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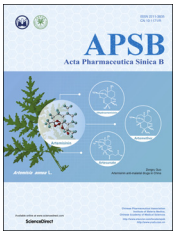

ORIGINAL ARTICLE

Flavonoid profiling of a traditional Chinese medicine formula of Huangqin Tang using high performance liquid chromatography

Tao Li[a,b,†], Shuaixing Zhuang[a,†], Yiwei Wang[c], Yanli Wang[a], Weihao Wang[a], Huihui Zhang[a], Li Chen[a], Dunfang Wang[a], Zhongming Zhou[a], Weipeng Yang[a,*]

[a]*Institute of Chinese Materia Medica, China Academy of Chinese Medical Sciences, Beijing 100700, China*
[b]*Experimental Research Center, China Academy of Chinese Medical Sciences, Beijing 100700, China*
[c]*China Food and Drug Administration, Beijing 100053, China*

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Abstract The quality control processes for herbal medicines have been problematic. Flavonoids are the major active components of Huangqin Tang (HQT, a traditional Chinese medicine formula). In this study, we used a combinative method approach consisting of chromatographic fingerprinting (high performance liquid chromatography; HPLC), quantitative methods and a pharmacodynamic evaluation model to analyze the flavonoids of HQT obtained from different sources. Ten batches of HQT were analyzed by the HPLC fingerprinting method and 26 common peaks were detected, of which 23 peaks corresponded with the chemical profile of HQT. In addition, 11 major compounds were identified by LC–MS analysis (liquid chromatography–tandem mass spectrometer; LC–MS^n) and quantified by the HPLC quantitative method approach. The studied 10 batches of HQT were found to be homogeneous in their composition with a similarity between 0.990 and 1.000. The distribution of the 11 identified compounds was found to be very similar among the batches. Only slight pharmacodynamic differences were detected between the different batches, confirming the homogeneity of HQT. The results of this study prove that the combination of chromatographic fingerprinting and quantitative analysis can be readily used for comprehensive quality control of herbal medicines.

Abbreviations: CFDA, China Food and Drug Administration; HPLC, high performance liquid chromatography; HQT, Huangqin Tang; ICH, International Conference on Harmonization; LC–MS^n, liquid chromatography–tandem mass spectrometer; LLOD, linearity, lower limit of detection; LLOQ, lower limit of quantification; PCA, principal component analysis; RSD, relative standard deviation; *S/N*, signal-to-noise ratio; TCM, traditional Chinese medicine.

*Corresponding author. Tel.: +86 10 64093014; fax: +86 10 64013996.
E-mail address: hrbywp@sina.com (Weipeng Yang).
†These authors made equal contributions to this work.

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1. Introduction

Traditional Chinese medicine (TCM), especially herbal medicine, is gaining increasing attention worldwide for its millennia-old practices and its potential all-natural therapeutic applications. Huangqin Tang (HQT), a well-known Chinese medicine formula, is a combination of four herbal medicines 3:2:2:2 by weight, namely Radix Scutellariae (*Scutellaria baicalensis* Georgi), Radix Paeoniae Alba (*Paeonia lactiflora* Pall.), Radix et Rhizoma Glycyrrhizae (*Glycyrrhiza uralensis* Fisch., *Glycyrrhiza inflata* Bat. or *Glycyrrhiza glabra* L.) and Fructus Jujubae (*Ziziphus jujuba* Mill). HQT has traditionally been used for the treatment of colds and gastrointestinal diseases with symptoms of fever, abdominalgia and diarrhea[1,2]. Recently, some studies have reported that PHY906, an extract derived from HQT, reduces gastrointestinal toxicity and enhances the anti-tumor effect of some anti-cancer drugs[3,4]. This extract has been studied in five clinical trials with patients suffering from different types of cancer in both the US and Taiwan with very encouraging results. Radix Scutellariae, the chief ingredient of HQT, has been widely used in TCM prescriptions[5]. It is known to be effective in the treatment of fever, inflammation, cancers and other gastrointestinal diseases[6–9]. The major functional constituents of Radix Scutellariae are flavonoid glycosides (*e.g.*, baicalin, wogonoside and oroxylin-A-glucoside) and flavonoids (*e.g.*, baicalein, wogonin and oroxylin-A)[10–12]. Phytochemical studies have shown that the main constituents of the other three herbs (Radix Paeoniae Alba, Radix et Rhizoma Glycyrrhizae and Fructus Jujubae) are flavones, isoflavones, terpenoids, volatile oils and polysaccharides with a wide range of pharmacological properties[13–15], such as anti-inflammatory, analgesic, antitussive, tumor suppressor and immunomodulatory[16–19]. Flavonoids are the important effective components from HQT.

Generally, the chemical composition of herbal formulations may vary greatly due to differences in plant origins, cultivation areas and practices, climate conditions and processing protocols among others[20–22]. This leads to wide disparities in quality among different samples. An effective and feasible method should take the complexity and variability of the chemical constituents of herbal medicines into consideration simultaneously. However, previous studies addressing HQT using fingerprinting analysis failed to include enough quality control markers or had a limited study sample population[23,24]. Here we use a combinatorial approach with chromatographic fingerprinting analysis, a multiple-component quantitative analysis and animal pharmacology to comprehensively assess the quality of different HQT samples.

2. Materials and methods

2.1. Chemicals and herbal materials

The reference standards for baicalin, wogonoside, oroxylin-A-glucoside, baicalein, wogonin, oroxylin-A, liquiritin, liquiritigenin, isoliquiritoside, isoliquiritigenin, liquiritin apioside and isoliquiritin apioside were purchased from Zelang Pharmaceuticals (Nanjing, China). The purity of these compounds was higher than 98% by HPLC analysis based on a peak area normalization method. Aspirin (Scientific Research Special) and carrageenin (Sigma Life Science), both in pure powdered form, were also used. Analytical-grade ethanol and acetic acid were purchased from Beijing Chemical Factory (Beijing, China). HPLC-grade methanol and acetonitrile were obtained from Fisher (Fisher Scientific, USA). Purified water was purchased from Wahaha Group (Hangzhou, China).

Crude Radix Scutellariae from three origins that met the requirements of Beijing Food and Drug Administration was collected from different pharmacies in Beijing. Crude Radix Rhizoma Glycyrrhizae, Radix Paeoniae and Fructus Jujubae (Herbal Pieces Co., Ltd.; originally from Anhui, Neimeng and Hebei, respectively) were collected from Beijing Wukesong Clinic. The source and labeling of the medicines is summarized in Table 1. All herbal medicines were identified by Professor Xianduan Li of the Institute of Chinese Materia Medica, China Academy of Chinese Medical Sciences and voucher specimens were deposited in the institute. HPLC analysis was used to confirm that the content of baicalin in crude Radix Scutellariaeis is $14.9 \pm 2.7\%$ in accordance with the quality level request of 4.0% established in the *Chinese Pharmacopoeia* 2010[5].

2.2. Preparation of HQT granule, HQT sample solutions and standard solutions

Ten batches of crude Radix Scutellariae (9 g) and one batch of Radix et Rhizoma Glycyrrhizae (6 g), Radix Paeoniae (6 g) and Fructus Jujubae (6 g) were weighed and thoroughly soaked in water for 30 min. For the first decoction, 10-fold water (1:10 *w/v*) was added to the crude drugs and the mixture was boiled for 1.5 h, after which the decoction was filtered. At this point, 8-fold water (1:8 *w/v*) was added to the crude drug residue. Then the mixture was boiled for additional 1 h. Following filtration, the decoction was combined with previous one and the sample was dried in a vacuum oven at 60 °C into a granulated powder which was

Table 1 Huangqin Tang samples used in the study and their calculated similarity values.

Sample No.	Batch No.	Collection site of Radix Scutellariae	Origin of Radix Scutellariae	Ratio extraction (%)	Similarity
S1	20120427	Beijing Tianheng Pharmacy	Neimeng	39.39	1.000
S2	20120519	Beijing Tongzhitang Pharmacy	Neimeng	33.22	0.998
S3	20120512	Beijing Tongrentang Pharmacy	Neimeng	32.56	0.990
S4	20120522	Beijing Hedantang Pharmacy	Neimeng	32.67	0.997
S5	20120523	Beijing Jinxiang Pharmacy	Hebei	32.11	0.996
S6	20120519	Beijing Yongantang Pharmacy	Hebei	33.78	0.999
S7	20120427	Beijing Xingainian Pharmacy	Hebei	38.89	0.999
S8	20120512	Beijing Wukesong Clinic	Hebei	36.00	0.998
S9	20120523	Beijing Jingzhitang Pharmacy	Shanxi	33.67	0.999
S10	20120426	Beijing Jinglongtang Pharmacy	Shanxi	33.56	0.999