



Characterizing the major morphological traits and chemical compositions in the bulbs of widely cultivated *Fritillaria* species in China

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ABSTRACT

Many *Fritillaria* species have long been exploited as the natural source of the widely used traditional medicine 'bulbus *Fritillaria*'. The major morphological traits and chemical compositions were characterized in the bulbs of nine widely cultivated *Fritillaria* species in China. LSD analysis revealed that the morphological traits differed significantly among nine species, which were clustered into four groups using PCA; the ZB-Ft and WY-Fwu were relatively distantly related to other species. Morphological traits could be used to identify species which differ significantly in bulb size from the others, and 'short diameter of bulb' could be used as the most important identification indicators. Based on the chemical composition data, all those species were clustered into two groups. The alkaloid and saponin contents could be effective in the identification of morphologically similar species. Our results have important implications for the source control of medication safety and the rational development of bulbus *Fritillaria*.

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1. Introduction

Bulbus *Fritillaria* (i.e., dry bulbs of *Fritillaria* species; 'Beimu' in Chinese) is derived from the bulbs of various *Fritillaria* species, and has long been used as one of the most important antitussive, expectorant, and antihypertensive drugs in traditional Chinese medicine (Pharmacopoeia Commission of the People's Republic of China, 2010). Five kinds of Bulbus *Fritillaria* are recorded in Pharmacopoeia Commission of the People's Republic of China (2010 edition), among which Bulbus *Fritillariae cirrhosae* and Bulbus *Fritillariae thunbergii* are believed to be superb in quality, and are both widely used (Cheng et al., 2002). As one of the geo-authentic crude drugs in Sichuan Province, Bulbus *F. cirrhosae*, which refers to dry bulbs of *F. cirrhosae* (CB-Fc), *Fritillariae unibracteata* (CB-Fun) and *Fritillariae przewalskii* (CB-Fp), is famous for its healing effects against chronic cough and asthma. Statistics showed that Bulbus *F. cirrhosae* has been used as an ingredient of more than 200 Chinese medicines, e.g., Nin Jiom Pei Pa Koa (Xie et al., 2000). As there is no breakthrough in artificial plantation technologies, the original plants of Bulbus *F. cirrhosae* are mainly obtained from its wild species, which are only distributed at high altitudes in the Qinghai-Tibet Plateau, slow in growth, and thus unable to satisfy the market demands (Jin et al., 2009). This has also resulted in surging price of Bulbus *F. cirrhosae* and the contradiction between supply and demand is very serious. Bulbus *F. thunbergii* (ZB-Ft), which refers to dry bulb of *F. thunbergii*, is a member of the geo-authentic crude drugs "Zhebawei" in Zhejiang Province, its production ranks first in the commodity Bulbus *Fritillaria* and has been approved as a national health functional food for relieving cough and reducing sputum (Xiao, 2001). The dry bulb of *F. thunbergii* var. *chekiangensis* (DB-Ftv) is functionally similar to ZB-Ft, and is also exploited as an alternative source of ZB-Ft (Li et al., 1999). Bulbus *Fritillariae*

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anhuiensis (WB-Fa), the dry bulb of *Fritillaria anhuiensis*, is similar to Bulbus *F. cirrhosae* in chemical composition, but has relatively more stable yield and lower price, which makes it a good substitute for Bulbus *F. cirrhosae* (Tang and Wu, 2004). Bulbus *Fritillariae ussuriensis* (PB-Fus), whose tropism of taste, main functions, usage and dosage are very similar to Bulbus *F. cirrhosae*, is another alternative of the latter. Bulbus *Fritillariae walujewii* (XJ-Fwa) and Bulbus *Fritillariae wuyangensis* (WY-Fwu) are another two counterparts, and are commonly used by local people in Xinjiang Uighur Autonomous Region and Henan Province, respectively.

Pharmacological study found that Bulbus *Fritillaria* has such effects as reducing blood pressure, anti-inflammatory analgesic, anti-tumor, etc. (Zhu and Liao, 2008). As revealed by modern studies, alkaloids and saponins are the major low-molecular-weight active chemical compositions, while starch constitutes the highest macromolecular composition in the Bulbus *Fritillaria*. Among the total extract compounds from different Bulbus *Fritillaria*, the majority belong to alkaloids (84.2%) (Ge et al., 2001).

Bulbus *Fritillaria* has extensive original plant sources, complex interspecific variation and obvious pharmacodynamic differences. Due to the effective treatment, limited resources and high price of famous species, there exist some phenomena such as mixing the fake with the genuine and taking substandard products as fine products in market, which has resulted in confused clinical utilization. For example, the small DB-Ftv has been fraudulently used as Bulbus *F. cirrhosae*, and the big DB-Ftv as ZB-Ft (Wen, 2002; Liu, 2001). As various Bulbus *Fritillaria* differ in drug properties and toxicity differences, it is important to illuminate the medicinal material basis of the differences, identify the Bulbus *Fritillaria* correctly and ensure medicinal quality. It is directly related to the clinical effects and people's health safety. Researches about morphological characteristics and chemical composition differences have very important applications to germplasm identification and quality evaluation (Wu et al., 2009; Zhang et al., 2010). But to date, no detailed efforts have been reported on the quantitative variations in their morphological characters within and among the Bulbus *Fritillaria*. On the other hand, previous studies on chemical composition have been mainly confined to the assay of the alkaloids in Bulbus *F. cirrhosae*, ZB-Ft etc., but ignored the saponins and starch, the highest macromolecular compositions in the Bulbus *Fritillaria*. No studies have been carried out to compare the micromolecular and macromolecular chemical compositions among the Bulbus *Fritillaria*.

In this study, the major morphological traits and chemical compositions were characterized in the bulbs of nine widely cultivated *Fritillaria* species in China. Our primary objectives were to: (i) determine the characteristic indices for these species; (ii) investigate their interspecific relationships; and (iii) eventually provide references for the crude drug identification, the formulation of quality standards and the safe medication practice.

2. Materials and methods

2.1. Experimental materials

In August 2010, mature bulbs of nine *Fritillaria* species were sampled from their respective geo-authentic production areas, including Sichuan, Zhejiang, Jilin, Henan, Anhui Provinces and Xinjiang Uygur Autonomous Region (Table 1; Fig. 1). For each species, 20 bulbs were randomly collected and kept in $-80\text{ }^{\circ}\text{C}$ freezer for the subsequent analyses.

2.2. Morphological traits of bulbs

After rinsing and air drying, the bulbs were further dried to constant weight in a $60\text{ }^{\circ}\text{C}$ incubator. Seven morphological traits were characterized in the bulbs, i.e., bulb weight, bulb height, long/short diameters of bulbs, height of central buds, and long/short diameters of central buds.

Table 1
Sampling localities and major chemical compositions in the bulbs of the nine studied *Fritillaria* species.

Code	Species	Sampling localities	Traditional medicine name	Chemical compositions (mean \pm SD)		
				Total alkaloids (%)	Total saponins (%)	Starch (mg/g)
CB-Fc	<i>F. cirrhosae</i>	Songpan, Sichuan Province	Chuan-Beimu	0.094g \pm 0.010	3.436a \pm 0.240	7.244bc \pm 1.250
CB-Fun	<i>F. unibracteata</i>	Ganzi, Sichuan Province	Chuan-Beimu	0.118f \pm 0.008	2.917b \pm 0.116	6.858bcd \pm 1.217
CB-Fp	<i>F. przewalskii</i>	Kangding, Sichuan Province	Chuan-Beimu	0.166cd \pm 0.008	2.593b \pm 0.309	7.738 ab \pm 0.752
ZB-Ft	<i>F. thunbergii</i>	Pan'an, Zhejiang Province	Zhe-Beimu	0.234b \pm 0.020	1.524cd \pm 0.059	5.147e \pm 1.022
DB-Ftv	<i>F. thunbergii</i> var. <i>chekiangensis</i>	Dongyang, Zhejiang Province	Dong-Beimu	0.148de \pm 0.029	1.751c \pm 0.158	6.536bcd \pm 0.966
XJ-Fwa	<i>F. walujewii</i>	Tuoli, Xinjiang Uygur Autonomous region	Xiniang-Beimu	0.126ef \pm 0.026	1.531cd \pm 0.088	5.879de \pm 0.983
PB-Fus	<i>F. ussuriensis</i>	Tonghua, Jilin Province	Ping-Beimu	0.163d \pm 0.010	1.951cd \pm 0.223	7.767bc \pm 1.291
WY-Fwu	<i>F. wuyangensis</i>	Wuyang, Henan Province	Wuyang-Beimu	0.186c \pm 0.022	1.313d \pm 0.032	6.335cd \pm 0.866
WB-Fa	<i>F. anhuiensis</i>	Bozhou, Anhui Province	Wan-Beimu	0.380a \pm 0.022	1.726c \pm 0.096	8.546a \pm 1.255

Mean values with alphabetical suffices within the same column are statistically different at the significance level of 0.01 based on the protected least significant difference test.

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