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Original article

Simultaneous Determination of Eight Constituents in Fruits of *Rubus chingii* by UPLC

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ABSTRACT

Objective To develop a simple, efficient, and reliable method for routine quantitative analysis of main constituents presented in the fruits of *Rubus chingii*, which is widely used in Chinese materia medica (CMM), known as Fupenzi (FPZ) in Chinese. **Methods** An ultra performance liquid chromatography-photo diode array (UPLC-PDA) system was employed for simultaneous quantification of eight compounds, i. e. adenosine, gallic acid, brevifolin carboxylic acid, ethyl gallate, ellagic acid, kaempferol-3-*O*-rutinoside, kaempferol-3-*O*-β-D-glucopyranoside, and tiliroside. The chromatographic analysis was performed on a C_{18} column using a gradient elution of acetonitrile -0.1% formic acid aqueous solution within a runtime of 25 min. **Results** All calibration curves were linear ($R^2 > 0.9997$) over the tested ranges. The intra- and inter-day precisions as determined from sample solutions were both less than 2.45% and 2.78%, respectively. The average recoveries for the eight constituents ranged from 94.77% to 101.35% with RSD ≤ 4.41%. The newly-developed method was applied to the quality assessment of various *R. chingii* samples, including both ripe and unripe fruits of *R. chingii* from different habitats. **Conclusion** The relative levels of the investigated compounds vary remarkably in the fruits of *R. chingii* collected from different habitats. As only two of the eight compounds, adenosine and ellagic acid, are determined in the ripe fruits of *R. chingii*, the results may explain the reason why only the unripe fruits can be used in CMM.

Key words

Adenosine; gallic acid; quantification; *Rubus chingii*; UPLC-PDA

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1. Introduction

The genus *Rubus* L. (Rosaceae) comprises about 700 species that are mainly distributed in Europe, North America, and Asia. In Chinese materia medica (CMM), the unripen fruits of *Rubus chingii* Hu, commonly known as Fupenzi (FPZ)

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in Chinese, have been used as food and tonic for many years. According to *Chinese Pharmacopoeia* 2015, the reasonable time to pick *R. chingii* fruits is the early summer when the color of unripen fruits turns yellow-green from green. FPZ is used as a liver and kidney tonic and to treat abnormal seminal discharge and polyuria (Pharmacopoeia Committee of P. R. China, 2015). Several types of secondary metabolites including flavonoids, terpenoids, sterols, phenolic acids, and lipids (Guo and Yang, 2005; Xiao et al, 2011; Han et al, 2012; Sun et al, 2013; Du et al, 2014) have been isolated and identified from FPZ, which possess some specific bioactivities. Modern researches have revealed that various pharmacological properties of *R. chingii* were attributed to flavonoids and phenolic acids, including anti-oxidant (Ding, 2011), antithrombotic (Han et al, 2012), anti-inflammatory (Sun et al, 2013), and other biological activities.

According to *Chinese Pharmacopoeia* 2015, ellagic acid and kaempferol-3-*O*-rutinoside were chosen as marker compounds of the content determination of FPZ, and tiliroside and kaempferol-3-*O*-rutinoside were chosen as the marker compounds for identification of Fupenzi (Pharmacopoeia Committee of P. R. China, 2015). Ellagic acid, kaempferol-3-*O*-rutinoside, and kaempferol-3-*O*-β-*D*-glucopyranoside were reported to possess hepatoprotective effects (Sohn et al, 2013; Wang et al, 2015). Tiliroside and gallic acid showed anti-oxidant activity (Buřičová et al, 2011; Ding, 2011). Ellagic acid, brevifolin carboxylic acid, and ethyl gallate exhibited anticancer activity (Lee et al, 1994; Cui et al, 2002). Adenosine showed neuroprotective effect (Olatunji et al, 2016).

At present, only a few methods have been reported for the quantitative analysis of FPZ, and these include HPLC (Chai, 2008; Li and Tan, 2008; Chen et al, 2009; Cheng et al, 2012; He et al, 2013; Zhong et al, 2014) and GC/MS (Dian et al, 2005). Each proposed technique possesses its own set of advantages and provides several choices for analysts. However, both methods suffer from long analysis time and low resolution and sensitivity, or are limited to a few analytes.

In this paper, a rapid and reliable UPLC method has been developed for the simultaneous quantification of eight main bioactive compounds in Fupenzi, such as adenosine, gallic acid, brevifolin carboxylic acid, ethyl gallate, ellagic acid, kaempferol-3-*O*-rutinoside, kaempferol-3-*O*-β-*D*-glucopyranoside, and tiliroside. The newly developed method was successfully applied to quality assessment of various FPZ samples.

2. Materials and methods

2.1 Materials

The deionized water used in the experiments was produced with a Milli-Q water purification system (USA). HPLC-grade formic acid and methanol were purchased from Concord Technology (Tianjin, China). Acetonitrile of HPLC-grade was purchased from Sigma-Aldrich (USA).

Constituents of brevifolin carboxylic acid, ethyl gallate, ellagic acid, kaempferol-3-*O*-rutinoside, kaempferol-3-*O*-β-*D*-glucopyranoside, and tiliroside were isolated and purified from FPZ by the authors. Their molecular structures were characterized by spectroscopic techniques (MS, ^{1}H- NMR and ^{13}C-NMR). Adenosine and gallic acid were purchased from the National Institutes for Food and Drug Control (Beijing, China). The purities of the standards were all > 98%.

Eight batches of FPZ were collected from different provinces in China and given to such designations as FPZ-1 (Zhejiang), FPZ-2 (Shandong), FPZ-3 (Henan), FPZ-4 (Anhui), FPZ-5 (Beijing), FPZ-6 (Hubei), FPZ-7 (Hebei), and FPZ-8 (Tianjin). FPZs-1−7 were unripen fruits, while FPZ-8 consisted of ripe fruits. These samples were identified by Dr. Tian-xiang Li, and voucher specimens were deposited in the Herbarium of Pharmacognosy, Tianjin University of Traditional Chinese Medicine, China.

2.2 Sample extraction

The fruits of *R. chingii* were pulverized into a fine powder (100 meshes). FPZ ground samples (1.0 g) were extracted with 70% methanol (50 mL) in an ultrasonic water bath at room temperature for 30 min. Additional 70% methanol was added to make up for lost weight. The solutions were centrifuged at 10 000 *g* for 10 min. An aliquot (3 μL) of each supernatant solution was used for UPLC analysis.

2.3 Preparation of standard solutions

The eight compounds were accurately weighed and dissolved in methanol to prepare stock solutions. A certain amount of each stock solution was placed in a 10 mL volumetric flask and diluted to volume with methanol to give the concentration of 23.20 μg/mL adenosine, 20.70 μg/mL gallic acid, 29.72 μg/mL brevifolin-carboxylic acid, 55.12 μg/mL ethyl gallate, 116.40 μg/mL ellagic acid, 63.30 μg/mL kaempferol-3-*O*-rutinoside, 22.95 μg/mL kaempferol-3-*O*-β-*D*-glucopyranoside, and 35.34 μg/mL tiliroside. The combined solution was then diluted stepwise with 85% methanol aqueous solution to give seven different concentration for construction of calibration curves.

2.4 UPLC analysis

UPLC analysis was carried out using Waters Acquity UPLC System (USA), composed of a column heater, a sample manager, a binary solvent manager, and a PDA detector. The chromatographic separation was performed on an Acquity UPLC^{TM} BEH C_{18} column (100 mm × 3.0 mm, 1.7 μm particle size; Waters) by fixing the column heater at 30 °C. The mobile phase consisted of acetonitrile (A) and water containing 0.1% formic acid (B) with the flow rate at 0.4 mL/min. A gradient elution program was employed as follows: 3%–5% A at 0–2 min, 5%–11.5% A at 2–8 min, 11.5%–15.5% A at 8–13.5 min, 15.5%–18% A at 13.5–15

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