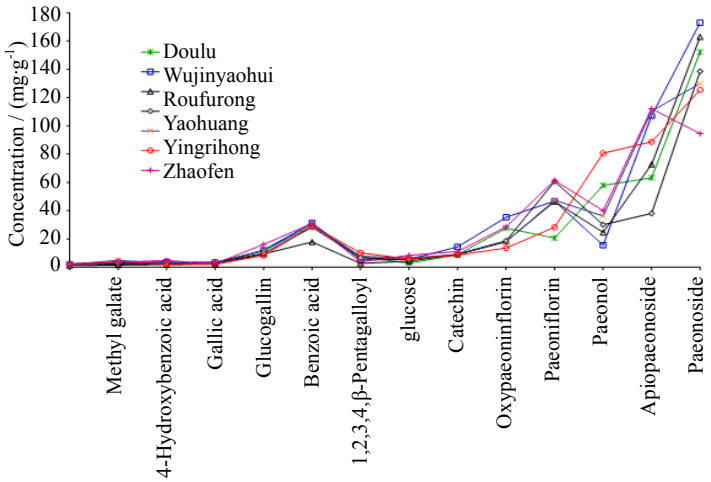


Figure 4 Concentration variation of metabolites in root bark extracts for seven tree peony cultivars ($n = 5$)

4. Conclusion

A total of 16 metabolites from root barks of seven tree peony cultivars were simultaneously identified and quantified based on NMR. The quantitative data revealed that sucrose and acetophenones were the major metabolites, rather than phenolics, monoterpene glycosides, flavonoids, and unsaturated fatty acids among tree peony cultivars. The significantly increasing levels of paeonoside with bioactivity were observed in "Xiangyu", "Wujinyaohui", "Roufurong", "Yaohuang", "Zhaofen", "Doulǘ", and "Yingrihong" in order. It can be inferred that "Yingrihong" with red flower has the highest medicine quality whereas "Xiangyu" with white flower has the lowest one. Opposite trends for the levels of paeonol and paeonoside were observed in both "Xiangyu"and "Yingrihong", suggesting that the changes in secondary metabolites in plants were influenced by primary metabolites, such as sucrose/glucose, and the different physiological processes occurred in different tree peony cultivars.

Conflict of interest statement

The authors declare no conflict of interest.

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