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Analysis of the HPLC fingerprint and QAMS from *Pyrrosia* species

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ABSTRACT

An effective and comprehensive evaluation method for identifying the origin and assessing the quality of *Pyrrosiae Folium* has been established, based on analysis of high performance liquid chromatography (HPLC) fingerprint combined with the similarity analysis, hierarchical cluster analysis (HCA), principal component analysis (PCA) and the quantitative analysis multi-components by single marker (QAMS) method. 20 peaks of the common model were collected and used for the similarity analysis, HCA, PCA and QAMS analysis. These methods drew a similar conclusion that 42 *Pyrrosia* samples were categorized into three groups by HCA and PCA, and the majority of the samples with similar ingredients were mainly concentrated in the areas of Shanxi, Guangxi and Sichuan provinces. When QAMS method was compared with the external standard method (ESM), it was feasible to evaluate the quality of *Pyrrosia* herbals by the values of relative correction factors (RCFs) from chlorogenic acid (the internal standard) versus mangiferin, rutin and kaempferol. In conclusion, these methods were successfully applied to identification of the herbs origin and evaluation the quality of *Pyrrosia* materials. Therefore, these evaluation methods are promising to be widely applied in the quality control of Traditional Chinese Medicines (TCMs).

1. Introduction

Pyrrosiae Folium from the aerial parts of various medicinal plants of *Polypodiaceae*, as the Traditional Chinese Medicines (TCMs), is recorded three species of *Pyrrosia*, including *Pyrrosia sheareri* (Bak.) Ching, *Pyrrosia lingua* (Thunb) Farwell and *Pyrrosia petiolosa* (Christ) Ching in the Chinese Pharmacopoeia, and in clinic is used for the treatment of acute pyelonephritis, chronic bronchitis and bronchial asthma (Pharmacopoeia Committee of PR China, 2010). Previous studies have demonstrated that *Pyrrosiae Folium* possesses various biological properties, such as antibacterial, anti-inflammatory, hyperglycemic and antitumor activities (Xin et al., 2015; Yang et al., 2003; Mi et al., 2012). These curative effects might be attributed to their active chemical constituents, including chlorogenic acid, mangiferin, isomangiferin, rutin and kaempferol, etc.

As is well known that chlorogenic acid possess anti-inflammatory (Francisco et al., 2013) and antibacterial properties (Lou et al., 2011) and clinically is applied to the treatment of brain damage (Lee et al., 2012), gastric ulcer treatment (Shimovama et al., 2013). Mangiferin shows certain antitumor activity (Li et al., 2013) and could mitigate hyperglycemia in diabetes (Muruganandan et al., 2005; Guo et al., 2011; Miura et al., 2001; Sellamuthu et al., 2013). Rutin could inhibit inflammatory cytokines generation, prevent neuronal apoptosis and improve antioxidant activity (Mascaraque et al., 2014; Niture et al., 2014; Chen et al., 2014). Kaempferol could be served as standard chemotherapy drugs (Ramos, 2007) for amending cellular signaling pathways and promoting apoptosis (Zhang et al., 2008). The above four ingredients together with other constituents, like saponins, volatile oils and polysaccharides in a certain proportion, as the active ingredients of *Pyrrosiae Folium*, have played a better curative effect.

In recent years, chromatographic fingerprint analysis has been accepted as a strategy for quality assessment of herbal medicines and preparations by the WHO, the FDA and the State Food and Drug Administration (SFDA) of China (Liu et al., 2007; Yu et al., 2007; Freischmidt et al., 2015; Jin et al., 2006). Since fingerprint is characterized by more chemical information, some substitutes and adulterants have been distinguished from the genuine medicinal materials by chromatographic fingerprint method based on the presence or absence of a limited number of peaks (Schaneberg et al., 2003). So construction and evaluation of chromatographic fingerprints are of importance for the quality control of TCMs (Kim et al., 2012; Yi et al., 2007; Xie et al., 2006). Furthermore, identification of crude drugs based upon principle component

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analysis (PCA), hierarchical cluster analysis (HCA) and similarity analysis is more conducive to analyzing the chemical profiles of herbal medicine (Pierce et al., 2006; Nyambaka and Ryley, 2004; Debeljak et al., 2005; Chen et al., 2008; Gan and Ye, 2006).

It is known that due to the complexity of chemical constitutions of TCMs, the multi-components analysis for quality control of TCMs is more scientific and reasonable. Hence, a new method for quality control of raw material, quantitative analysis multi-components by single marker (QAMS) method was proposed and then widely used in the quantitative analysis of raw material and compound preparations in TCMs, and it was obtained using the relative correction factors (RCFs) calculated by the calibration curves of coexistent ingredients (Gao et al., 2009; Wang et al., 2015). However, the quality control of *Pyrrosia* herbal was evaluated only using the index parameter of chlorogenic acid (>2%) and ignored the other active ingredients, resulting in the flooding of *Pyrrosia* substitutes, adulterants and in inconsistent clinical effects. Considering the active multi-components of *Pyrrosia* raw materials, QAMS method was to be adopted to determine more active components.

In the present study, a comprehensive HPLC fingerprint method for *Pyrrosia* samples was to be constructed, which was characterized by more chemical information and could better reflect quality of *Pyrrosia* materials. The differences and similarities of the HPLC fingerprints were visually compared using HCA, PCA and similarity analysis. In the meantime, QAMS method was adopted to quantify the main active components by comparing with the external standard method (ESM) in all the *Pyrrosia* samples.

Table 1
Different species and geographical locations of 42 *Pyrrosia* samples in China.

No.	Species	Origins	Date of collection	Voucher number
A_1	*P. petiolosa*	Shaoxing, Zhejiang	2014.6	201460701B
A_2	*P. petiolosa*	Yangxian, Shanxi	2014.7	201460708B
A_3	*P. petiolosa*	Rizhao, Shandong	2014.9	201411003B
A_4	*P. petiolosa*	Deyang, Sichuan	2014.6	201460714B
A_5	*P. petiolosa*	Bozhou, Anhui	2014.8	201460715B
A_6	*P. petiolosa*	Pingdingshan, Henan	2014.8	201460717B
A_7	*P. petiolosa*	Hechi, Guangxi	2014.8	201460718B
A_8	*P. petiolosa*	Yibing, Sichuan	2014.8	2014110401B
A_9	*P. petiolosa*	Chengdu, Sichuan	2014.8	201460716B
A_{10}	*P. petiolosa*	Guiyang, Guizhou	2014.9	2014102602B
A_{11}	*P. petiolosa*	Kunming, Yunnan	2014.7	2014103101B
A_{12}	*P. petiolosa*	Ningqiang, Shanxi	2014.8	2014110302B
A_{13}	*P. petiolosa*	Chengdu, Sichuan	2014.6	201460711B
A_{14}	*P. petiolosa*	Changbaishan, Jilin	2014.8	2014607101B
A_{15}	*P. petiolosa*	Wuhan, Hubei	2014.8	201460713B
A_{16}	*P. petiolosa*	Fuyang, Anhui	2014.8	2014607105B
A_{17}	*P. petiolosa*	Wenzhou, Zhejiang	2014.8	201460701B
B_1	*P. shearери*	Xuzhou, Jiangsu	2014.8	201460704A
B_2	*P. shearери*	Yangxian, Hubei	2014.8	2014607103A
B_3	*P. shearери*	Bozhou, Anhui	2014.8	201460703A
B_4	*P. shearери*	Wenzhou, Zhejiang	2014.8	2014102901A
B_5	*P. shearери*	Chengdu, Sichuan	2014.6	2014607104A
B_6	*P. shearери*	Yibin, Sichuan	2014.8	201460702A
B_7	*P. shearери*	Baoding, Hebei	2014.9	201460709A
B_8	*P. shearери*	Anshun, Guizhou	2014.8	201460707A
B_9	*P. shearери*	Chengdu, Sichuan	2014.9	201460701A
B_{10}	*P. shearери*	Yulin, Guangxi	2014.8	201411002A
C_1	*P. lingua*	Weixian, Hebei	2014.8	201460708C
C_2	*P. lingua*	Longyan, Fujian	2014.8	201460705C
C_3	*P. lingua*	Yizhou, Guangxi	2014.8	201460707C
C_4	*P. lingua*	Guilin, Guangxi	2014.8	201460702C
C_5	*P. lingua*	Anshun, Guizhou	2014.8	2014102302C
C_6	*P. lingua*	Kunming, Hunan	2014.9	201403103C
C_7	*P. lingua*	Wenzhou, Zhejiang	2014.8	2014110501C
C_8	*P. lingua*	Hanyin, Shanxi	2014.8	201460701C
D_1	*P. tonkinensis*	Yizhou, Guangxi	2014.7	201460702E
D_2	*P. tonkinensis*	Yinzhou, Guangxi	2014.8	201460701E
E_1	*P. porosa*	Maoming, Guangxi	2014.7	2014103102E
E_2	*P. porosa*	Anshun. Guizhou	2014.9	2014102301E
F_1	*P. subfurfuracea*	Huangshan, Anhui	2014.8	201460703F
F_2	*P. subfurfuracea*	Yichang, Hubei	2014.8	201460703F
G	*P. drakeana*	Nanchang, Jiangxi	2014.8	201460701D

2. Experimental

2.1. Instruments

The chromatographic system was controlled by a Daojin LC-10 ATVP N2000 series HPLC chromatograph with diode-array detector. The data were processed using the software of computer aided similarity evaluation system (CASES, version 2004 A), and the SPSS software (Windows 19.0, USA) was used to execute cluster analysis and PCA analysis. The KQ-250E-type ultrasonic bath (Kunshan,

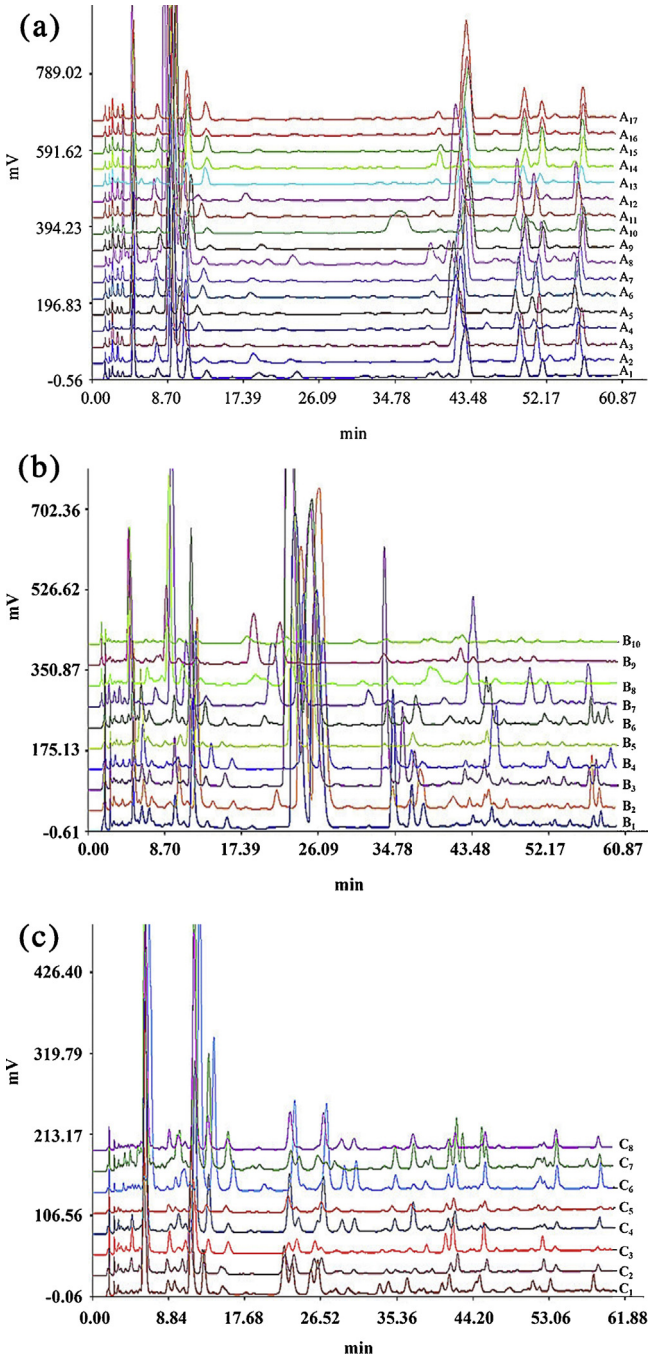

Fig. 1. HPLC Fingerprints for 17 *P. petiolosa* samples (a), 10 *P. shearери* samples (b) and 8 *P. lingua* (c) samples, respectively.