Phytochemistry 146 (2018) 16–24

Contents lists available at ScienceDirect

Phytochemistry

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

Phytochemical variation among the traditional Chinese medicine Mu Dan Pi from *Paeonia suffruticosa* (tree peony)

Shan-Shan Li [a], Qian Wu [a, b], Dan-Dan Yin [a, b], Cheng-Yong Feng [a, b], Zheng-An Liu [a, **], Liang-Sheng Wang [a, b, *]

[a] Key Laboratory of Plant Resources/Beijing Botanical Garden, Institute of Botany, The Chinese Academy of Sciences, Beijing 100093, China
[b] University of Chinese Academy of Sciences, Beijing 100049, China

ARTICLE INFO

Article history:
Received 16 May 2017
Received in revised form
6 November 2017
Accepted 22 November 2017

Chemical compounds studied in this article:
Oxypaeoniflorin (PubChem CID: 21631105)
Paeonolide (PubChem CID: 442923)
Paeonoside (PubChem CID: 52952637)
Apiopaeonoside (PubChem CID: 46883331)
Paeoniflorin (PubChem CID: 442534)
Galloyloxypaeoniflorin (PubChem CID: 101636348)
Paeonol (PubChem CID: 11092)
1,2,3,4,6-Penta-O-galloyol-β-D-glucose (PubChem CID: 65238)

Keywords:
Mu Dan Pi
Paeonia
UPLC
Metabolomics
Monoterpene glycoside
Tannin
Paeonol
Flavonoid

ABSTRACT

Mu Dan Pi is a traditional Chinese medicine used to treat inflammation, cancer, allergies, diabetes, angiocardiopathy, and neurodegenerative diseases. In this study, the metabolome variation within Mu Dan Pi collected from 372 tree peony cultivars was systematically investigated. In total, 42 metabolites were identified, comprising of 14 monoterpene glucosides, 11 tannins, 8 paeonols, 6 flavonoids, and 3 phenols. All cultivars revealed similar metabolite profiles, however, they were further classified into seven groups on the basis of their varying metabolite contents by hierarchical cluster analysis. Traditional cultivars for Mu Dan Pi were found to have very low metabolite contents, falling into clusters I and II. Cultivars with the highest amounts of metabolites were grouped in clusters VI and VII. Five potential cultivars, namely, 'Bai Yuan Qi Guan', 'Cao Zhou Hong', 'Da Zong Zi', 'Sheng Dan Lu', and 'Cheng Xin', with high contents of monoterpene glycosides, tannins, and paeonols, were further screened. Interestingly, the majority of investigated cultivars had relatively higher metabolite contents compared to the traditional medicinal tree peony cultivars.

1. Introduction

The cortex of the tree peony root (*Paeonia suffruticosa* Andrews) is used as a traditional Chinese medicine commonly known as Mu Dan Pi, and is part of the compendium in the Pharmacopoeia of the People's Republic of China (Chinese Pharmacopoeia Commission, 2015). The complex botanical history of *P. suffruticosa* discerns its existence as a hybrid with other *Paeonia* species (Hong, 2010). Consequently, botanical variations are observed in this genus. Nine wild *Paeonia* species and more than 1345 tree peony cultivars are found in China (Wang et al., 2015). All *Paeonia* species, except *P. ludlowii*, exhibit pharmacological properties similar to those of

Abbreviations: ANOVA, analysis of variance; LOD, limit of detection; LOQ, limit of quantitation; PGG, 1,2,3,4,6-penta-*O*-galloyol-*β*-D-glucose; RSD, relative standard deviation; TMC, total metabolite content; UHPLC, ultra-high performance liquid chromatography.

* Corresponding author. Key Laboratory of Plant Resources/Beijing Botanical Garden, Institute of Botany, The Chinese Academy of Sciences, Beijing 100093, China.
** Corresponding author.
E-mail addresses: liuzhengan@ibcas.ac.cn (Z.-A. Liu), wanglsh@ibcas.ac.cn (L.-S. Wang).

https://doi.org/10.1016/j.phytochem.2017.11.008
0031-9422/

P. suffruticosa (He et al., 2010). The remainder of *Paeonia* species constitute Chinese tree peony cultivars. Theoretically, the root bark of most tree peony cultivars can be used as Mu Dan Pi. However, few tree peony cultivars are actually used in Mu Dan Pi production. Although Mu Dan Pi originated from China, this traditional medicine has <5% of the international market share. The price of high-quality Mu Dan Pi produced in China and sold in Japan is only a quarter of the price of those sourced from Korea (Liu et al., 2005). This observation may indicate that the quality of Chinese tree peony root cortices largely varies as a result of the diverse tree peony resources. To uncover Chinese-derived Mu Dan Pi variability, secondary metabolite analysis from various tree peony cultivars should be comprehensively compared.

The secondary metabolites of the tree peony have been intensively evaluated, and 43 compounds have been identified in tree peony species based on root cortex metabolic fingerprinting (He et al., 2014). Generally, monoterpene glycosides, paeonols, and tannins are considered to be the main bioactive metabolites in Mu Dan Pi extracts (Han, 2008; He et al., 2010). However, the variability of Mu Dan Pi remains unclear. In recent years, the underlying mechanisms related to specific bioactive compound were identified and investigated, in which paeonol, paeoniflorin, 1,2,3,4,6-penta-*O*-galloyol-β-D-glucose (PGG), methyl gallate, and gallic acid were the target compounds (Lin et al., 2015; Wu et al., 2016; Zhao et al., 2015). Therefore, potential cultivars with high contents of target compounds and excellent pharmacological functions could be identified. Mu Dan Pi has been subjected to qualitative analysis, chemical fingerprint analysis, and metabolomic variation studies in terms of different root parts, growth years, habitats, and harvest periods (Fan et al., 2012; Hu et al., 2013; Xiao et al., 2015). Although metabolomic variations among five Chinese tree peony cultivars have been characterized, their sample size used in the study is too small to provide a valuable reference for Mu Dan Pi variety selection (Xiao et al., 2014). Phytochemical investigation, systematic evaluation, and pharmacological research on various tree peony cultivars have yet to be performed.

HPLC-MS-based metabolomics are an effective tool used to perform comparative analyses on phenotypic cultivar chemical constituents to determine novel and potential chemotaxonomic characteristics (He et al., 2014). In this study, metabolites in 372 tree peony cultivars were systematically and quantitatively assessed. The phytochemical characteristics of Mu Dan Pi were determined by using the UHPLC system, and the cultivars were then classified into seven clusters. The major metabolites were quantitatively characterized using corresponding standards with an ACQUITY UPLC™ I-Class system coupled with a Xevo G2-S QTOF mass spectrometer. The findings herein suggested that many tree peony cultivars may be utilized as an alternative source for Mu Dan Pi production. Additionally, variety selection was found to be an important factor influencing metabolite content, and Chinese tree peony cortex quality likely varies based on the different Mu Dan Pi resources used in production.

2. Results and discussion

2.1. Characterization of metabolites in the root cortex of the tree peony

Mu Dan Pi metabolites are difficult to separate because of their complex compositions and similar physicochemical properties. The retention behavior and resolution of metabolites are also significantly affected by the pH of the mobile phases. As such, 0.1% formic acid was used in this study to obtain the desired separation performance and maximum peak capacity. Some representative chromatograms were shown in Fig. 1. We found that the metabolic profiles within cultivars were similar. A total of 42 metabolites were detected and identified in Mu Dan Pi collected from 372 tree peony cultivars. These were examined according to the UV absorption spectra, fragment ions, and retention time of the corresponding standard compounds (Table 1, Supplementary File 1). Of these metabolites, 14 were monoterpene glucosides (peaks **2**, **4**, **6**, **14**, **25**, **26**, **30**, **32**, **33**, **36**, **37**, **39**, **40**, and **41**), 11 were tannins (peaks **3**, **5**, **11**, **15**, **16**, **18**, **24**, **27**, **31**, **34**, and **35**), 8 were paeonols (peaks **8**, **10**, **12**, **17**, **20**, **21**, **23**, and **42**), 6 were flavonoids (peaks **7**, **13**, **19**, **22**, **28**, and **38**), and 3 were phenols (peaks **1**, **9**, and **29**) (Fig. 2). These 42 phytochemicals found in Mu Dan Pi were identified as the bioactive compounds for further quantification.

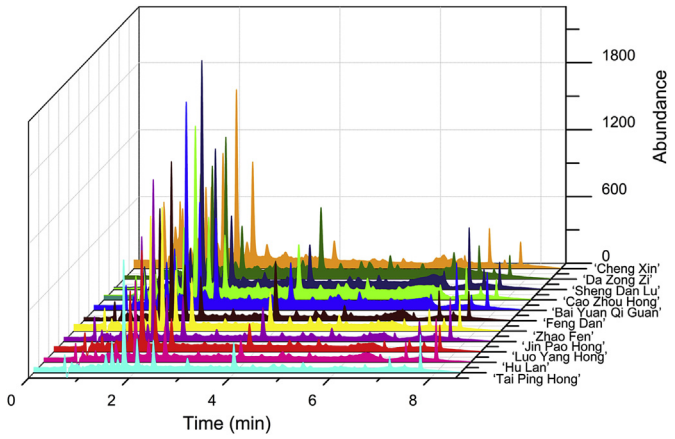

Fig. 1. UHPLC profiles from the traditional medicinal tree peony cultivars and potential cultivars for Mu Dan Pi. The traditional medicinal cultivars are 'Tai Ping Hong', 'Hu Nan', 'Luo Yang Hong', 'Jin Pao Hong', 'Zhao Fen', and 'Feng Dan', while the potential cultivars are Bai Yuan Qi Guan', 'Cao Zhou Hong', 'Sheng Dan Lu', 'Da Zong Zi', and 'Cheng Xin'.

Ten analytical standards were used to verify the sensitivity of the separation method (Table A1). The calibration curves showed good linearity over relatively wide concentration ranges at their maximum absorption wavelengths (λ_{max}, nm) ($r^2 \geq 0.9974$). The limit of detection (LOD) and the limit of quantitation (LOQ) were defined as the signal-to-noise ratios of 3:1 and 10:1, respectively. As shown in Table A1, the lowest and highest LOD and LOQ were obtained for paeoniflorin (peak **14**, 0.19 and 0.64 μg mL^{-1}) and rutin (0.94 and 3.15 μg mL^{-1}), respectively. The precision of our analytical procedure was evaluated by examining the intra-day precision (n = 6) and inter-day precision (n = 3) of the compounds separated from the Mu Dan Pi extracts. The results showed that the relative standard deviations (RSDs) of the 42 compounds were less than 2.83% in the inter-day test and less than 3.60% in the intra-day analysis (Table A2). Their high repeatability and intermediate precision strongly suggested high reliability of the proposed methods.

2.2. Determination of metabolites in various cultivars

The functional compounds in Mu Dan Pi were characterized through UHPLC. The metabolites (peak **1**, gallic acid; peak **4**, oxypaeoniflorin; peak **7**, (+)-catechin; peak **11**, 1,3,6-tri-*O*-galloyl-β-D-glucose; peak **14**, paeoniflorin; peak **26**, albiflorin; peak **27**, PGG; peak **40**, benzoylpaeoniflorin; and peak **42**, paeonol) in each sample were quantified in absolute terms through linear regression with their corresponding standards, while the remaining metabolites were analyzed semi-quantitatively by using corresponding analogs. Although the UHPLC chromatograms among the cultivars were similar, total metabolite contents (TMCs) varied and ranged from 18.4 mg g^{-1} to 122.2 mg g^{-1} (Table A3). The results indicated