

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 “Zang Yin Chen” (*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.

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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 “Zang Yin Chen”, 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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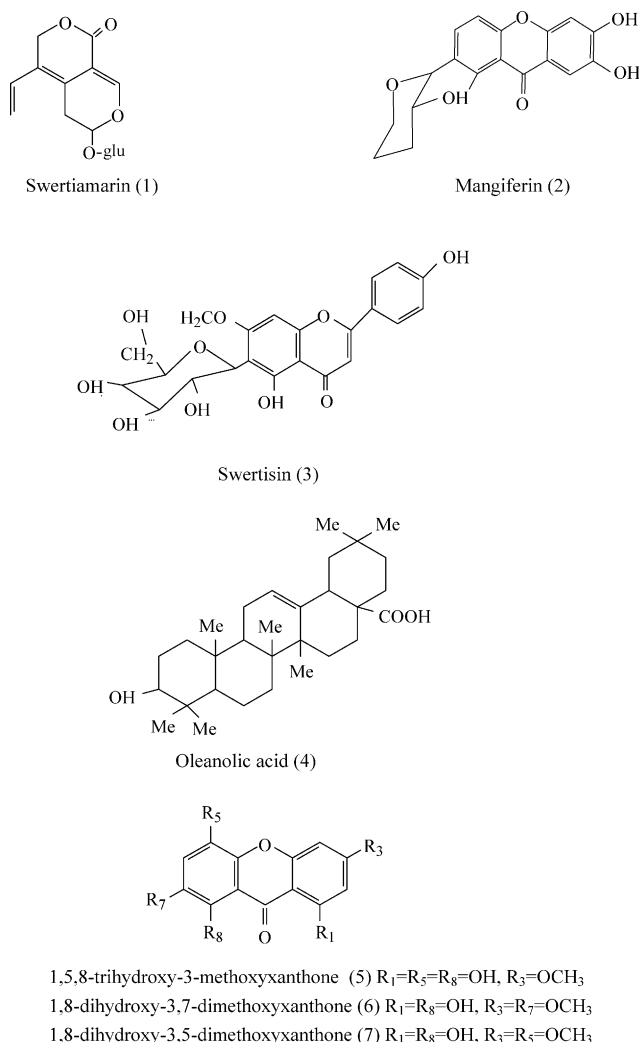


Fig. 1. Chemical structures of the active constituents (one to seven) quantified in this study.

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

leased seeds. The remaining samples from natural localities and the other two cultivation localities were sampled at the fruiting stage. From each population or collection, we randomly chose 30 individuals that were spaced more than 1 m apart. The collected individuals were air-dried and the whole plants were cut and mixed for analyses.

2.2. Analytical methods

All analyses were repeated at least four times, using methods suggested by Demizu et al. (1986) and Menković et al. (2000a,b). The compounds were extracted in two batches, from equal amounts of material. The optimized methods suggested by Ding and Sun (1980) by using 70% methanol were used to extract swertiamarin, mangiferin and swertisin, and those optimized processes suggested by Sun and Ding (1981) by using 100% ethanol to extract oleanolic acid and the three xanthenes. All extracts were analyzed using an HPLC chromatograph (Waters 600E) equipped with an ultraviolet detector (Waters 486) and a Phenomenex kromasil C₁₈, 5 μm, 250 mm × 4.60 mm column. The HPLC protocol developed for analyzing the bitter-tasting swertiamarin, flavonoids (swertisin) and xanthenes by Demizu et al. (1986) was adopted. Chemical structures of seven compounds are depicted in Fig. 1. Five different HPLC runs were used to measure these compounds, respectively. Typical HPLC chromatograms were given in Fig. 2. The mobile phases were methanol and water in the following proportions: 32:68 for swertiamarin and mangiferin; 45:55 for swertisin; 96:4 for oleanolic acid and 78:22 for the three xanthenes. The same

proportion mobile phase run is used to measure one compound in sample for once time, next mobile phase began after one over at different time. Swertiamarin and mangiferin did not run one time, with the same concentration mobile phase because of much different contents in one sample. We diluted the same sample of 10% for measuring mangiferin.

Standards for all seven compounds were isolated and purified in previous studies (Ding and Sun, 1980; Sun and Ding, 1981; Sun et al., 1991). To calculate the concentrations of the seven compounds present in the samples, the previously separated standards were weighed, dissolved in 1 ml of methanol or ethanol and diluted to give a series of concentration (0.1, 0.2, 0.3, 0.4 and 0.5 mg/ml). Three injections were performed for each dilution. Concentrations were determined by injecting 10 μl of the standard solutions or sample extracts at a flow rate of 1 ml/min at room temperature. The absorbance peaks of the compounds were at 227 nm for swertiamarin, 220 nm for oleanolic acid (Wang, 1994) and 254 nm for mangiferin, swertisin and the three xanthenes. A standard curve was generated for each compound using linear least-square regression equations derived from the resulting peak areas.

2.3. Statistics

All statistical analyses were performed using Microsoft Excel 2000 or the SPSS 10.0 for Windows software package. The mean values obtained in the different groups were compared by one-way ANOVA, post hoc LSD and *t*-tests, assuming that differences between means were significant at probability levels <0.05. Simple linear correlation analysis was used to obtain indications of the correlations and strength of the relationships between pairs of variables.

3. Results

Table 2 shows the concentrations of the seven active constituents: swertiamarin (1), mangiferin (2), swertisin (3), oleanolic acid (4), 1,5,8-trihydroxy-3-methoxyxanthone (5), 1,8-dihydroxy-3,7-dimethoxyxanthone (6) and 1,8-dihydroxy-3,5-dimethoxy-xanthone (7); found in the four natural populations and four cultivated collections at the fruiting stage. Levels of two of the most abundant and important constituents, mangiferin and oleanolic acid, were found to vary significantly among the four natural populations ($P < 0.001$) and the former showed a negative correlation with altitude (Table 3). No distinct difference was detected for the remaining constituents among populations ($P > 0.05$). However, all of the constituents were found to vary greatly among the four cultivation localities ($P < 0.001$), except for swertiamarin ($P > 0.05$).

The comparative analyses of active constituents at the fruiting stage showed that levels of mangiferin, the most abundant of the active constituents in *Swertia mussotii*, were significantly lower in the plants cultivated at low altitude than in the plants from the natural, high-altitude populations

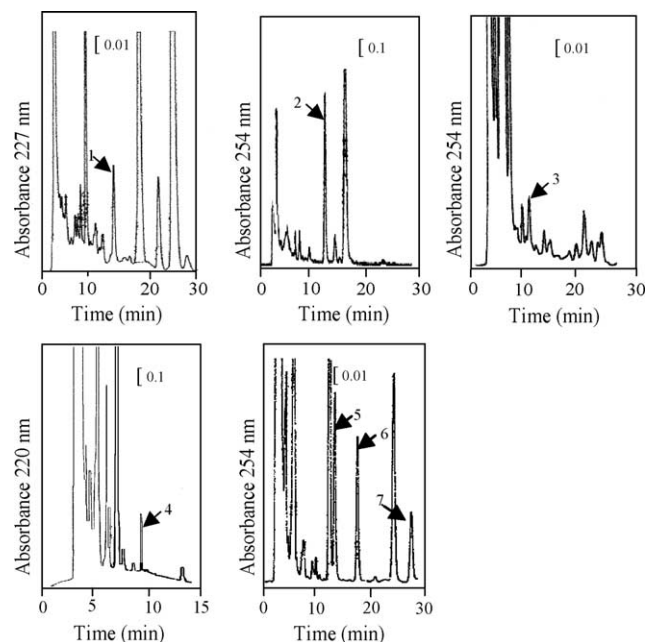


Fig. 2. The typical chromatogram of the seven compounds swertiamarin (1), mangiferin (2), swertisin (3), oleanolic acid (4), 1,5,8-trihydroxy-3-methoxyxanthone (5), 1,8-dihydroxy-3,7-dimethoxyxanthone (6) and 1,8-dihydroxy-3,5-dimethoxyxanthone (7).

Table 2

Concentrations of swertiamarin (1), mangiferin (2), swertisin (3), oleanolic acid (4), 1,5,8-trihydroxy-3-methoxyxanthone (5), 1,8-dihydroxy-3,7-dimethoxyxanthone (6) and 1,8-dihydroxy-3,5-dimethoxyxanthone (7) at the fruiting stage from four artificial cultivated samples and four natural populations

	Xining (mg/g)	Nanshan (mg/g)	Datong (mg/g)	Huangzhong (mg/g)	Jiangda (mg/g)	Yushu (mg/g)	Chengduo (mg/g)	Nangqian (mg/g)
1	1.52 ± 0.17	1.80 ± 0.63	1.52 ± 0.54	1.67 ± 0.59	1.56 ± 0.42	1.73 ± 0.37	1.98 ± 0.56	1.72 ± 0.62
2	15.10 ± 0.54	23.38 ± 0.53	18.08 ± 0.69	15.46 ± 1.48	21.54 ± 0.28	31.25 ± 7.03	44.21 ± 3.72	33.07 ± 0.70
3	0.76 ± 0.05	0.21 ± 0.03	0.17 ± 0.05	0.49 ± 0.22	0.97 ± 0.63	0.41 ± 0.20	0.71 ± 0.61	0.38 ± 0.05
4	3.09 ± 0.25	2.53 ± 0.06	2.49 ± 0.30	3.07 ± 0.30	5.03 ± 0.27	1.94 ± 0.66	2.56 ± 0.68	2.88 ± 0.30
5	1.57 ± 0.01	1.84 ± 0.25	0.78 ± 0.05	0.69 ± 0.12	7.17 ± 2.47	1.95 ± 0.49	1.93 ± 1.36	1.39 ± 0.02
6	0.95 ± 0.03	0.52 ± 0.04	0.61 ± 0.01	0.93 ± 0.05	0.08 ± 0.26	0.21 ± 0.01	0.23 ± 0.86	0.69 ± 0.01
7	0.25 ± 0.01	0.24 ± 0.01	0.12 ± 0.01	0.23 ± 0.01	1.63 ± 0.14	0.24 ± 0.02	0.22 ± 0.14	0.22 ± 0.01

Values are means ± S.E.M. ($n \geq 4$).

Table 3

Matrix correlation of altitude and analyzed constituents: swertiamarin (1), mangiferin (2), swertisin (3), oleanolic acid (4), 1,5,8-trihydroxy-3-methoxyxanthone (5), 1,8-dihydroxy-3,7-dimethoxyxanthone (6) and 1,8-dihydroxy-3,5-dimethoxyxanthone (7)

	1	2	3	4	5	6	7
Altitude	0.529	0.827*	0.243	0.200	0.191	0.137	0.195
1		0.859*	-0.09	0.258	-0.153	-0.070	-0.288
2			0.485	-0.338	0.850**	0.863**	0.826*
3				0.052	0.658	0.638	0.087
4					-0.077	-0.049	-0.139
5						0.987**	0.980**
6							0.945**

* Significant at the 0.05 probability level.

(Fig. 3). The other constituents showed neither a positive nor a negative correlation with cultivation at the low altitude. This finding is corroborated by the matrix correlation for all analyzed parameters (Table 3), in which mangiferin shows a negative correlation with altitude, but no correlation between the concentration and altitude for the remaining constituents. The concentration of mangiferin shows a positive correlation with the production of swertiamarin, but the latter shows no correlation with altitude. The matrix further reveals significant correlations among the three xanthenes, and between the xanthenes and oleanolic acid.

Table 4 shows the variation in levels of the active constituents with growth stages. All constituents peaked at the bud stage ($P < 0.05$ for all tests) and then decreased. Significant differences ($P < 0.05$) were also found between the flowering stage and fruiting stage in concentrations

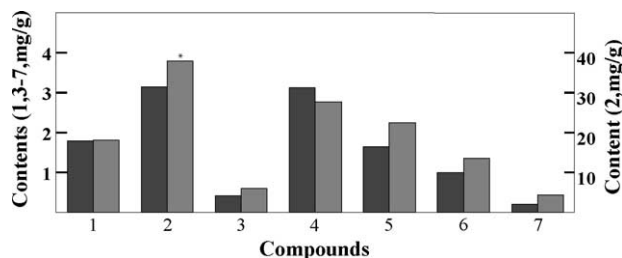


Fig. 3. Mean levels of swertiamarin (1), mangiferin (2), swertisin (3), oleanolic acid (4), 1,5,8-trihydroxy-3-methoxyxanthone (5), 1,8-dihydroxy-3,7-dimethoxyxanthone (6) and 1,8-dihydroxy-3,5-dimethoxyxanthone (7) in four cultivated samples (black bars) and four natural populations (gray bars). S.E.M. values (not shown for clarity), were all $\leq 10\%$ of the means, $n \geq 4$. *Significant at the 0.05 probability level.

Table 4

Contents of swertiamarin (1), mangiferin (2), swertisin (3), oleanolic acid (4), 1,5,8-trihydroxy-3-methoxyxanthone (5), 1,8-dihydroxy-3,7-dimethoxyxanthone (6) and 1,8-dihydroxy-3,5-dimethoxyxanthone (7) at different growth stages

	Rosette stage (mg/g)	Bud stage (mg/g)	Flowering stage (mg/g)	Fruiting stage (mg/g)
1	1.66 ± 0.12	3.63 ± 0.15	2.59 ± 0.76	1.80 ± 0.63
2	89.17 ± 3.99	144.11 ± 14.01	38.73 ± 2.39	23.38 ± 0.53
3	0.25 ± 0.18	1.04 ± 0.30	0.57 ± 0.31	0.21 ± 0.03
4	2.49 ± 0.85	3.97 ± 2.49	2.60 ± 0.92	2.53 ± 0.06
5	5.16 ± 0.93	5.90 ± 0.34	1.53 ± 0.04	3.84 ± 0.25
6	1.62 ± 0.48	3.39 ± 0.08	0.99 ± 0.03	1.52 ± 0.04
7	0.36 ± 0.31	0.25 ± 0.03	0.09 ± 0.00	0.24 ± 0.01

Values are means ± S.E.M. ($n \geq 4$).

of 1,5,8-trihydroxy-3-methoxyxanthone and 1,8-dihydroxy-3,7-dimethoxyxanthone (both higher in the latter), but not for any of the other compounds.

4. Discussion

For most plants used in herbal medicines, e.g., rhubarb (Iizuka et al., 2004) the concentration of the active components has been found to vary greatly among plants from different origins. Therefore, before attempting to cultivate *Swertia mussotii* for medicinal purposes at low altitude, it is essential to compare concentrations of its active constituents in cultivated plants and samples from natural, high-altitude populations. Our study demonstrated that concentrations of mangiferin, the most abundant of the active constituents in *Swertia mussotii*, were distinctly lower in the plants cultivated

in the low-altitude habitats. However, this habitat-related shift is consistent with the substantial variation found in levels of mangiferin among the different natural populations. In contrast, other constituents, such as the xanthenes, which show less variation among natural populations, were found to change less. Our results indicate that the quality of both cultivated and naturally collected *Swertia mussotii* should be carefully monitored when using it to make the related folk medicines. Because of the distinct decrease in mangiferin, the cultivated *Swertia mussotii* is likely to be less effective than those from natural populations.

Swertia mussotii has traditionally been harvested to make the folk medicine derived from it at the flowering stage (Yang, 1991). However, we found that the active constituents peaked at the bud stage in the early summer, rather than the flowering or fruiting stages in August or September. This is in marked contrast to roots of *Radix astragali*, in which concentrations of the major active constituents peak (and they are best collected) in September to October (Ma et al., 2002). However, the phenology of the detected variations in constituents in *Swertia mussotii* is similar to that of reported changes in quercetin levels in *Apocynum venetum* and *Poacynum hendersonii*. The content of quercetin in the leaves increases significantly during vegetative growth of the plants and peaks in the summer when they blossom (Ma et al., 2003). The developmental patterns of active constituents in *Swertia mussotii* imply that the best stage for harvesting this species for making the medicine is at the bud stage. However, the biomass at the bud stage is only half that at the flowering and fruiting stages. Since levels of the active constituents at the fruiting stage are not significantly different from those at the flowering stage, our results imply that it is better to harvest *Swertia mussotii* at the fruiting stage, leaving seeds to produce further offspring for this biennial herb in the future.

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