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Variation of active constituents of an important Tibet folk medicine *Swertia mussotii* Franch. (Gentianaceae) between artificially cultivated and naturally distributed

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Abstract

Concentrations of seven phytochemical constituents (swertiamarin, mangiferin, swertisin, oleanolic acid, 1,5,8-trihydroxy-3-methoxyxanthone, 1,8-dihydroxy-3,7-dimethoxyxanthone and 1,8-dihydroxy-3,5-dimethoxyxanthone) of "ZangYinChen" (*Swertia mussotii*, a herb used in Tibetan folk medicine) were determined and compared in plants collected from naturally distributed high-altitude populations and counterparts that had been artificially cultivated at low altitudes. Levels of mangiferin, the most abundant active compound in this herb, were significantly lower in cultivated samples and showed a negative correlation with altitude. The other constituents were neither positively nor negatively correlated with cultivation at low altitude. Concentrations of all of the constituents varied substantially with growth stage and were highest at the bud stage in the cultivars, but there were no distinct differences between flowering and fruiting stages in this respect.

Keywords: Tibetan folk medicine; *Swertia mussotii*; Active constituents

1. Introduction

Swertia mussotii Franch. is a biennial herb of the family Gentianaceae that has been widely used for a long time in Tibetan folk medicine, under the name "ZangYinChen", to treat various conditions, including gall and liver disorders (Yang, 1991). Recent investigations have revealed that its major phytochemical constituents are mangiferin, swertiamarin, swertisin, oleanolic acid and three xanthones (Fig. 1) (Ding and Sun, 1980; Sun and Ding, 1981; Sun et al., 1991). These active constituents, especially mangiferin, have been found separately or collectively to have hepatoprotective (Liu et al., 1993; Komatsu et al., 1997), hypoglycemic (Song, 1986), anti-inflammatory (Banerjee et al., 2000), antioxidative (Ashida et al., 1994), antitubercular (Ghosal and Chaudhuri, 1975; Bian et al., 1998) and antifungal activities (Rodriguez et al., 1995), together with various other pharmacological properties (Rafatullah et al., 1993; Singha et al., 1993; Ji, 1995).

This species is strictly restricted to the high alpine lands of the Tibetan Plateau, at altitudes ranging between 3200 and 3800 m (Ho, 1988). The natural resources of *Swertia mussotii* have been declining in recent years because increasing numbers of flowering plants of the species have been harvested across its entire distribution, without leaving sufficient seeds to maintain its populations (Yang, 1991). This species has now been listed as endangered by the local governments and further harvesting has been prohibited in some parts of its natural distribution (Liu et al., 2001). However, the species has been successfully cultivated in agricultural areas at low altitude using a recently developed technique to break seed dormancy (Yang and Liu, 2005).

The purpose of this paper is to compare statistically the concentration of seven active constituents in *Swertia mussotii* using materials collected from natural, high-altitude popula-

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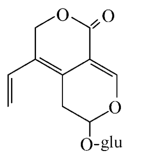
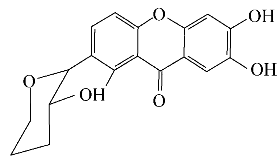
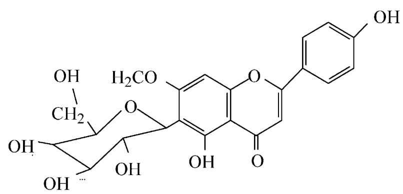


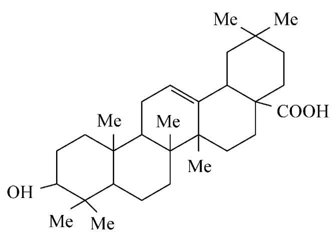
Swertiamarin (1)



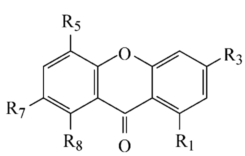
Mangiferin (2)



Swertisin (3)



Oleanolic acid (4)



1,5,8-trihydroxy-3-methoxyxanthone (5) R_1=R_5=R_8=OH, R_3=OCH_3
1,8-dihydroxy-3,7-dimethoxyxanthone (6) R_1=R_8=OH, R_3=R_7=OCH_3
1,8-dihydroxy-3,5-dimethoxyxanthone (7) R_1=R_8=OH, R_3=R_5=OCH_3

Fig. 1. Chemical structures of the active constituents (one to seven) quantified in this study.

tions and plants that were cultivated at low altitudes. This evaluation was undertaken to determine whether the accumulation of these compounds is affected by artificial cultivation at low altitude and assess the potential value of these cultivated plants. Traditionally, harvesting *Swertia mussotii* at the flowering stage is believed to give the highest yields of active constituents (Yang, 1991), but the variations of these constituents with growth conditions remains unknown. Thus, another objective of this research was to investigate the variation of the active constituents at different growth stages, in relation to cultivated controls.

2. Materials and methods

2.1. Materials

The geographical origin and altitude of both the cultivated plants and those from areas within the species' natural distribution are listed in Table 1. For the natural samples, 30 individuals, spaced more than 1 m apart, were randomly collected at the fruiting stage from each of four high-altitude localities from August to September 2002. The whole plants were used for analyses. Voucher specimens (Yang HL 0001-0023) were authenticated by Professor Ho Tingnong (Ho, 1988) and deposited in the herbarium at the Northwest Plateau Institute of Biology, Chinese Academy of Sciences (HNWP).

The seeds for cultivation were harvested from Chengduo in late September 2000 and planted in four localities in a low-altitude agricultural area after their dormancy had been broken in spring 2001. In summer 2001, the seeds germinated formed rosettes and their above-ground parts died back in winter. In April 2002, individuals re-emerged, formed buds in July, flowered in August and began to set fruit in late August. Each individual produced more than 30 flowers. Usually, the terminal flower on each cyme flowered first, but apart from this the inflorescences did not appear to follow any particular flowering sequence. In order to analyze the correlation (if any) between the concentrations of the active constituents and growth stages, cultivated samples from Nanshan were collected representing five stages: the rosette stage in August 2001, the bud stage in June 2002, the flowering stage in July, the post-flowering stage in early August 2002 and the fruiting stage in late August 2002. The bud stage is defined as the time when half of the buds have appeared. The samples in the flowering stage were collected when more than 50% of the plants' flowers had flowered. We collected individuals in the post-flowering stage when more than 70% of their flowers had been pollinated, but no fruit had matured. For fruiting samples, we collected individuals where more than 70% of the fruits had matured and the top fruit had opened and re-

Table 1
The location and altitude of *Swertia franchetiana* populations sampled in this study

Sample locality	Altitude (m)	Longitude	Latitude	Patterns	Growth stages
Xining	2200	36°36.684′	101°45.009′		Fruiting
Nanshan	2300	36°35.188′	101°11.290′	Artificially cultivated	Different growth stages
Datong	2500	36°37.177′	101°10.950′		Fruiting
Huangzhong	2620	36°32.211′	101°25.150′		Fruiting
Jiangda	3240	31°35.362′	98°23.090′		Fruiting
Yushu	3520	32°15.402′	96°56.703′	Naturally distributed	Fruiting
Nangqian	3600	32°25.347′	96°24.370′		Fruiting
Chengduo	3660	33°19.979′	98°52.139′		Fruiting