Pharmacogn. Mag.
A multifaceted peer reviewed journal in the field of Pharmacognosy and Natural Products
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ORIGINAL ARTICLE

Qualitative Analysis and the Profiling of Isoflavonoids in Various Tissues of *Pueraria Lobata* Roots by Ultra Performance Liquid Chromatography Quadrupole/Time-of-Flight-Mass Spectrometry and High Performance Liquid Chromatography Separation and Ultraviolet-Visible Detection

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Submitted: 13-04-2017 Revised: 19-05-2017 Published: 14-08-2018

ABSTRACT

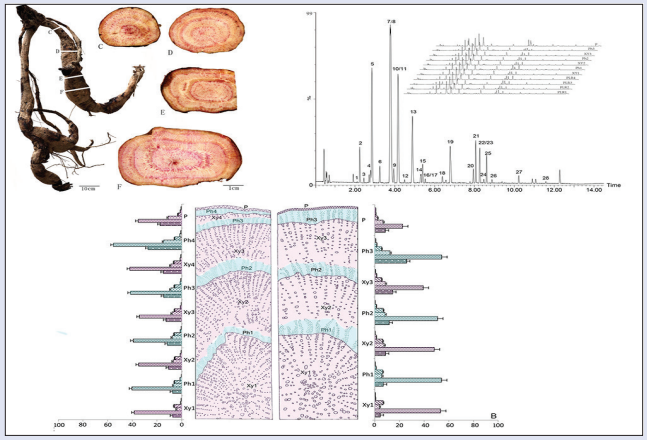
Background: Puerariae Lobatae Radix is widely used in the pharmaceutical, food, and cosmetic industries. For these applications, when grading roots according to diameter, thick roots are always considered to be of better quality. **Objective:** The objective of this study is to qualitative and quantitative analysis of isoflavonoids profiling in various tissues of *P. lobata* roots. **Materials and Methods:** Ultraperformance liquid chromatography quadrupole/time-of-flight-mass spectrometry (UPLC-QTOF/MS) and high-performance liquid chromatography separation and ultraviolet-visible detection were used to identify and profile the isoflavonoids detected in various tissues of *P. lobata* roots. **Results:** Consequently, 82 peaks were detected by UPLC-QTOF/MS, and 28 isoflavonoid compounds were identified in the various tissue samples. According to the results of this study, the thick roots of *P. lobata* are of better quality for medical use than the thin ones. The fine roots and the periderm of roots contained slightly lower isoflavonoid abundances. The results indicated that isoflavonoids, particularly puerarin, mainly accumulated in the xylem and phloem. **Conclusions:** This study provides a new and practical method for evaluating the quality of *P. lobatae* Radix.

Key words: Isoflavonoids, *Pueraria lobata*, root, various tissues, vascular bundles

SUMMARY

- Eighty-two peaks were detected by ultra-performance liquid chromatography quadrupole/time-of-flight-mass spectrometry, and 28 isoflavonoid compounds were identified in various tissue samples
- The fine roots and the periderm of roots contained slightly lower isoflavonoid abundances
- The isoflavonoids, in particular, puerarin, mainly accumulated in the xylem and phloem.

Abbreviations used: ANOVA: One-way analysis of variance; CNKI: Chinese National Knowledge Infrastructure; HPLC-UV: High performance liquid chromatography separation and ultraviolet-visible detection; PPRC: Pharmacopoeia of the People's Republic of China; RSD: Relative standard deviation; UPLC-QTOF/MS: Ultraperformance liquid chromatography quadrupole/time-of-flight-mass spectrometry.



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DOI: 10.4103/pm.pm_139_17

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INTRODUCTION

Puerariae Lobatae Radix, also known as *Gegen* in China, has been a commonly used Traditional Chinese Medicine for about 2000 years. It was first recorded in Shen Nong Ben Cao Jing. *Gegen* is the root of *Pueraria lobata* (Willd.) Ohwi, according to Pharmacopoeia of the People's Republic of China (PPRC).[1] It has very high medicinal value in applications such as muscle pain relief (by virtue of its antifebrile properties), venting measles and rashes, and preventing spleen-deficiency

Cite this article as: Duan H, Cheng M, Yang J, Lai C, Zha L, Hu Y, *et al*. Qualitative analysis and the profiling of isoflavonoids in various tissues of *Pueraria Lobata* roots by ultra performance liquid chromatography quadrupole/time-of-flight-mass spectrometry and high performance liquid chromatography separation and ultraviolet-visible detection. Phcog Mag 2018;14:418-24.

syndrome.[1] Modern pharmacological studies have showed that P. Lobatae Radix has anti-inflammatory, antioxidant, antineoplastic, antiarrhythmic, and central inhibition of alcohol activities.[2-4] P. Lobatae Radix is characterized by its puerarin abundance, and it is commonly used in China, Korea, and Japan for the treatment of fever, diarrhoea, diabetes and cardiovascular diseases.[5] In addition to its medicinal uses, *P. lobata* is also used as a food plant, and its starch content has been incorporated into health care products, food, beverage, etc.[6] Thus, *P. lobatae* radix has a huge demand in the domestic and international market, and its supply is attracting increasing attention. The quality of *P. lobatae* radix is also increasingly of interest since it is not only the prerequisite for its medical and nutraceutical effects but also an important determinant of its price.

Isoflavonoids such as puerarin, daidzin, and daidzein are the main bioactive components in *P. lobatae* radix.[2] Puerarin is used as the index component for *P. lobatae* radix quality control according to the PPRC.[1] Apart from the morphological characteristics, researchers have also used chemical methods of analysis such as high-performance liquid chromatography (HPLC) and high-performance thin-layer chromatography, which is recommended for medicinal herb authentication.[7-10] In most cases, puerarin was selected as the index component in these studies.

Based on a previous study of the developmental anatomy of *P. lobata* root, it has been established that the root is formed by internal development of abnormal vascular tissue.[11] In general, the transverse sections of *P. lobata* roots have 4–6 concentric circular permutations of abnormal vascular bundles [Figure 1]. The medicinal components of plants are often distributed in particular tissues.[12] The previous studies of medical herbs such as *huangqi*,[13,14] *shaoyao*,[15,16] *danshen*,[17] and *fuzi*[18] have demonstrated that the macroscopic characteristics are linked to the structures and chemical compositions of inner tissues and have inferred the relationship between anomalous structures and the distribution of effective components.[19,20] Most publications have focused on analytical methodology studies and genuine authentication. There is limited information about the complete quality assessment of *P. lobatae* radix and very few research studies about the profiling of isoflavonoids in *P. lobata* root.[21]

In recent years, the HPLC-quadrupole/time-of-flight-mass spectrometry (UPLC-Q/TOF-MS) technique, with high-detection sensitivity and short analysis time, has been widely applied to analyze trace chemical composition since it can provide accurate molecular weights and structural information for metabolite identification, and provide both qualitative and quantitative data.[22-26] Recently, researchers have successfully applied morphological characterization of internal structure to analyze bioactive components in the tissues of medical herbs, such as *shaoyao*,[15] *huangqi*[27] and *fuzi*.[18]

Thus, the objective of this paper is to determine isoflavonoid composition in various tissues of *P. lobata* root, to reveal the distribution patterns of isoflavonoids in tissues. The relationship between morphological characteristics and chemical component distribution in various tissues is also discussed. The results present a basis for the rational use and quality evaluation of the root of *P. lobata,* especially suitable for industrial use.

MATERIALS AND METHODS

Plant materials

P. lobata is a widespread wild species, and Dabie Mountain is the main producing area in China. Seven fresh root systems of *P. lobata* were collected from Yangtou village in Tongcheng (N31°06'54.76" E116°50'54.91" Anhui, China) on October 31, 2014. All materials were authenticated as the root of *P. lobata* (Willd.) Ohwi by the authors and a voucher specimen of each batch was deposited in the Department of Traditional Chinese Medicine Resources in Anhui University of Chinese Medicine. Collected materials were stored in dry conditions, the ecological environment of the root system of *P. lobata* is shown in Figure 2A.

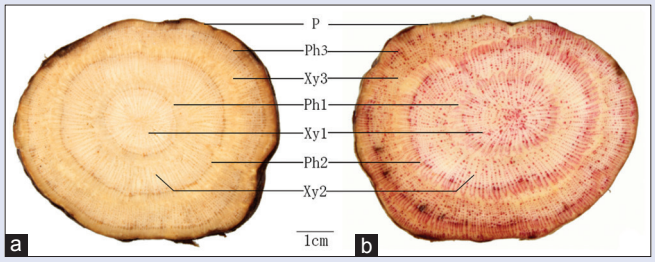

Figure 1: Macroscopic transverse sections characterization of *Pueraria lobata* root. (a) Root tuber cross-section without phloroglucinol–HCl staining; (b) root transverse sections with phloroglucinol–HCl staining; Xy1: Xylem from the first ring of vascular bundle; Ph1: Phloem from the first ring of vascular bundle; Xy2: Xylem from the second ring of vascular bundle; Ph2: Phloem from the second ring of vascular bundle; Xy3: Xylem from the third ring of vascular bundle; Ph3: Phloem from the third ring of vascular bundle

Sample preparation

Based on a previous anatomy study,[11] the fresh roots of *P. lobata* were classified into four parts according to their diameter, namely, labeled PLR1-4, corresponding to roots with short- and long-axial lengths of 4.60 mm × 5.47 mm, 5.52 mm × 7.32 mm, 5.07 mm × 8.97 mm, and 7.15 mm × 11.34 mm, respectively [Figure 2]. These roots were then cut into segments around 0.5 cm long. These segments were divided into several parts ordinarily depending on their organizational structure [Figure 1].

To provide an example, PLR1 has 4 concentric circular permutations of vascular bundles, and this sample was divided into 9 parts (Xy1, Ph1, Xy2, Ph2, Xy3, Ph3, Xy4, Ph4, and P, from inside to outside) depending on the organisational structure. Samples PLR2, PLR3, and PLR4 all have 3 concentric circular permutations of vascular bundles and these samples were divided into 7 parts, labeled Xy1, Ph1, Xy2, Ph2, Xy3, Ph3, and P, from inside to outside. Thus, the final number of various tissue samples is 30.

Chemicals and reagents

Reference substances of 3'-hydroxypuerarin (1), puerarin (2), 3'-methoxypuerarin (3), daidzin (4), genistin (5), and daidzein (6) were purchased from Wuhan Chem Faces Biochemical Co., Ltd., China, with respective batch numbers of CFN90236, CFN99169, CFN90780, CFN99101, CFN90250, and CFN98774. The purity of each reference substance was above 98%. The methanol was HPLC grade, purchased from TEDIA (TEDIA Company, Inc. USA), and the ethanol was analytical grade purchased from Tianjin Fuyu Company (Tianjin, China). Water was prepared by a Mill-Q purification system (Millipore, Bedford, MA, USA). All other chemicals were analytical grade.

Sample extraction

All 30 various tissue samples were dried at 40°C and then ground into a fine powder before passing through a No. 3 sieve (355 ± 13 μm). The collected powders were stored in dry conditions until future use.

The sample extraction procedure was improved by minor modification according to the PPRC.[1] For isoflavonoid analysis of each sample, 0.1 g of the fine powder was suspended in 25 mL of 30% (v/v) ethanol in pure water for 40 min. The extract solution was then heated under reflux in a

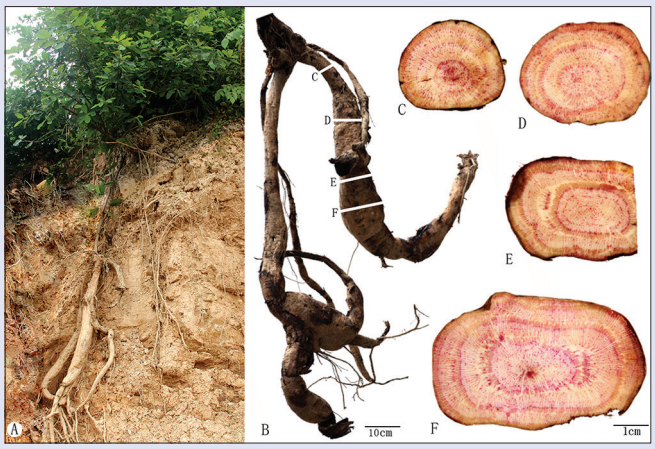

Figure 2: The root system and different diameter transverse sections of *Pueraria lobata* samples. A, B: The root system of *Pueraria lobata*; C: PLR1; D: PLR2; E: PLR3; F: PLR4. C, D, E, F represents the transverse sections of roots with phloroglucinol–HCl staining

water bath for 30 min, chilled to room temperature of 25°C and weighed. The sample weight loss was corrected with 30% (v/v) ethanol in pure water and filtered before HPLC injection.

Ultra Performance liquid chromatography quadrupole/time-of-flight-mass spectrometry analysis

The UPLC separation was performed using a Waters Acquity UPLC-1-Class system (Waters Corp., Milford, MA), with an acquity HSS T3 column (100 mm × 2.1 mm, 1.8 μm, Waters, U. S. A.), at a temperature of 40°C. The mobile phase was a mixture of acetonitrile (A) and water (B), both containing 0.1% formic acid, with an optimized linear gradient elution as follows: 0–0.5 min, 5% A; 0.5–1.0 min, 5%–10% A; 1.0–3.0 min, 10%–13% A; 3.0–7.0 min, 13%–23% A; 7.0–7.2 min, 23%–30% A; 7.2–12 min, 30%–55% A; 12–14.4 min, 55%–80% A; 14.4–14.5 min, 80%–98% A; 14.5–17.0 min, 98% A; and 17.0–17.1 min, 98%–95% A. The flow rate was set at 0.5 mL/min. The sample injection volume was 1 μL. The temperature of the auto-sampler was set at room temperature of 25°C.

The TOF/MS experiments were performed using a Xevo G2-S Q-TOF/MS system (Waters Corp., Milford, MA). Qualitative analysis was performed in positive electrospray ionization (ESI) mode. High- and low-energy MS scans were acquired simultaneously. The source temperature and desolvation gas (N_2) temperature were 100 and 450°C, respectively, and the desolvation gas flow rate was 900 L/h. The capillary voltage was 0.5 and 2.0 kV for the ESI (+) and ESI (−) experiments, respectively. The cone voltage was 40 V. The collision energy was set for the low-energy scans, and 20–45 V and 40–75 V ramps were employed for high-energy ESI (−) and ESI (+) scans, respectively. The coeluents and some minor compounds were reanalyzed in MS/MS mode to collect high-quality MS/MS data. In positive-mode experiments, the mass spectra were acquired by scanning over a mass-to-charge ratio (m/z) from 50 to 1500. The scan time was 0.1 s.

Data analysis

The data acquisition and analysis were performed with Mass Lynx 4.1 software (Waters Corp., Milford. MA, USA). By searching databases including PubMed of the U. S. National Library of Medicine and the National Institutes of Health, SCI Finder Scholar of the American Chemical Society, and Chinese National Knowledge Infrastructure of Tsinghua University, all the chemicals reported in the literature as derived from *P. lobata* roots were summarized in a Microsoft Office Excel table to establish a database, which includes the name, molecular formula, and molecular weight of each chemical.

High-performance liquid chromatography separation and Ultraviolet-visible detection analysis

The HPLC method was performed on a Waters 1525 automatic HPLC system (Waters Corporation) equipped with a solvent-delivery system and a 2489 UV Visible Detector. A Zorbax Eclipse XDB HPLC C_{18} analytical column (250 mm × 4.6 mm, 5 μm, Agilent, USA) was used for separation at 30°C. The mobile phase consisted of methanol (solvent A) and water (solvent B) with the following gradient elution: 0–12 min, 23% A; 12–28 min, 23%–40% A; 28–42 min, 40%–65% A; 42–45 min, 65%–23% A. The injection volume was 10 μL and the flow rate was set at 0.8 mL/min. The UV detection wavelength was 250 nm.

Statistical analysis was performed using SPSS 19.0 (IBM Corporation, Armonk, NY, USA). The differences among samples of xylem, phloem, and periderm were evaluated by one-way analysis of variance, and the results were considered statistically significant when the $P < 0.05$. The isoflavonoid abundances were analyzed using factor analysis with principal component extraction. A regression method was used to calculate the factor scores.

Method validation

Calibration curves and intraday precision were assessed under the optimized conditions. The investigated analytes were dissolved in 50% (v/v) methanol in pure water, and a series of diluted stock solutions were prepared accordingly for validation. Calibration curves were obtained by linear regression of the peak areas of the six reference standards versus concentration. The contents of the six reference substances in each of the 30 samples from various tissues were then calculated from the corresponding calibration curves.

The calibration curves for the six reference substances were prepared for the present study. A triplicate injection was carried out from each standard solution concentration. The linearity of each calibration curve was confirmed by plotting the peak area versus the concentration. The correlation coefficients, slopes, and intercepts obtained from the linearity studies are shown in Table 1.

The intraday precision was evaluated by analyzing a standard solution mixture of the six markers under the optimized conditions 6 times a day. As shown in Table 1, the intraday relative standard deviations (RSDs) are in the range of 0.20%–0.43%.

For sample preparation and analysis, the whole experiment was repeated twice, and the average values were determined from these repeats.

Isoflavonoid stabilities were determined in the methanol extracts of the sample solutions. All analytes were basically stable over 24 h, with RSDs of <4.76% achieved between the measurements at the start and end of the stability test.

RESULTS AND DISCUSSION

Extraction and elution optimization

Guided by the PPRC, the choice of extraction solvent was optimized on the basis of its ability to extract the maximum amount of isoflavonoids from the root tissue samples of *P. lobata*. Pure methanol and ethanol, the most commonly used solvents, as well as their various concentrations in pure water were chosen for optimization, and 70% (v/v) ethanol in pure water was finally chosen since its extract exhibited the greatest number of high-resolution chromatographic peaks.

The extraction times and extraction method were also optimized according to the maximum extraction efficiency of all isoflavonoids in the samples. Extract solution reflux in 70% (v/v) ethanol in pure water in a water bath for 30 min was chosen as the optimum procedure, which, in the present study, was considered to be acceptable for all the samples including those with different root diameters and tissue types.

To form better peak shapes and avoid tailing, 0.1% formic acid was added to both components (acetonitrile and water) of the mobile phase for UPLC-Q-TOF/MS analysis. The elution gradient was also optimized to maximize the overall resolution across all the peaks. High $[M+H]^+$ and $[M-H]^-$ ion responses for the analytes were detected in the sample solutions.

Identification of isoflavonoids in samples with different root diameters and various tissue types

The extract solutions were obtained from *P. lobata* samples with different root diameters and various tissue types, and analyzed by UPLC-QTOF/MS. A total of 82 common peaks were detected in chromatograms of all the samples. Isoflavonoid peaks, as the major type of secondary metabolites in *P. lobata* roots, can be easily recognized by their generated $[M + H]^+$ and/or $[M-H]^-$ molecular ions. Finally, 28 peaks were identified to be isoflavonoids, 6 of which were confirmed by comparing their mass, ion abundance and retention time with the reference substances (3'-hydroxypuerarin, puerarin, 3'-methoxypuerarin, daidzin, genistein, and daidzein). The others were tentatively identified by matching the empirical molecular formula with those of known reported isoflavonoids, and further confirmed by elucidating the collision-induced dissociation fragmentations.[28-40] The detailed results are shown in [Table 2]. Moreover, the chromatographic behaviors of isoflavonoids reported in the literature provided complementary information to confirm isomer identities.

The chromatograms of whole-sectioned tissues from the four samples with different root diameters were quite similar. A total of 82 peaks were detected in samples, regardless of root diameter, of which 28 peaks were identified as isoflavonoids [Figure 3].

Chemical profile analysis of samples with various tissue types was carried out by comparing them with the respective chemical profiles from the whole-sectioned tissues. The chromatograms of the various tissue samples were quite similar. A total of 82 peaks were detected in various tissue samples, of which 28 peaks were identified as isoflavonoids. The previous studies have found that different herbal tissues have distinct chemical profiles.[19,41] Therefore, according to their tissue structure, the qualitative

Table 1: Linearity studies of six reference substances (n=6)

Reference substance	Calibration curve	R^2	Range (μg)	Precision (%)
3'-hydroxypuerarin	Y=5×10⁶X+313,736	0.9998	0.1-1.9	0.20
Puerarin	Y=5×10⁶X−995,216	0.9996	0.3256-6.1864	0.25
3'-methoxypuerarin	Y=5×10⁶X−254,951	0.9996	0.0784-1.4896	0.27
Daidzin	Y=6×10⁶X−235,264	0.9997	0.0612-1.1628	0.28
Genistin	Y=6×10⁶X−28,099	0.9998	0.00792-0.15084	0.29
Daidzein	Y=8×10⁶X−8836.6	0.9995	0.0025-0.0475	0.43

Y: Peak area; X: Content

Table 2: Identified secondary metabolites of various tissue samples from *Pueraria lobata* root by UPLC-Q-TOF/MS

Peak number	Retention time (min)	$[M+H]^+$(m/z) Mass error (ppm)	Molecular formula	Identification
1	2.11	595.1665 (0.22)	$C_{27}H_{30}O_{15}$	3'-hydroxyl-4'-O-glucosyl purearin
2	2.25	579.1708 (0.09)	$C_{27}H_{30}O_{14}$	Puerarin-4'-O-glucoside
3	2.45	609.1813 (0.11)	$C_{28}H_{32}O_{15}$	Kakkalide
4	2.72	579.1710 (0.36)	$C_{27}H_{30}O_{14}$	Daidzein-4',7-di-O-glucoside
5	2.86	433.1130[a] (0.13)	$C_{21}H_{20}O_{10}$	3'-hydroxy puerarin
6	3.26	565.1553 (1.60)	$C_{26}H_{28}O_{14}$	Genistein 8-C-apiosyl (1→6) glucoside
7	3.73	433.1124 (1.14)	$C_{21}H_{20}O_{10}$	Daidzein 4'-O-glucoside
8	3.78	417.1182[a] (0.52)	$C_{21}H_{20}O_{9}$	Puerarin
9	3.95	549.1602 (0.20)	$C_{26}H_{28}O_{13}$	Puerarin xyloside
10	4.17	447.1287[a] (0.23)	$C_{22}H_{22}O_{10}$	3'-methoxy puerarin
11	4.19	549.1604 (0.28)	$C_{26}H_{28}O_{13}$	6"-O-xylosylpuerarin
12	4.50	579.1707 (0.14)	$C_{27}H_{30}O_{14}$	3'-Methoxy-6"-O-xylosy-puerarin
13	4.89	417.1178[a] (0.10)	$C_{21}H_{20}O_{9}$	Daidzin
14	5.32	447.1283 (0.57)	$C_{22}H_{22}O_{10}$	3'-methoxy daidzin
15	5.40	565.1552 (0.09)	$C_{26}H_{28}O_{14}$	Genistein-8-C-apiosyl (1→6) glucoside
16	5.53	503.1183 (0.14)	$C_{24}H_{22}O_{12}$	6"-O-malonyl daidzin
17	5.56	607.2027 (0.40)	$C_{29}H_{34}O_{14}$	Pueroside A
18	6.39	433.1127[a] (0.59)	$C_{21}H_{20}O_{10}$	Genistein
19	6.78	563.1758 (0.16)	$C_{27}H_{30}O_{13}$	Formononetin-8-C-apiosyl (1→6) glucoside
20	7.95	475.1592 (1.37)	$C_{24}H_{26}O_{10}$	Sophoraside A
21	8.05	579.1716 (0.10)	$C_{27}H_{30}O_{14}$	6"-O-xylosyl-tectoridin
22	8.27	431.1333 (0.93)	$C_{22}H_{22}O_{9}$	Ononin
23	8.27	255.0651[a] (0.21)	$C_{15}H_{10}O_{4}$	Daidzein
24	8.47	285.0755 (0.84)	$C_{16}H_{12}O_{5}$	Glycitein
25	8.62	517.1332 (0.58)	$C_{25}H_{24}O_{12}$	Formononetin 7-O-glucoside-6"-O-malonate
26	8.88	447.1281 (1.06)	$C_{22}H_{22}O_{10}$	5-hydroxyl ononin
27	10.23	269.0808 (0.17)	$C_{16}H_{12}O_{4}$	Formononetin
28	11.59	323.1275 (0.96)	$C_{20}H_{18}O_{4}$	8-preyl daidzein

[a]Which have compared with reference substance and used to determine the peak area when determining the component abundances

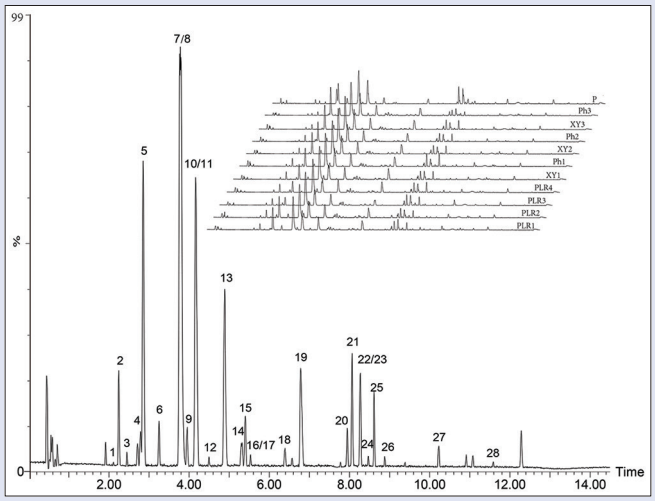

Figure 3: Representative total ion current chromatograms of various tissues of *Pueraria lobata* roots

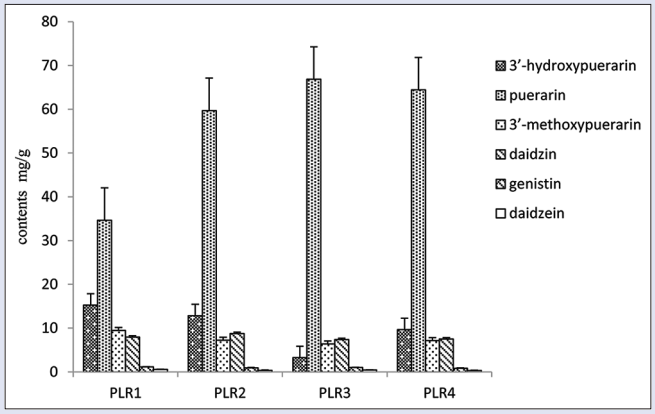

Figure 4: Determination of six isoflavonoids in *Pueraria lobata* roots with different diameters

analysis of various isoflavonoids in tissues of *P. lobata* root in this study provides further evidence on the accumulation of these compounds in periderm, phloem, and xylem. Further, transcript analysis of these tissues might reveal more detail about the isoflavonoid compositions.

Determination of isoflavonoids in samples with different root diameters

The compositions of six isoflavonoids in samples with different root diameters were determined by peak area and linear equation analysis [Figure 4]. In general, the abundance of one type of isoflavonoid, puerarin was much higher than the other five components. In fact, the puerarin abundance is twice as much as the sum of the other five isoflavonoid compounds in all the samples with different root diameters [Figure 4]. The daidzein abundances were the lowest [Figure 4]. Importantly, all the samples with different root diameters met the minimum puerarin content requirement described by the PPRC.

Morphological features and internal composition are key quality factors for Chinese medicinal materials. Since it is well known that the grade of some medicines is mainly based on the diameter, the analysis of the distribution of secondary metabolites in different anatomical structures can establish a relationship between secondary metabolite abundance and diameter. The previous studies have attempted to reveal the relationship between root diameter and secondary metabolite abundance, demonstrating that morphological features do correlate with secondary metabolite concentrations and that the former can, therefore, be used to evaluate quality.[14,15,34,42] In short, Chinese medicine can be evaluated by appearance. The present quantitative analysis study has revealed that the average isoflavonoid abundance does slightly increase with increasing diameter, especially for puerarin. This is consistent with the traditional differential experience, which assumes that thick roots have good quality.[43] These results provide a theoretical basis for the quality evaluation and commodity specification and grading of *P. lobatae* radix.

Determination of isoflavonoids in various tissues

The various tissues of *P. lobata* roots, which are related to periderm, xylem, and phloem were separated for quantitative analysis according to their tissue structures [Figure 1]. The abundance of each isoflavonoid in the various tissue types is plotted in Figure 5.

In the individual tissue types, as in the samples with different root diameters, there was more abundance of puerarin in all the tissues than any of the other five isoflavonoids. The average abundances of puerarin, daidzin, and genistin in the periderm were lower than in the phloem and xylem in all tissues, which were consistent with the qualitative analysis results.[21] However, when *gegen* is prepared from *P. lobata* roots, the periderm is peeled off.[43] In other words, the present study suggests that the process of peeling periderm off *P. lobata* roots reduces the isoflavonoid abundance for medicinal use. In particular, daidzein is mainly distributed in the periderm. The abundances of 3'-hydroxypuerarin and 3'-methoxypuerarin varied little across the periderm, phloem, and xylem. For individual tissues, no significance differences in isoflavonoid composition were found in periderm, xylem, and phloem ($P > 0.05$), or between xylem and phloem ($P > 0.05$). In contrast, a significant difference was found between periderm and xylem ($P < 0.05$), and between periderm and phloem ($P < 0.05$).

For vascular bundles, the isoflavonoid abundances in normal vascular bundles were similar to those in abnormal vascular bundles. In other words, there was no significant difference between the isoflavonoid abundances in normal and abnormal vascular bundles. The highest puerarin abundances were found in the outer abnormal vascular bundle (Ph3, Xy3 and Ph4, Xy4), close to the periderm.

A previous study has indicated that the secondary metabolites of medicinal plants are often distributed across certain organs, tissues, and cells.[12] In various research studies, the distribution of secondary metabolites of medical plants has indicated that there are certain relationships between the distribution of secondary metabolites and the internal structures of medicinal plants.[15-17,44-46] The root is formed by internal development of abnormal vascular bundles of *P. lobata*, and the xylem and phloem of abnormal vascular bundles form the main body of the root.[11] The results reveal that isoflavonoids are mainly distributed in xylem and phloem and found at their lowest levels in the periderm. Isoflavonoid compounds have different functions in various tissues. With an increase in the root diameter, the isoflavonoid abundances increase as well. Thus, isoflavonoid accumulation in *P. lobata* root is related to the internal structure.

CONCLUSIONS

In the present study, UPLC-QTOF/MS and HPLC-UV were established as convenient and effective methods to chemically profile in various tissues from different root diameters of *P. lobata*. The chromatograms of samples with different root diameters and various tissue types were quite similar. A total of 82 common peaks were detected in the chromatograms of all the samples and 28 peaks were identified to be isoflavonoids.

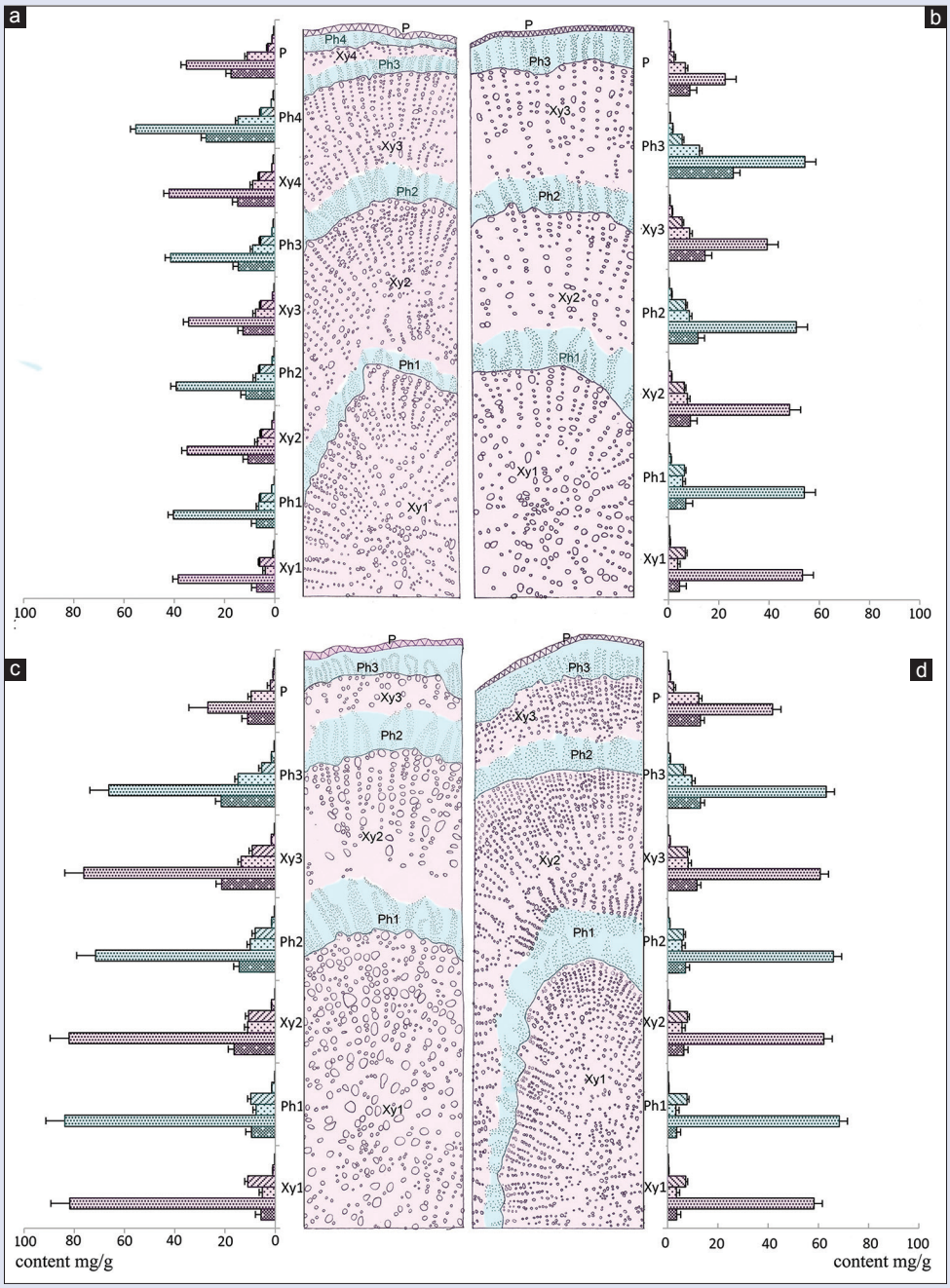

Figure 5: Determination of six isoflavonoids in various tissues separated from *Pueraria lobata.* Roots. (a) Transverse ink-drawing section and column diagram of PRL1; (b) Transverse ink-drawing section and column diagram of PRL2; (c) Transverse ink-drawing section and column diagram of PRL3; (d) Transverse ink-drawing section and column diagram of PRL4

The study indicated that all the samples contained the six reference substances, both in samples with different root diameters and various tissue types. The distribution of isoflavonoids in various tissues was consistent between samples, however, samples from various tissues from the same sample have different profiles. The study showed that isoflavonoids were mainly accumulated in normal and abnormal xylem and phloem. That is, periderm has the lowest isoflavonoid abundance. Therefore, there is some theoretical basis for the removal of the periderm of herbal material before it is processed into herbal medicine.[43] The isoflavonoid composition varied in samples of *P. lobata* root with different diameters. Thin root tuber has lower isoflavonoid abundance while thick root has higher isoflavonoid abundance. That is to say, the isoflavonoid abundance of *P. lobata* root with larger diameter is consistent with the traditional macroscopic descriptions, which are used for quality authentication in producing areas. In addition, the results suggest that the periderm and small-diameter *P. lobata* roots deserve further study into their potential clinical effectiveness.

Acknowledgement

The National Key Research and Development Plan 2017YFC17039501.

Financial support and sponsorship

This work was supported by the National Natural Science Foundation of China (NSFC:81573543, 81773853), the central project significant

increase or decrease at the corresponding level "Rare traditional Chinese medicine resources sustainable utilization of capacity building" (No. 2060302) and the Natural Science Foundation of the Colleges and Universities in Anhui province (KJ2017A296).

Conflicts of interest

There are no conflicts of interest.

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