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Characterization of molecular signature of the roots of *Paeonia lactiflora* during growth

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[ABSTRACT] The roots of *Paeoniae lactiflora* Pall. are widely consumed as crude drugs in Asian countries due to their remarkable beneficial health effects. The present research was undertaken to illuminate the dynamic changes in metabolites and enzymes and facilitate selection of the harvesting time when the herb can provide optimum health benefits. *P. lactiflora* roots were analyzed at 12 stages of growth for monoterpenoid glycosides, phenols, nucleosides, nucleobases, amino acids, and polysaccharides by high-performance liquid chromatography with photodiode array detector, ultra-high pressure liquid chromatography coupled with tandem mass spectrometry, and UV spectrophotometry. The enzyme activities of plant *β*-glucosidases and esterases were determined by UV methods. The total content of monoterpenoid glycosides and phenols peaked in December. For nucleosides and nucleobases, the highest content appeared in April. The maximum phasic accumulation of the total amino acids took place in March, and the content of total polysaccharides reached a peak value in September. December, April, and March were selected as the appropriate harvesting times for producing natural medicinal or health food products. Plant *β*-glucosidases and esterases showed the highest activity in December and May, respectively. When the activity of *β*-glucosidase increased, esterase activity decreased, while the contents of oxypaeoniflora and paeoniflorin increased. When esterase activity increased, the contents of benzoylpaeoniflorin, paeoniflorin, and gallic acid decreased. In conclusion, the results from the present study would be useful in determination of the suitable time for harvesting *P. lactiflora* roots for medicinal purposes.

[KEY WORDS] *Paeonia lactiflora*; Primary and secondary metabolites; Plant enzymes; Dynamic change; Growth stage

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Introduction

Paeoniae lactiflora Pall. is an important ornamental and medicinal plant found in both China and Japan [1]. The roots of cultivated *P. lactiflora* from Anhui, Zhejiang, and Sichuan provinces in China are used as "Baishao" after they are processed by boiling and peeling [2]. It is one of the most popular sources of bioactive material in traditional Chinese medicine, with claims of antispasmodic, tonic, astringent, and analgesic properties [3].

Many studies have reported the chemical constituents in *P. lactiflora* roots, mainly including monoterpenoid glycosides and phenols [4-6]. As vegetative organs, some nutrients, such as nucleosides, amino acids and sugars, are also found [7]. In previous publications, the above metabolites are determined in *P. lactiflora* roots and their preparations, both *in vitro* and *in vivo* [8-14]. These components significantly contribute to the nutritional and functional values of *P. lactiflora*, as well as their medicinal and edible value. For example, paeoniflorin, one of the principal bioactive components isolated from *P. lactiflora* roots, accounts for > 90% of total paeony gluco-

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sides [15]. It exhibits various pharmacological activities in the cardiovascular system [16], as well as displaying sedative [17], anti-inflammatory [18], analgesic [19], osteoporosis preventative [20], and other pharmacologic activities. It can also be used as functional food or functional food ingredients [21].

Bioactive compounds can be both synthesized and degraded as the plant matures, and the induction of some enzymes is considered significant to plant metabolism during growth. *β*-Glucosidase selectively catalyzes the hydrolysis of *β*-glycosidic bonds between glycone residues or those between glucose and anaryl or alkyl aglycone, and plays key roles in a variety of essential physiological processes. Plant *β*-glucosidases are involved in regulation of the physiological activity of phytohormones through hydrolysis of their inactive hormone-glucoside conjugates [22] and provide plants with an immediate chemical defense against protruding herbivores and pathogens [23]. Plant esterase is a kind of esterase that exists in many medicinal plants and crops [24]. It plays key roles in many biological processes, including activation of signal molecules [25] and regulation of the bioactivity of endogenous products [26].

Zha *et al*. [15] have reported that paeoniflorin content in *P. lactiflora* roots is correlated with cultivar and slowly declined with increasing age. Except for paeoniflorin, no information is available regarding the dynamic changes of other compounds and plant enzymes during *P. lactiflora* growth. Investigation of compounds and plant enzymes would be helpful in evaluating the functional and nutritional values of *P. lactiflora* roots at different growth stages. Thus, there is a need to define the contents of *P. lactiflora* roots at different stages of maturity. Such research may facilitate the selection of the optimal maturity stage and maximize its benefits as a crude drug and functional food.

In the present study, the contents of monoterpenoid glycosides and phenols in *P. lactiflora* roots collected at different stages of growth were determined using high performance liquid chromatography coupled with photodiode array detector (HPLC-PAD). Amino acids, nucleosides, and nucleobases were determined using ultra-high performance liquid chromatography coupled with tandem mass spectrometry (UHPLC-MS/MS). The contents of polysaccharides and the enzyme activities of plant *β*-glucosidases and esterases were determined by ultraviolet (UV) spectrophotometry. Furthermore, the variations in tendencies of these compounds and enzyme activities were described from these samples.

Materials and Methods

Chemicals

Reference compounds of gallic acid (1), oxypaeoniflora (2), catechin (3), albiflorin (4), paeoniflorin (5), benzoic acid (6), benzoylpaeoniflorin (7), adenine (8), uridine (9), hypoxanthine (10), adenosine (11), inosine (12), and glucose were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Guanosine (13), phenylalanine (14), tryptophan (15), leucine (16), *γ*-aminobutyric acid (17), methionine (18), valine (19), proline (20), tyrosine (21), alanine (22), threonine (23), glutamic acid (24), glutamine (25), serine (26), and asparagine (27) were obtained from Sigma (St. Louis, MO, USA). The reagents of *p*-nitrophenyl *β*-D-glucopyranoside (pNPG), and *α*-naphthyl acetate were obtained from Sigma. The acetonitrile, methanol, and ammonium acetate were all of HPLC grade and purchased from Merck (Darmstadt, Germany). Deionized water was purified by an EPED purification system (Eped, Nanjing, China). Other reagent solutions were of analytical grade and obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

Sampling of P. lactiflora roots

A local cultivar of *P. lactiflora* called ‘bobaishao’ planted in Bozhou, China, was used in the present study. The crops were managed according to the integrated cultivation protocols, and the roots were harvested from October 12, 2012, to September 23, 2013, in 12 stages (BS1–BS12; Table 1). All the crops were planted during August 19–21, 2009, collected from the same field, and stored in a freezer at −80 °C until analysis. Their botanical origins were identified by the corresponding author, and voucher specimens (FORMULAE-ORT-2013-PL9) were deposited at the Herbarium in Nanjing University of Chinese Medicine, Nanjing, China.

Table 1 Sample list of *P. lactiflora* roots at various stages during growth

No.	Harvesting time
BS1	October 12, 2012
BS2	November 12, 2012
BS3	December 20, 2012
BS4	January 20, 2013
BS5	February 20, 2013
BS6	March 23, 2013
BS7	April 20, 2013
BS8	May 25, 2013
BS9	June 26, 2013
BS10	July 21, 2013
BS11	August 20, 2013
BS12	September 23, 2013

HPLC analysis of monoterpenoid glycosides and phenols

P. lactiflora roots at each growth stage were sliced in 0.3–0.5-cm thickness, and a total of 1 g of the slices were extracted with 50 mL of aqueous ethanol (1 : 1, *V*/*V*) solution in a cooled ultrasonic bath (50 kHz) for 30 min. The extract was filtered through a 0.45-μm nylon 66 membrane, and the subsequent filtrate was transferred to a vial.

The contents of paeoniflorin, catechin, gallic acid, benzoic acid, albiflorin, oxypaeoniflora, and benzoylpaeoniflorin in the samples were analyzed by the modified procedure of the reported liquid chromatography method in the present