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Simultaneous analysis of flavonoids from *Hypericum japonicum* Thunb.ex Murray (Hypericaceae) by HPLC-DAD–ESI/MS

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Abstract

A novel and sensitive HPLC-UV method has been developed for the simultaneous determination of five major flavonoids in *Hypericum japonicum* hydroalcoholic extract. The chemical profile of five flavonoids, including taxfolin-7-*O*-α-L-rhamnoside (**1**), isoquercitrin (**2**), quercitrin (**3**), quercetin-7-*O*-α-L-rhamnoside (**4**) and quercetin (**5**) was acquired by using high-performance liquid chromatography-diode array detector coupled to an electrospray tandem mass spectrometer (HPLC-DAD–ESI/MS). The analysis was performed on a ZORBAX SB-C18 analytical column (5 μm, 250 mm × 4.6 mm, i.d.) with a gradient solvent system of acetonitrile-0.5% aqueous formic acid. The validation was carried out and the linearities ($r^2 > 0.9997$) and recoveries (ranged from 98.4% to 99.8%) were acceptable. The limits of detection (LOD) of these flavonoids ranged from 0.5 to 7.5 ng. The results indicated that the contents of investigated flavonoids in *H. japonicum* varied significantly from habitat to habitat with contents ranging from 2.00 to 34.18 mg/g. The proposed method is simple, effective and suitable for the quality control of this traditional Chinese medicine (TCM).
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Keywords: Flavonoids; *Hypericum japonicum*; Hypericaceae; HPLC-DAD–ESI/MS; Simultaneous analysis

1. Introduction

Hypericum japonicum, locally called 'tianjihuang', is prepared from the entire herbs of *H. japonicum* Thunb.ex Murray (Hypericaceae). It is one of traditional Chinese medicine (TCM), widely distributed in south drainage area of the Changjiang River, China [1]. *H. japonicum* has been used for the treatment of bacterial diseases, infectious hepatitis, gastrointestinal disorder, internal hemorrhage and tumors [2–6]. As reported previously, *H. japonicum* mainly contains xanthones [6,7], chromenes [8], flavonoids [9,10], dipeptide derivatives [11] and phloroglucinol derivatives [12]. Of these flavonoids are commonly considered as the major bioactive constituents.

In our previous study [13], eight flavonoids had been isolated from *H. japonicum* by column chromatography (CC). Their chemical structures were identified by spectral analysis (^{1}H NMR, ^{13}C NMR, 2D NMR, MS, UV and IR). Most of the flavonoids were found to have antihepatitis activity [14]. Isoquercitrin, quercitrin, quercetin-7-*O*-α-L-rhamnoside were found to have hepatoprotective and jaundice-relieving effects [15]. Therefore, the simultaneous determination of the flavonoids is significant to ensure the quality of *H. japonicum*. The presently employed quality control methods for the flavonoids in *H. japonicum* and its preparations are mainly based on thin layer chromatography (TLC) [16,17], and high-performance liquid chromatography (HPLC) [17–20]. Until now, to the best of our knowledge, no data have been reported on the simultaneous determination of these flavonoids in *H. japonicum*.

In this paper, a simple and efficient HPLC–ESI/MS method is proposed for the quantification of the major flavonoids in twenty batches of *H. japonicum* from different habitats.

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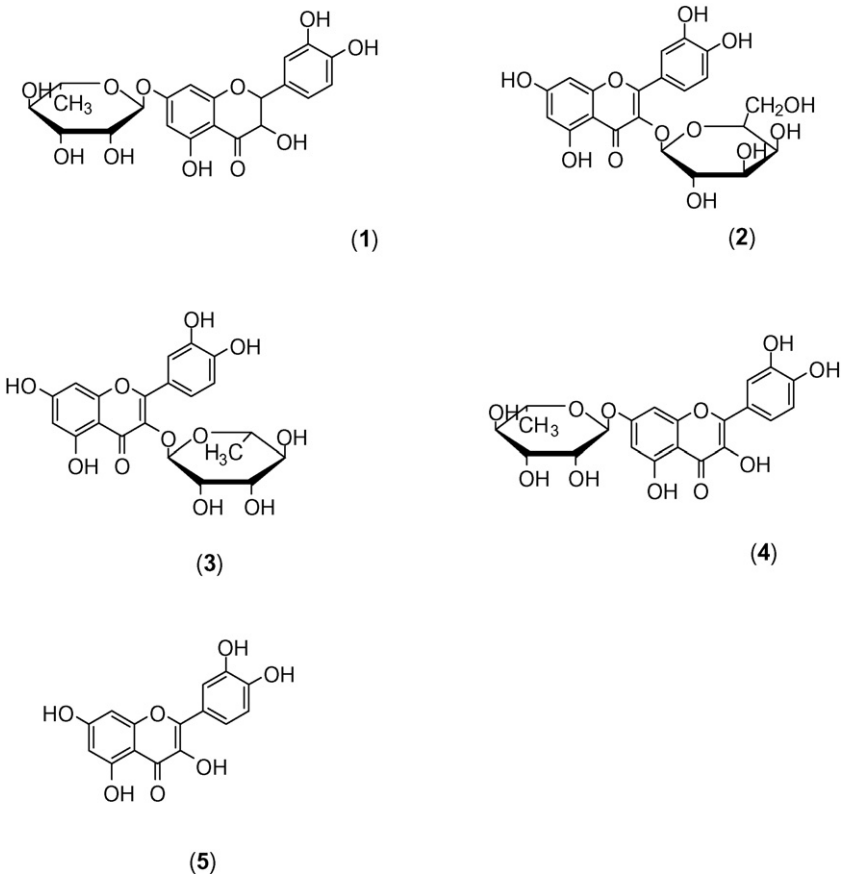

Fig. 1. Chemical structures of the investigated flavonoids in *H. japonicum*. (**1**) taxfolin-7-O-α-L-rhamnoside, (**2**) isoquercitrin, (**3**) quercitrin, (**4**) quercetin-7-O-α-L-rhamnoside and (**5**) quercetin.

2. Experimental

2.1. Reagents and materials

HPLC-grade acetonitrile, methanol and formic acid were purchased from Merck Company (Merck, Darmstadt, Germany). Ultrapure water was prepared by Milli-Q50 SP Reagent Water System (Millipore Corporation, MA, USA). All other solvents used in this study were of analytical grade from Shanghai Chemical Reagent Corporation (Shanghai, China).

The reference standards of the five flavonoids (taxfolin-7-*O*-α-L-rhamnoside, isoquercitrin, quercitrin, quercetin-7-*O*-α-L-rhamnoside, quercetin) were isolated in our laboratory (over 99.5% purity) and their chemical structures (Fig. 1) were identified by spectral analysis [21–24]. The ethanol extract of the whole plant of *H. japonicum* was evaporated *in vacuo*. The residue was suspended in water, and then partitioned with petroleum ether, $CHCl_3$ and EtOAc. The EtOAc extract was repeatedly submitted to column chromatography over silica gel, reverse phase gel (ODS), and Sephadex LH-20 to yield the five flavonoids.

Twenty batches of *H. japonicum* were collected from various habitats as follows: Wuhan City, Hubei Province (lot no. 060705), Tiandeng City, Guangxi Province (lot no. 060802), Zigong City, Sichuan Province (lot no. 060715), Chongqian City (lot no. 060814), Dabie Mountain, Anhui Province (lot no. 060901), Yingtan Ctiy, Jiangxi Province

Table 1
HPLC-ESI/MS data of the MeOH extract of *H. japonicum*

Peak	R_t (min)	MS (*m*/*z*)	MS^2 fragment ion (*m*/*z*)	Identification
1	21.5	451[*M*+H]$^+$	305[*M*+H-Rhamnose]$^+$	Taxfolin-7-O-α-L-rhamnoside
2	30.5	465[*M*+H]$^+$ 487[*M*+Na]$^+$	303[*M*+H-Glucose]$^+$	isoquercitrin
3	37.9	449[*M*+H]$^+$	303[*M*+H-Rhamnose]$^+$	Quercitrin
4	48.6	449[*M*+H]$^+$	303[*M*+H-Rhamnose]$^+$	Quercetin-7-O-α-L-rhamnoside
5	61.0	303[*M*+H]$^+$	285[*M*+H–H_2O]$^+$, 257[*M*+H–H_2O–CO]$^+$, 137[*M*+H–$C_6H_4O_2$–$C_2H_2O_2$]$^+$	Quercetin

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