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Comparative analysis of sixteen flavonoids from different parts of *Sophora flavescens* Ait. by ultra high-performance liquid chromatography–tandem mass spectrometry

Zebin Weng[a,b], Fei Zeng[a], Zhenhua Zhu[a], Dawei Qian[a], Sheng Guo[a,*], Hanqing Wang[c], Jin-ao Duan[a,b,*]

[a] *Jiangsu Collaborative Innovation Center of Chinese Medicinal Resources Industrialization, National and Local Collaborative Engineering Center of Chinese Medicinal Resources Industrialization and Formulae Innovative Medicine, and Jiangsu Key Laboratory for High Technology Research of TCM Formulae, Nanjing University of Chinese Medicine, Nanjing 210023, China*
[b] *School of Traditional Chinese Pharmacy, China Pharmaceutical University, Nanjing 210009, China*
[c] *College of Pharmacy, Ningxia Medical University, Yinchuan, Ningxia 750004, China*

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ABSTRACT

The root of *Sophora flavescens* Ait. has long been used as a crude drug in China and other Asian countries for thousands of years. The quinolizidine alkaloids and flavonoids are considered as the main bioactive components in this plant. To determine the distribution and content of the flavonoids in different organs of this plant, a rapid, sensitive and reproducible method was established using ultra-high-performance liquid chromatography coupled with a triple quadrupole electrospray tandem mass spectrometry. A total of sixteen flavonoids including five different types (isoflavones, pterocarpans, flavones, flavonols and prenylflavonoids) were simultaneously determined in 10 min. The established method was fully validated in terms of linearity, sensitivity, precision, repeatability as well as recovery and successfully applied in the methanolic extracts of *S. flavescens* parts (root, stem, leaf, pod and seed). The analysis results indicated that the distribution and contents of different type of flavonoids showed remarkable differences among the five organs of *S. flavescens*. This study might be useful for the rational utilization of *S. flavescens* resource.

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1. Introduction

The root of *Sophora flavescens* Ait. is a widely used crude drug and medicinal material in many countries including China, Japan, Korea, India and some European countries [1]. In traditional Chinese medicine (TCM), *S. flavescens* has been used extensively in treating dysentery [2], hepatitis [3], asthma [4], cancer [5], inflammatory disorders [6], and cardiovascular disease [7]. Decades of research have proved that quinolizidine alkaloids are the main bioactive components related to these bioactivities. Furthermore, recent studies revealed that flavonoids in *S. flavescens* also exhibited good bioactivities of anti-bacteria [8], anti-angiogenesis [9], anti-tumor [10], anti-inflammatory [11] and tyrosinase inhibition effect [12]. Previous studies have found that *S. flavescens* contains multiple types of flavonoids including flavones, isoflavones, flavonols, pterocarpans and prenylflavonoids [13–15]. However, most studies were focused on the quality control of the root, which is the traditional medicinal part of *S. flavescens*. According to our knowledge, up to now, there were few analytical methodologies for the quality control of other organs of *S. flavescens* such as stem, leaf, pod and seed. Such situation was not conducive for the quality control of *S. flavescens* and limiting its further application. In addition, most previous studies only measured two or three types of flavonoids like flavones, pterocarpans and prenylflavonoids of *S. flavescens* in a single run [16,17]. The method for the simultaneous determination of these five classes of compounds (isoflavones, pterocarpans, flavones, flavonols and prenylflavonoids) in *S. flavescens* on one separation procedure has not been found. It is well known that the curative effect of TCM is related to the synergic activities of their multiple bioactive compounds [18,19]. Therefore, it is imperative to establish a reliable analytical method for the simultaneous determination of multiple pharmacologically active compounds, which could be utilized in the quality control of these herb drugs.

So far, various analytical methods have been utilized to analyze flavonoid constituents. Among them, high performance liquid chro-

* Corresponding authors at: Jiangsu Collaborative Innovation Center of Chinese Medicinal Resources Industrialization, Nanjing 210023, China.
E-mail addresses: guosheng@njucm.edu.cn (S. Guo), dja@njucm.edu.cn (J.-a. Duan).

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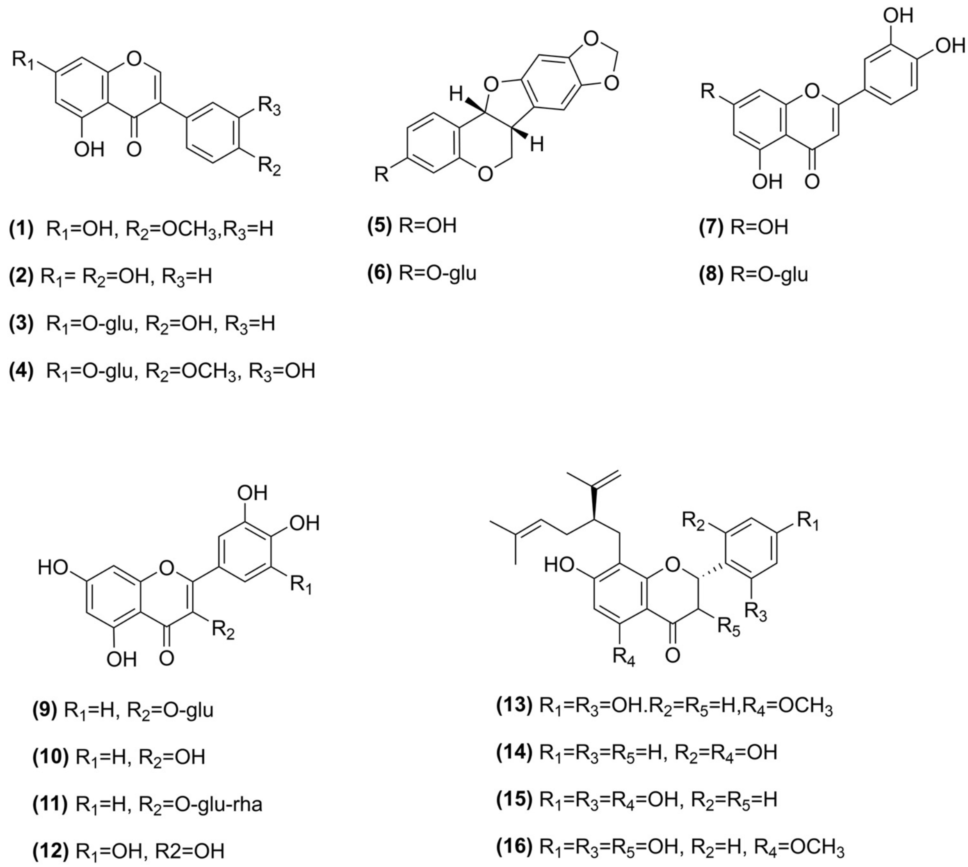

Fig. 1. Chemical structures of 16 markers: biochanin A (**1**), daidzein (**2**), daidzin (**3**), calycosin-7-glucoside (**4**), maackiain (**5**), trifolirhizin (**6**), luteolin (**7**), luteoloside (**8**), isoquercitrin (**9**), quercetin (**10**), rutin (**11**), myricetin (**12**), kurarinone (**13**), kushenol A (**14**), sophoraflavanone G (**15**) and kushenol N (**16**).

matography (HPLC) is the most commonly used method [20–22]. However, the long analysis time and lower sensitivity make it difficult to simultaneous determine multiple constituent especially some trace components in a short time. Recent success in the utilization of liquid chromatography coupled with triple quadrupole mass spectrometry (LC–MS/MS) for characterizing and quantifying the constituents in complex samples especially in medical plants provides an appropriate way to determine different types of chemical components in a single run [23–25]. To date, few attempts have been made to simultaneously determine different types of flavonoids in *S. flavescens* using UPLC–MS/MS. Previous studies have established methods for the characterization of flavonoids in the root of *S. flavescens* [26,27]. However, the quantitative UPLC–MS/MS method for the determination of different types of flavonoids in different plant parts of *S. flavescens* have not been reported yet.

In this study, an accurate and rapid UPLC–MS/MS method with high sensitivity has been developed for the first time for simultaneous determination of sixteen flavonoids of five types including four isoflavones, two pterocarpans, two flavones, four flavonols and four prenylflavonoids in different organs of *S. flavescens*. The established method could be utilized to further enrich the quality control and rational utilization of *S. flavescens* resource.

2. Experimental

2.1. Chemicals, reagents and materials

Chemical standards of biochanin A, daidzein, daidzin, calycosin-7-glucoside, maackiain, trifolirhizin, luteolin, luteoloside, isoquercitrin, quercetin, rutin, myricetin, kurarinone, kushenol A, sophoraflavanone G and kushenol N were purchased from Bomei biotechnology Co., Ltd. (Hefei, China). The purity of each compound was determined to be over 98% by HPLC detection. Their structures are presented in Fig. 1.

Acetonitrile was HPLC-grade from Merck (Darmstadt, Germany) and deionized water was purified by a Milli-Q water purification system (TGI Pure Water Systems, Greenville, SC, USA). Other reagents, such as methanol and formic acid, were of analytical grade (TCI Chemical Reagent Co., Ltd., Shanghai, China).

Three *S. flavescens* plants were collected from the medical plants garden in Nanjing University of Chinese Medicine in September 19, 2017. Their botanical origin was identified by Prof. Jin-ao Duan from Nanjing University of Chinese Medicine. Then the whole plant was divide into root, stem, leaf, seed, and pod. All the samples were stored at −80 °C and protected from light until analysis.

2.2. Preparation of standard solutions

A certain amount of biochanin A (**1**), daidzein (**2**), daidzin (**3**), calycosin-7-glucoside (**4**), maackiain (**5**), trifolirhizin (**6**), luteolin (**7**), luteoloside (**8**), isoquercitrin (**9**), quercetin (**10**), rutin (**11**), myricetin (**12**), kurarinone (**13**), kushenol A (**14**), sophoraflavanone G (**15**) and kushenol N (**16**) was dissolved in methanol to obtain 16 reference compound stock solutions. The working standard solutions were prepared by diluting the standard solution with methanol to a series of proper concentrations within the ranges: **1**, $1.257–5.150 \times 10^3$ ng/ml; **2**, $3.662–1.500 \times 10^4$ ng/ml; **3**, $2.490–1.020 \times 10^4$ ng/ml; **4**, $7.434–3.045 \times 10^4$ ng/ml; **5**, $1.343–5.500 \times 10^3$ ng/ml; **6**, $12.30–5.040 \times 10^4$ ng/ml; **7**, $6.116–2.505 \times 10^4$ ng/ml; **8**, $2.441–1.000 \times 10^4$ ng/ml; **9**, $3.735–1.530 \times 10^4$ ng/ml; **10**, $3.796–1.555 \times 10^4$ ng/ml; **11**,

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