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Chemical taxonomy of tree peony species from China based on root cortex metabolic fingerprinting

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ABSTRACT

The section *Moutan* of the genus *Paeonia* consists of eight species that are confined to a small area in China. A wide range of metabolites, including monoterpenoid glucosides, flavonoids, tannins, stilbenes, triterpenoids, steroids, paeonols, and phenols, have been found in the species belonging to section *Moutan*. However, although previous studies have analyzed the metabolites found in these species, the metabolic similarities that can be used for the chemotaxonomic distinction of section *Moutan* species are not yet clear. In this study, HPLC–DAD-based metabolic fingerprinting was applied to the classification of eight species: *Paeonia suffruticosa*, *Paeonia qiui*, *Paeonia ostii*, *Paeonia rockii*, *Paeonia jishanensis*, *Paeonia decomposita*, *Paeonia delavayi*, and *Paeonia ludlowii*. In total, of the 47 peaks that exhibited an occurrence frequency of 75% in all 23 tree peony samples, 43 of these metabolites were identified according to their retention times and UV absorption spectra, together with combined HPLC–QTOF-MS. These data were compared with reference standard compounds. The 43 isolated compounds included 17 monoterpenoid glucosides, 11 galloyl glucoses, 5 flavonoids, 6 paeonols and 4 phenols. Principal component analysis (PCA), and hierarchical cluster analysis (HCA), showed a clear separation between the species based on metabolomics similarities and four groups were identified. The results exhibited good agreement with the classical classification based on the morphological characteristics and geographical distributions of the subsections *Vaginatae* F.C. Stern and *Delavayanae* F.C. Stern with the exception of *P. decomposita*, which was found to be a transition species between these two subsections. According to their metabolic fingerprinting characteristics, *P. ostii* and *P. suffruticosa* can be considered one species, and this result is consistent with the viewpoint of medicinal plant scientists but different from that of classical morphological processing. Significantly large variations were obtained in the metabolic profiles of *P. delavayi*, whereas no significant difference was found between *P. delavayi* and *P. ludlowii*. This indicates that these two species have a close genetic relationship. In conclusion, the combination of HPLC–DAD and multivariate analyses has great potential for guiding future chemotaxonomic studies to examine the potential pharmaceutical value of the effective constituents of tree peony species and appears to be able to clarify the confusion and skepticism associated with the reported morphology- and molecular phylogenetics-based taxonomy of tree peonies.

1. Introduction

Paeonia, a genus in the Paeoniaceae family, comprises approximately 35 species that are divided into three sections: *Moutan*, *Oneapia*, and *Paeonia*. The section *Moutan* (named tree peony), which consists of eight woody species, is native to China, extending from Yunnan and Xizang (Tibet) to Anhui and Shanxi. According to the morphology of their flowers (floral disks), tree peonies can be divided into two subsections: *Vaginatae* (flower disk is leathery) and *Delavayanae* (flower disk is fleshy) (Fang, 1958).

In China, tree peonies, which are designated the king of flowers, have been used as a medicine for more than 2000 years and as ornamental flowers for approximately 1500 years. At present, there are approximately 600 Chinese tree peony cultivars (Li, 1999). After their introduction abroad in A.D. 724, several other unique cultivar groups have been bred in France, Britain, the United States, and other countries (Haw, 2001). The root cortex of

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Paeonia suffruticosa (Cortex Moutan) is one of the well-known Chinese Materia Medica and has been recorded in the Pharmacopoeia of the People's Republic of China. All of the species, with the exception of *Paeonia ludlowii*, exhibit almost the same medical properties as *P. suffruticosa*.

As one of the earliest and most well-known horticultural and medicinal plants worldwide, the genetic resources of wild tree peonies have been under heavy threat in the past several decades due to climate change, the reduction in biodiversity, and human activities (Zhou, 2006). Their distribution area and the size of natural populations have become increasingly smaller in China. Several wild species are listed as either rare or endangered taxa in the China Plant Red Data Book (Fu, 1992). To achieve a better understanding and sustainable use of the genetic resources of tree peonies, scientists, particularly Chinese botanists, have conducted extensive studies on the taxonomy and ecology of wild tree peonies since 1990. However, taxonomists have not yet reached a consensus on the precise number of species in the genus *Paeonia* due to a lack of comprehensive investigations. Confusion and skepticism regarding the taxonomy of tree peonies still exist (Halda, 1997), and the phylogenic positions of the species in each subsection are still disputed and incomplete. Considerable inconsistency exists in the taxonomic treatments of these plant revisions due to the diverse interpretations of the morphological variability of tree peonies. One way to circumvent these difficulties is to use markers that are independent of the morphological variations, and molecular markers have often been the optimal choice in this type of study in recent years. However, there are difficulties associated with the use of molecular markers, such as the choice of general molecular markers, and DNA barcoding (a single DNA fragment) cannot be used to accurately distinguish different species (Zhang et al., 2009; Zou et al., 1999).

The secondary metabolites of tree peonies has been intensively studied, and more than 130 highly diverse constituents (He et al., 2010), including monoterpenoid glucosides (Lin et al., 1996; He et al., 2012), flavonoids, tannins, stilbenes, triterpenoids (Ikuta et al., 1995) and steroids, paeonols, and phenols, have been isolated (Fig. 1). There are few reports on the chemotaxonomy of tree peonies. The phytochemical studies performed in our group on the seven constituents from the roots of 23 *Paeonia* taxa, including three tree peony species, as well as studies conducted by other groups, support the sectional division of *Paeonia* (Yu et al., 1985, 1986; Yu and Xiao, 1985; He et al., 1980; Guo et al., 2002, 2008). A high content of paeonol (**44**) was found in the woody section *Moutan*, whereas notably low contents are present in the herbaceous section *Paeonia*, and none of these constituents were isolated from the species belonging to section *Oneapia*. The accumulation of four components, paeoniflorin (**16**), albiflorin (**12**), oxypaeoniflorin (**9**), and paeonol (**44**), in 53 samples of *Paeonia lactiflora* is mainly controlled by genetic factors. It has been shown that the chemical characteristics associated with the morphological features, which are not easily changed, can be used as characteristic chemical constituents of *Paeonia* (Nishizawa et al., 1979). Furthermore, the contents of monoterpene glycosides and stilbenes exhibit a regular distribution in the seeds of *Paeonia* (He et al., 2013a, b). However, the secondary metabolites in the root cortex of tree peonies have not been used in a comprehensive medicinal comparison.

Thus, a comprehensive comparative analysis of the main secondary metabolites and chemical constituents was performed to identify the similarities and differences between all of the plant samples and to find the distribution of secondary metabolites in peonies. In addition, a taxonomic study was performed to provide complementary data to the pharmacophylogenetic study of the genus *Paeonia* (Chen et al., 2005), which was conducted to obtain experimental evidence. Therefore, it was necessary to conduct a comprehensive comparison between the secondary metabolites in the species belonging to section *Moutan* to provide experimental evidence for the taxonomy of tree peonies.

This paper addresses the phenetics of eight tree peony species (23 accessions) collected in China. The PCA provided the eigenvectors from the chemical fingerprint analysis, which was performed using high-performance liquid chromatography (HPLC), the major metabolites were identified according to the reference standard compounds combined HPLC–QTOF-MS. The eight tree peony species were readily divided into four clusters. The study demonstrates that the investigation of the metabolic fingerprints enables the chemical classification of tree peony species.

2. Results

2.1. The common pattern of the section Moutan species

The common patterns of the HPLC profiles of the 23 tree peony samples showed that 138 peaks were detected, and 27 peaks were common ones (Supplementary Material 1). Of the total 138 peaks, 47 peaks that exhibited an occurrence frequency of 75% were used in the PCA and cluster analyses (Supplementary Material 2). Except that *Paeonia delavayi* var. *angustiloba* (PMG-053 and 068) were more than 70%, the 47 chromatographic peak areas of all samples accounted for more than 90% of the total peak area. 43 metabolites were identified according to the retention time and the UV absorption spectra of the reference standard compounds combined LC–QTOF-MS, including 17 monoterpenoid glucosides, 11 galloyl glucoses, 5 flavonoids, 6 paeonols and 4 phenols (Fig. 2 and Table 1).

2.2. Principal component analysis of 23 samples of section Moutan

Principal component analysis (PCA), which is an unsupervised pattern recognition method, was performed to analyze 23 tree peony samples based on the areas of their 47 peaks (Supplementary Material 3). PCA uses an *N*-dimensional vector approach to separate samples based on the cumulative correlation of all metabolite data and then identifies the vector (eigenvector) that yields the greatest separation among the samples without any prior knowledge of the datasets (Zhang and Zhang, 2010).

The PCA provided ten eigenvalues, which were analyzed to obtain ten factors (Z_1–Z_{10}) (Supplementary Material 4). The proportion of each eigenvalue was computed, and the cumulative proportion of the ten factors was found to total 91.44%. The ten factors were retained for further analyses because their cumulative proportions were higher than 90%, which is considered adequate for the estimation of metabolic fingerprinting patterns. The eigenvectors of the patterns of the 47 peaks used to obtain the ten factors are summarized in Supplementary Material 5.

A three-component PCA model cumulatively accounted for 61.5% of the total variance. The plot of the PCA scores, which is shown in Figure 3A, was readily divided into seven relative clusters, which indicates that the content and distribution of the secondary metabolites is highly varied in the different tree peony species.

The plots of the PCA loadings were utilized to identify the differential metabolic compositions for the discrimination of groups. The metabolic fingerprinting patterns, monoterpenoid glucosides (**43**, mudanpioside A; **42**, mudanpioside B; **37**, mudanpioside J; **35**, mudanpioside C; **34**, paeoniflorigenone; **26**, mudanpioside H), paeonols (**44**, paeonol; **22**, suffruticoside A/B/C/D/E; **21**, suffruticoside A/B/C/D/E), and quercetin (**41**)are largely influenced by the first factor (PC1) (Fig. 3B), whereas monoterpenoid glucosides (**45**, benzoylpaeoniflorin; **40**, benzoyloxypaeoniflorin; **32**, 3″-methoxy-4″-hydroxy-6′-benzoyl-paeoniflorin; **10**, mudanpioside E), galloyl glucoses (**29**, hexagalloyl glucose; **27**, 1,2,3,4,6-penta-*O*-galloyl-*β*-D-glucose; **25**, dihydroxymethylbenzoyltetragalloyl