Biochemical Systematics and Ecology 76 (2018) 46–51

Contents lists available at ScienceDirect

Biochemical Systematics and Ecology

journal homepage: www.elsevier.com/locate/biochemsyseco

Rapid identification of Fritillariae Cirrhosae Bulbus and its adulterants by UPLC-ELSD fingerprint combined with chemometrics methods

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ARTICLE INFO

Article history:
Received 7 September 2017
Received in revised form
7 December 2017
Accepted 9 December 2017

Keywords:
Fritillariae Cirrhosae Bulbus
UPLC-ELSD
Fingerprint analysis
Chemometrics
Adulterants

ABSTRACT

Fritillariae Cirrhosae Bulbus (FCB) is a well-known and precious antitussive traditional Chinese medical herb. It is prone to be accidentally contaminated, deliberately substituted or admixed with other species of similar morphological characters. These adulterations might cause quality confusion and safety issues. In this study, a UPLC-ELSD fingerprint analysis with chemometric methods, including similarity analysis, hierarchical clustering analysis, and principal component analysis, were applied for the identification of FCB and its adulterants. Our results indicated that the combined method could efficiently identify and distinguish FCB from its adulterations. The proposed method provides a potential perspective for the quality control of traditional Chinese medicine.

1. Introduction

Fritillariae Cirrhosae Bulbus (FCB), known as Chuanbeimu in Chinese, has been used as one of the most important antitussive and expectorant drugs in traditional Chinese medicine (TCM) for thousands of years (Wang et al., 2016). According to the Pharmacopoeia of the People's Republic of China (Chinese Pharmacopoeia Commission, 2015), FCB origins from six species (*Fritillaria cirrhosa* D.Don, *F. unibracteata* Hsiao et K.C.Hsia, *F. przewalskii* Maxim., *F. delavayi* Franch., *F. taipaiensis* P.Y.Li, *F. unibracteata* Hsiao et K.C.Hsia var. *wabuensis* (S.Y.Tang et S.C.Yue) Z.D.Liu, S.Wang et S.C.Chen). Recently, the limited supply and high demand for FCB have triggered a noticeable jump in price. It was reported that the price of high-quality FCB products can reach as high as ¥ 3800 per kilogram in several major Chinese herbal medicine markets in November 2017 (http://www.zgycsc.com). Due to its high demand and price, adulterations have been admixed with the crude drugs of FCB unintentionally or for economic gain, such as *F. thunbergii* Miq., *F. hupehensis* P.K.Hsiao & K.C.Hsia and *F. anhuiensis* S.C.Chen & S.F.Yin (Jiang, 2010; Li et al., 2013). However, the components are different between FCB and its adulterants, and different components will lead to different therapeutic effects. Thus, the adulteration of herbal products is a threat to consumer safety. To date, gas chromatography (GC), Fourier transform infrared (FT-IR) spectroscopy, two-dimensional infrared (2D-IR) spectroscopy, liquid chromatography/mass spectrometry (LC-MS), high-pressure liquid chromatography/evaporative light scattering detector (HPLC-ELSD) and DNA barcoding technology have been applied to distinguish *Fritillaria* species (Li et al., 2000, 2010; Xiang et al., 2016; Ye and Wang, 2014; Zhou et al., 2010). These previously developed methods can be used for the authentication of *Fritillaria* samples, but they require long operating times and demands high level of expertise. Accordingly, developing a rapid and reliable method to distinguish FCB from its adulterations is urgently needed.

In the present study, by combining the chemometric methods such as similarity analysis (SA), hierarchical clustering analysis (HCA) and principal component analysis (PCA), we used UPLC-ELSD to develop a specific, practical and valid chromatographic fingerprint analysis approach for quality assessment and species differentiation of FCB and its adulterations. The proposed method provides a potential perspective for the quality control of traditional Chinese medicine.

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https://doi.org/10.1016/j.bse.2017.12.007
0305-1978/

2. Materials and methods

2.1. Materials and reagents

All samples were collected from different areas of China and are listed in Table 1. These samples were authenticated by Prof. Baozhong Duan (Dali University). The voucher specimens were deposited at the Herbarium of Dali University. The peimisine was isolated in our laboratory, as published earlier (Cao, 2008). Hupehenine, imperialine, peimine and peiminine (purity > 98%) were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). The chemical structures of these alkaloids are shown in Fig. 1. HPLC-grade methanol and acetonitrile were purchased from Fisher (Fisher Scientific, Fair lawn, NJ, USA). Deionized water was prepared using a Purelab Plus UV System (ELGA, UK). Triethylamine was obtained from Sigma-Aldrich (Saint Louis, MO, USA). Other chemicals (Beijing Analytic Co., ltd, Beijing, China) were of reagent grade.

2.2. Instrument

Chromatographic analysis was performed on a Waters ACQUITY

Table 1
Information and similarity analysis results of 26 batches of *Fritillaria* samples.

Sample No.	Species	Source areas	Parts	Similarity
S1	*F. cirrhosa* D.Don	Kangding, Sichuan	Bulbus	0.985
S2		Luding, Sichuan	Bulbus	0.943
S3		Kangding, Sichuan	Bulbus	0.958
S4		Daocheng, Sichuan	Bulbus	0.940
S5		Luding, Sichuan	Bulbus	0.961
S6		Kangding, Sichuan	Bulbus	0.971
S7		Daocheng, Sichuan	Bulbus	0.984
S8		Xiaojin, Sichuan	Bulbus	0.985
S9		Gongshan, Yunan	Bulbus	0.969
S10		Gongshan, Yunan	Bulbus	0.945
S11	*F. taipaiensis* P.Y.Li	Wuxi, Chongqing	Bulbus	0.920
S12		Wuxi, Chongqing	Bulbus	0.957
S13		Wuxi, Chongqing	Bulbus	0.943
S14	*F. delavayi* Franch	Kangding, Sichuan	Bulbus	0.941
S15		Gongshan, Yunan	Bulbus	0.924
S16		Luding, Sichuan	Bulbus	0.985
S17	*F. thunbergii* Miq.	Ninbo, Zhejiang	Bulbus	0.685
S18		Ninbo, Zhejiang	Bulbus	0.809
S19		Ninbo, Zhejiang	Bulbus	0.860
S20		Yinzhou, Zhejiang	Bulbus	0.859
S21		Yinzhou, Zhejiang	Bulbus	0.369
S22	*F. hupehensis* Hsiao et K.C.Hsia	Lichuan, Hubei	Bulbus	0.185
S23		Lichuan, Hubei	Bulbus	0.435
S24		Lichuan, Hubei	Bulbus	0.744
S25	*F. anhuiensis* S.C.Chen & S.F.Yin	Bozhou, Anhui	Bulbus	0.795
S26		Bozhou, Anhui	Bulbus	0.833

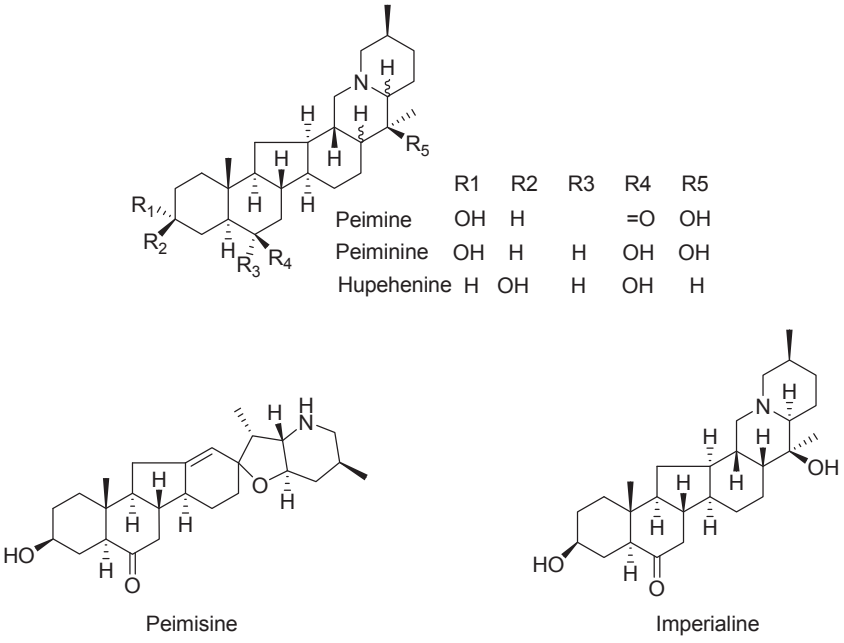

Fig. 1. The structure of the five compounds.