Journal of Pharmaceutical and Biomedical Analysis 103 (2015) 7–16

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

Whole transverse section and specific-tissue analysis of secondary metabolites in seven different grades of root of *Paeonia lactiflora* using laser microdissection and liquid chromatography-quadrupole/time of flight-mass spectrometry

Qiuling Wang[a], Zhitao Liang[b], Yong Peng[a], Jun Ling Hou[c], Sheng Li Wei[c], Zhong Zhen Zhao[a,b,**], Wen Quan Wang[a,c,*]

[a] *Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences, Peking Union Medical College, Beijing 100193, People's Republic of China*
[b] *Chinese Academy of Medical Sciences, Peking Union Medical College, Hong Kong Baptist University, Kowloon Tong, Kowloon 999077, Hong Kong, China*
[c] *School of Chinese Pharmacy, Beijing University of Chinese Medicine, Beijing 100102, People's Republic of China*

ARTICLE INFO

Article history:
Received 5 August 2014
Received in revised form 17 October 2014
Accepted 19 October 2014
Available online 6 November 2014

Keywords:
Paeonia lactiflora Pall.
UPLC-Q/TOF–MS
Laser microdissection
Grade

ABSTRACT

The root of *Paeonia lactiflora* Pall. is widely used in the pharmaceutical, food and cosmetic industries. For these purposes, roots are graded according to diameter, with larger roots considered to be of better quality. To assess the inherent quality of different grades and of different tissues in roots of *P. lactiflora*, here laser microdissection coupled with UPLC-Q/TOF–MS was applied. The results show the quantity of pharmaceutically important components decreased with increase in root diameter from 0.3 cm to 0.7 cm. Above 0.7 cm of diameter, quantity and diversity of these components increased proportionally with increase in root diameter. The tissue-specific study indicated that the high content of paeoniflorin and albiflorin are mainly distributed in the cork and cortex. According to the results of this study, the roots of *P. lactiflora* greater than 1.7 cm in diameter are of better quality medicinal use than smaller, and the commercial grades chose was best cutoff points. The fine roots and the outer bank of roots, which besides the commercial grades, contain such significant amounts of chemical components too. This study provides a new and practical method for evaluating the different grades of *P. lactiflora*.

1. Introduction

Due to natural variation in agricultural products and uncontrollable factors in their production, these products are often divided into different grades according to morphological characteristics that are believed to correspond to gradations in quality, and then sold at different prices according to grade [1]. Additionally, in some agricultural commodities, different grades are used for different purposes. For example, fruit in good condition is used for direct consumption, while damaged or overly ripe fruit is processed [2]. The situation is similar for medicinal plant materials. For example, the thick roots of *Paeonia lactiflora*, which are considered to be of higher quality, are used for prescription medicinal materials, while the thin roots are used for industrial extraction. The question arises as to whether external morphology does in fact accurately reflect medicinal value in terms of chemistry. Studies of some agricultural products have shown correlation between external shape and internal quality, but the correlation is not consistent [3]. Morphologically, external forms are created by different tissues, and tissues are comprised of specific cells. The relative proportions of different tissues and their component cells may be different in different grades. If these differences are consistent, and if specific tissues have specific chemical profiles, then morphological form can be a reliable indicator of chemical and pharmaceutical quality. But very little research has been done on whether differences in tissues and cells correlate with differences in medicinal value, due both to limited technology and limited interest making such research worthwhile.

In recent years, the technique of ultra-performance liquid chromatography-quadrupole/time-of-flight-mass spectrometry (UPLC-Q/TOF–MS), with high detection sensitivity and short

* Corresponding author at: Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences, Peking Union Medical College, Beijing 100193, People's Republic of China. Tel.: +86 10 57833140; fax: +86 10 57833140.
** Corresponding author at: Chinese Academy of Medical Sciences, Peking Union Medical College, Hong Kong Baptist University, Kowloon Tong, Kowloon 999077, Hong Kong, China. Tel.: +852 34112424; fax: +852 34112461.
E-mail addresses: zzzhao@hkbu.edu.hk (Z.Z. Zhao), wqwang@implad.ac.cn (W.Q. Wang).

http://dx.doi.org/10.1016/j.jpba.2014.10.020
0731-7085/

analysis time, has been widely applied to analyze the trace chemical composition of herbal medicines. This technique can quantify chemical components and can provide accurate molecular weights and structural information by which metabolites can be identified, and provide both quantitative and qualitative data [4]. Additionally, laser microdissection (LMD) has been used for tissue and cell separation in life science research for many years. Recently, we have successfully applied the combination of LMD and UPLC-Q/TOF–MS to analyze bioactive components in the tissues of two medicinal herbs, Sinomenii Caulis and Radix et Rhizoma Rhei [5,6]. This is our first study applying this technique to investigate the inherent quality of different grades of an agricultural product.

For this first study we have chosen the root of *P. lactiflora* because it is widely used in pharmaceutical industries and because the different grades command great differences in price. The question of whether these grades are worth the money is significant. In China, as recorded in the Pharmacopoeia of the People's Republic of China, the root of *P. lactiflora* Pall. Radix Paeonia Alba and Radix Paeonia Rubra, known as '*Baishao*' or '*Chishao*' is used as an herbal medicine for cooling and tonifying the blood [7]. Modern pharmacological studies have demonstrated that its protective effects of the cardiovascular system and central nervous system derive primarily from its monoterpenoid glycosides, tannins and phenols, which are secondary metabolites [8,9]. In addition, its component paeoniflorin possesses a protective effect against optic nerve crush, while its tannins and phenols have antioxidant, antitumor and immune system modulation activities [10,11]. *P. lactiflora* is also widely used in the food and cosmetic industries [12]. Today, *P. lactiflora* is widely cultivated in Anhui, Zhejiang, and Sichuan Provinces of China. Before entering the market, it is graded according to length and diameter: grade 1 represents roots 8 cm long and more than 1.7 cm in diameter; grade 2, roots 6 cm long and more than 1.3 cm in diameter; grade 3, roots 4 cm long and more than 0.8 cm in diameter; and grade 4, regardless of length and thick. A number of researchers have attempted to evaluate the inherent quality of different grades of roots of *P. lactiflora*, but their reported results are inconsistent [13,14], and only focused on the comparison of monoterpenoid glycosides. No study analyzing the chemical profiles of different tissues and cells from various grades of roots of *P. lactiflora* has been carried out.

Thus, the objective of this paper is to determine the inherent quality of different grades of *P. lactiflora* by analyzing the chemical profiles of its different tissues and cells. The results represent the basis for the rational use and quality evaluation of roots of *P. lactiflora*, especially for industrial use.

2. Materials and methods

2.1. Chemicals

Standard compounds of paeoniflorin, oxypaeoniflorin, benzoylpaeoniflorin, methyl gallate, catechin, albiflorin, and 1,2,3,4,6-o-pentagalloylglucose were purchased from Shanghai Rong He Co., China, batch nos. 130524, 130603, 130619, 120629, 130609, 130513 and 130702, respectively. The purity of each chemical was above 98%. The solvents, acetonitrile and methanol, were of HPLC grade from E. Merck (Darmstadt, Germany) and formic acid with a purity of 96% was also of HPLC grade (Tedia, Fairfield, OH, USA). Water was obtained from a Mili-Q water purification system (Millipore, Bedford, MA, USA).

2.2. Plant materials

Three *P. lactiflora* plants (PLC-Bo-1, 2 and 3) were planted in Beijing University of Chinese Medicine resources nursery on October 12, 2009. Roots for this study were harvested from these plants in July 23, 2013. Specimens were examined and identified by Prof. Wen-Quan Wang of the Institute of Medicinal Plant Development.

2.3. Sample preparation

The fresh roots of PLC-Bo-1, 2 and 3 were cut into segments approximately 3 cm long. These segments were then divided into seven grades according to diameter, namely grades 1–7, corresponding to roots with diameters of approximately 2, 1.7, 1.2, 1, 0.7, 0.5, and 0.3 cm in diameter, respectively. We used three more grades than the commercial (four grades) in order to do a more detailed analysis of the relationship between quality and grade. Three samples were randomly selected from each grade, and each was cut into segments 1–2 cm long. The smaller material was embedded in tissue freezing medium (Leica Microsystems, Germany), and frozen in blocks. The blocks were then cut with a cryostat (Thermo Shandon As620 Cryotome, UK) at −20 °C in serial slices of 30 μm in thickness. Slices were mounted directly on PET slides with steel frames (76 mm × 26 mm, 1.4 μm in thickness, Leica Microsystems, Germany). After the freezing medium was defrosted and coagulated to make slices adhere firmly, the slides were mounted on a Leica LMD7000 system (Leica, Benshein, Germany). Laser dissection was accomplished using a DPSS laser beam at aperture of 6, speed of 3, and power of 50–60 μJ under a Leica LMD-BGR fluorescence filter system consisting of an excitation filter (blue light), dichromatic mirror (green light) and suppression filter (red light) with intensity of 17%, and auto exposure time mode. Observations were made at ×6.3, ×10, or ×20 magnifications, and microdissection was performed at ×20 magnification. Tissue parts within an area of around 1×10^6 μm^2 were determined as the investigated size and dissected separately according to their autofluorescence color, and collected in the caps of 0.5 mL microcentrifuge tube (Leica, Germany). The cutting areas were accurately recorded, and this measurement was used to calculate the component content per unit area. Samples were then centrifuged for 2 min, thereby transferring the tissues and cells to the bottoms of the tubes (Centrifuge 5415R, Eppendorf, Hamburg, Germany). For representing raw material, one whole transverse section, composed of all tissue parts obtained through cryostat sectioning, was put on a glass slide. The area of the whole transverse section was measured using a Leica LMD7000 system.

2.4. Sample extraction

The tissue parts sample was ultrasonic-extracted with 100 μL of 50% methanol for 30 min (Crest 1875HTAG Ultrasonic Processor, Crest Trenton, NJ), and centrifuged to obtain the supernatant centrifugation at 12,000 rpm for 10 min. The final supernatant was transferred to a glass flat bottom insert (400 μL, Grace, HK) in a brown HPLC vial (Grace, HK), and stored in a refrigerator at 4 °C for analysis. The whole sectioned tissue slice was scraped from a glass slide, transferred to a 1.5 mL microcentrifuge tube and then treated in the same way as above except that 1 mL 50% methanol was used for the extraction.

2.5. UHPLC–MS analysis

The ultra-performance liquid chromatography was performed on an Agilent 6540 accurate-mass Q-TOF LC/MS system (Agilent Technologies, USA). A UPLC C_{18} analytical column (2.1 mm × 100 mm, I.D. 1.8 μm, ACQUITY UPLC® BEH, Waters, USA) was used for separation, coupled with a C_{18} pre-column (2.1 mm × 5 mm, I.D. 1.7 μm, VanGuard™ BEH, Waters, USA) at room temperature of 20 °C. The mobile phase was a mixture of water (A) and acetonitrile (B), both containing 0.1% formic acid,