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ORIGINAL PAPER

Spatial variation profiling of four phytochemical constituents in *Gentiana straminea* (Gentianaceae)

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Abstract *Gentiana straminea* is the famous Tibetan folk medicine thought to cure various diseases. Historically, the Qinghai–Tibetan region has been considered as the geo-authentic production area of "Mahua Jiao," where large quantities of the medicine are grown. However, there is still little known about the phytochemical constituent spatial variation of this species. In order to find the differences between the main phytochemical constituents of *G. straminea* and to provide comprehensive information for quality evaluation, four main bioactive compounds (loganic acid, swertiamarin, gentiopicroside and sweroside) were analysed in 26 populations grown in areas with elevations ranging from 2320 to 4720 m across the Qinghai–Tibetan Plateau. The results showed that the four phytochemical constitutes' concentrations varied greatly in the spatial profiling of the Qinghai–Tibetan region. Throughout the range of distribution of this species, no altitudinal, latitudinal or longitudinal trends have proven to be significant in any of the four constitutes' concentrations or their summation. Furthermore, hierarchical clustering analysis and statistical tests showed that four populations (Liu0609-18, Liu0609-15, Liu2006-13-9 and Liu0609-22) had total constitute contents that were higher than other populations. The spatial profiling of the four phytochemical constituents suggests that the geo-authentic producing area of this species exists at a few regions within the Qinghai province, which could be attributed to specific environmental or genetic factors.

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Introduction

Gentiana straminea, a well-known Tibetan folk medicine also known as "Mahua Jiao," of the family Gentianaceae, has been used to treat fungal and bacterial infections, hepatitis, constipation, rheumatism, pain and hypertension for thousands of years [1, 2]. The main active constituents of *G. straminea* comprise four different iridoid glycosides (i.e. gentiopicroside, loganic acid, swertiamarin and sweroside) [3–5]. As a member of the *Gentiana* genus, *G. straminea* not only has close relations with the *Gentiana macrophylla* Pall [6], but it also has similar active constituents with other species in the *Gentiana* sect. *Cruciata* [7]. *G. straminea* is one of three representational herbal medicines of "Qinjiao" [8].

Although endemic to the Qinghai–Tibetan Plateau (QTP), the species is widely distributed in areas ranging in altitude from 2600 to 5000 m [9]. Historically, the Qinghai and Tibet provinces were thought to be the "geo-authentic" producing regions of "Mahua Jiao". The geo-authentic herbs are believed to have the highest quality of all the samples throughout different regions and to be the most effective for pharmaceutical use [10]. At present, the QTP is still the best known region of the "Mahua Jiao" producing area. The QTP covers nearly 2.5 million km^2 and is the largest continuous high-altitude ecosystem in the world [11]. The climate and environment on the QTP are extremely harsh; thus, genetic diversity [12, 13] and physiological character [14, 15] may suffer from these factors. Although analysis methods for the main active compounds have been set up for several years [16, 17] and the chemical constituents have been determined

[7], comprehensive spatial profiling of the active constituents of the species is still lacking.

As the natural resources of "Qinjiao" have become scare in recent years due to increasing market demand, overexploitation and habitat loss [18], many regions of western China are interested in developing and exploring the wild *G. straminea* in the Qinghai–Tibetan region as a way to meet the increasing "Qinjiao" market demand [19, 20]. Therefore, there is a pressing need to study the chemical constituent variation of the species for quality evaluation, protection and genetic utilisation in the Qinghai–Tibetan region.

In this study, the quantities of four main marker constituents (gentiopicroside, loganic acid, swertiamarin and sweroside) from the roots of *G. straminea* collected from 26 different populations across the QTP were determined by high-performance liquid chromatography (HPLC). The phytochemical variation profiles were also then discussed. The results provide the first comparison between the different populations of this species across the QTP based on quantitative measurements of their main active constituents, as well as useful information on the development, conservation and further exploration of this species.

Materials and methods

Plant materials

Plant roots were collected from 26 populations growing at different locations during the harvest season (Table 1), covering the entire QTP. All samples were collected during the 2–3 years of the plant's ingathering stage in order to avoid yearly differences. In each population, 20 individual roots were air-dried, crushed and mixed completely. All samples were authenticated by the second and third authors. Voucher specimens of all samples were deposited at the Herbarium of the Northwest Plateau Institute of Biology, the Chinese Academy of Sciences, in the People's Republic of China (HNWP).

Analytical methods

Standard compounds

Standards of gentiopicroside and swertiamarin were purchased from the National Institute for the Control of

Table 1 The location of *Gentiana straminea* population samples in this study

Population	Location	Longitude	Latitude	Altitude (m)
Liu2006183	Yanshiping, Qinghai	92°03′482″	33°33′944″	4720
Liu1901	Dangxiong, Tibet	92°34′057″	31°46′000″	4700
Liu2006193	Luomaxiang, Tibet	91°49′909″	31°17′978″	4620
Liu2006110	Zaduo, Qinghai	94°46′016″	32°54′201″	4370
Liu2006092	Nangqian, Qinghai	96°02′626″	32°57′003″	4220
Liu2006088	Zaduo, Qinghai	95°35′735″	32°52′968″	4100
Liu1798	Dari, Qinghai	100°25′089″	33°18′001″	4020
Liu0609-26	Xinghai, Qinghai	99°99′054″	35°06′156″	3950
Liu0609-11	Zeku, Qinghai	101°05′412″	35°03′058″	3860
Liu0609-12	Henan, Qinghai	101°62′021″	34°75′564″	3750
Liu0609-19	Maqin, Qinghai	100°26′854″	34°49′245″	3600
Liu0609-7	Maixiushan, Tongren, Qinghai	102°63′005″	35°55′036″	3500
Liu0609-15	Hebei, Tongde, Qinghai	100°48′721″	34°47′195″	3450
Liu2006129	Riyueshan, Qinghai	101°05′114″	36°26′245″	3480
Liu2006330	Hongyuan, Sichuan	102°32′709″	32°06′722″	3440
Liu2006152	Delingha, Qinghai	97°20′058″	37°27′273″	3400
Liu0609-18	Tongde, Qinghai	100°63′023″	35°25′045″	3385
Liu2006139	Qinghai Lake, Qinghai	99°40′751″	36°46′729″	3380
Liu0609-22	Banma, Qinghai	100°53′254″	32°42′658″	3350
Liu0609-21	Wosai, Dari, Qinghai	100°68′351″	32°74′254″	3350
Liu2006138	Jiangxi Sea, Qinghai	99°58′395″	36°39′595″	3210
Haibei Station	Haibei Station, Qinghai	101°12′052″	37°29′002″	3200
Liu1218	Bomi, Tibet	96°12′29″	29°39′267″	3160
Liu0609-6	Maixiu, Tongren, Qinghai	102°63′154″	35°55′021″	3000
Liu Duoba	Duoba, Huangzhong, Qinghai	101°25′150″	36°32′211″	2620
Liu Jinchuan	Jinchuan, Sichuan	102°03′235″	31°48′056″	2320

Pharmaceutical and Biological Products (NICPBP, no. 200308, 0785-200303, Beijing, China). Loganic acid and sweroside were provided by Professor Yulin Li (Northwest Plateau Institute of Biology, the Chinese Academy of Sciences). The purity of all standard compounds was ≥97 % (determined by HPLC).

Extraction

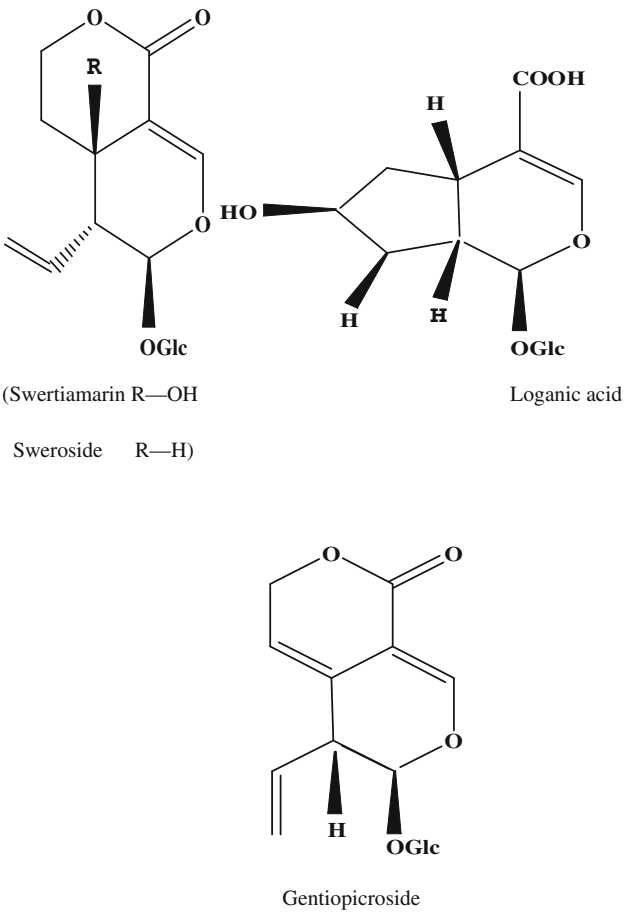
The materials were extracted at 25 °C for 30 min with 25 ml of methanol in an ultrasonic bath. All extracts were filtered through a 0.45-μm nylon filter and prepared for analysis.

HPLC analysis

An HPLC chromatograph (Waters 515E) equipped with an ultraviolet detector (Waters 2996), chromatograph workstation (Waters Empower Pro) and Alltech® C_{18} 5 μm (4.6 × 250 mm) column was used to analyse all samples, and all analyses were repeated at least four times using the same methods [16, 20]. The absorbance peak wavelengths were 238 nm for loganic acid and swertiamarin, and 246 nm for gentiopicroside and sweroside. An absorbance wavelength of 240 nm was chosen as the determined wavelength because, at this condition, the peaks were symmetrical and the baselines were smooth. The injection volume was 10 μl and the flow rate was 1.0 mL/min at 240 nm [16]. The gradient mobile phases were a mixture of (A) methanol and (B) water from methanol:water (0.5 % acetic acid) 19:81 to 25:75 (v/v). The solvent gradient in volumetric ratios of solvents A and B was as follows: 0–10 min, 19A/81B; 10–20 min, 25A/75B. Detection was carried out using 240 nm as the preferred wavelength. The chemical structures of the four compounds are depicted in Fig. 1. Typical HPLC chromatograms are depicted in Fig. 2.

Statistical analyses

Statistical analysis of the data was performed using Microsoft Excel 2003 and the SPSS 16.0 software package for Windows. The mean values obtained in the different groups were compared by one-way analysis of variance (ANOVA), post hoc least significant difference (LSD) and *t*-tests. Differences between the means were assumed to be statistically significant at probability levels of $p < 0.05$. Simple linear correlation analysis was used to obtain a measure of the correlation and strength of the relationships between the variables. Hierarchical clustering analysis of samples from 26 batches was performed based on the variation patterns of the four active constituents of each batch.



(Swertiamarin R—OH Loganic acid

Sweroside R—H)

Gentiopicroside

Fig. 1 Chemical structures of the four determined iridoid glycosides

Results

Contents of the four active constituent variations

Using the available methods [16, 21], the four constituents of *G. straminea* across the QTP were measured. As shown in Table 2, there was significant variation in the quantities of the four constituents in different regions. The content of loganic acid ranged from 0.479 to 1.298 % (Fig. 3). The highest concentration of loganic acid was about 1.74-fold of the average content (0.381 %). Similarly, the highest content of swertiamarin in a sample varied from 0.128 to 0.865 %. Liu0609-18 (3385 m) had a swertiamarin concentration 6.7 times higher than that of the population Liu2006138 (3210 m) ($p < 0.001$). The gentiopicroside concentration ranged from 3.035 to 15.592 %. The sample containing the highest content of sweroside was Liu0609-22 (1.9669 %), which was 32 times higher than that of the lowest content of Liu2006193 (0.0581 %).

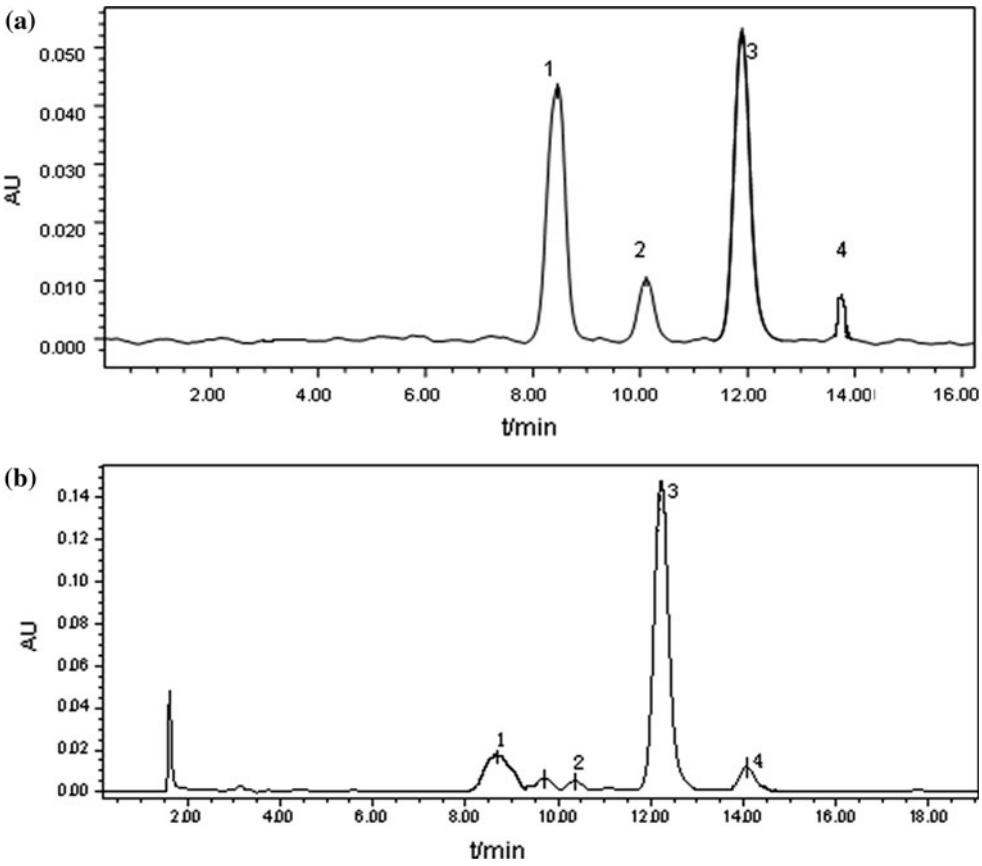


Fig. 2 High-performance liquid chromatography (HPLC) chromatograms of reference substance (**a**) and samples (**b**). **a** Standard. **b** *Gentiana straminea*. *1* loganic acid, *2* swertiamarin, *3* gentiopicroside, *4* sweroside

Hierarchical clustering analysis

The *Gentiana* genus is divided into 12 sections [9]. Some studies show common characteristic peak clustering among the section of *Cruciata* [22]. In order to identify the diverse chemical characteristics of the populations, 26 batches of samples were analysed with the SPSS software. The results showed that the population could be divided into four groups (Fig. 4). Groups A and B each contained 11 samples, while groups C and D each had two samples. Interestingly, the C and D populations appeared separate from groups A and B. Population D in Tongde (3340 m) had the highest constituent concentrations of all the populations ($p < 0.05$), and population C also had a higher total constituent concentration than the average A and B groups. In order to identify the difference between these groups, one-way ANOVA was performed (Table 2). The results showed that the total content of groups C and D was significantly higher than that of groups A and B (Table 3). In addition, the average content of group B was also higher than that of group A, and the average content of group D had significantly more loganic acid and sweroside than that of group C (Table 3). In fact, group D had more than 13.78-fold of the sweroside content than that of group C. These results show that there are different quality *G. straminea* populations on the QTP and "geo-authentic" production areas exist in several regions.

Correlation analysis of the four constituents

The relationship between constituent concentration and altitude was also analysed. Although the four marker compound concentrations fluctuated greatly between different altitudes (Fig. 3a–d), the Pearson correlation showed no distinct relationship between the four constituents and the four total concentrations and altitude. The correlation coefficients were 0.243, −0.081, −0.135 and 0.089, respectively (Table 4). In the same way, there was no significant correlation between the four active constituent concentrations and longitudes or latitudes; the coefficient is also small ($p < 0.05$) (Table 4).

In addition, the results showed that there is a significant positive correlation among the four constituents (Table 4). Except for gentiopicroside and sweroside, the concentration correlation of the four constituents is significant ($p < 0.05$) or extremely significant ($p < 0.01$). This might suggest that the four constituents are synthesised by the genetic controls in a conserved related pathway.

Table 2 Content of the four constituents in different populations

Population	Loganic acid (% ± SD)	Swertiamarin (% ± SD)	Gentiopicroside (% ± SD)	Sweroside (% ± SD)
Liu2006183	0.907 ± 0.007	0.329 ± 0.004	8.710 ± 0.193	0.072 ± 0.030
Liu1901	0.779 ± 0.004	0.317 ± 0.005	4.121 ± 0.048	0.129 ± 0.006
Liu2006193	0.509 ± 0.008	0.212 ± 0.019	4.300 ± 0.079	0.058 ± 0.006
Liu2006110	1.034 ± 0.061	0.570 ± 0.099	9.927 ± 0.426	1.396 ± 0.074
Liu2006092	0.540 ± 0.003	0.280 ± 0.008	5.975 ± 0.018	0.072 ± 0.002
Liu2006088	0.920 ± 0.004	0.395 ± 0.004	7.319 ± 0.013	0.315 ± 0.054
Liu1798	0.897 ± 0.002	0.570 ± 0.064	7.785 ± 0.123	0.317 ± 0.019
Liu0609-26	0.862 ± 0.077	0.346 ± 0.051	6.465 ± 0.177	0.560 ± 0.123
Liu0609-11	0.922 ± 0.060	0.349 ± 0.028	6.070 ± 0.322	0.426 ± 0.029
Liu0609-12	1.041 ± 0.015	0.565 ± 0.013	9.740 ± 0.133	0.243 ± 0.006
Liu0609-19	0.617 ± 0.002	0.423 ± 0.032	6.804 ± 0.089	0.732 ± 0.068
Liu0609-7	0.813 ± 0.019	0.378 ± 0.004	9.368 ± 0.157	0.208 ± 0.002
Liu0609-15	0.764 ± 0.009	0.655 ± 0.007	11.868 ± 0.126	0.067 ± 0.002
Liu2006129	0.502 ± 0.003	0.211 ± 0.010	3.823 ± 0.037	0.745 ± 0.030
Liu2006330	0.701 ± 0.003	0.231 ± 0.005	4.895 ± 0.043	0.162 ± 0.007
Liu2006152	0.572 ± 0.007	0.208 ± 0.009	4.389 ± 0.007	0.328 ± 0.003
Liu0609-18	0.626 ± 0.009	0.865 ± 0.017	15.592 ± 0.179	0.176 ± 0.003
Liu2006139	0.649 ± 0.024	0.322 ± 0.023	5.403 ± 0.120	0.232 ± 0.019
Liu0609-22	1.298 ± 0.003	0.808 ± 0.002	11.768 ± 0.024	1.967 ± 0.017
Liu0609-21	0.663 ± 0.012	0.452 ± 0.015	7.711 ± 0.020	0.253 ± 0.011
Liu2006138	0.479 ± 0.003	0.128 ± 0.0004	3.035 ± 0.073	0.103 ± 0.008
Haibei Station	0.797 ± 0.006	0.452 ± 0.001	8.242 ± 0.085	0.243 ± 0.005
Liu1218	0.748 ± 0.011	0.326 ± 0.035	5.477 ± 0.054	0.066 ± 0.004
Liu0609-6	0.536 ± 0.006	0.320 ± 0.007	5.713 ± 0.057	0.072 ± 0.001
Liu Duoba	0.749 ± 0.012	0.454 ± 0.017	8.239 ± 0.021	0.093 ± 0.004
Liu Jinchuan	0.612 ± 0.002	0.363 ± 0.008	8.892 ± 0.080	0.075 ± 0.019

Discussion

G. straminea is a well-known traditional Tibetan medicine which can be found in abundance across the QTP. Historically, the QTP was thought of as the "geo-authentic" producing region of this herb. However, spatial chemical constituent variation profiling has yet to be thoroughly completed. Through the analysis of 26 populations of *G. straminea* growing at altitudes of 2230–4730 m across the QTP, the results indicate that all of the wild populations contain the four marker compounds, while the concentration of each of the four compounds vary greatly among the populations. In Pharmacopoeia, *G. straminea* is treated as one of the "Qinjiao", and its lowest amount of gentiopicroside must be 2.0 % in dry root [8]. According to our results, the lowest amount of gentiopicroside in the *G. straminea* population is 3.035 % (Table 2); this is 1.518-fold of the national standard. This finding supports the use of all of these populations as a crude medicinal resource.

However, the constituent concentrations are not consistent in quality across differing populations. The dendrogram of clustering analysis showed that the *G. straminea* populations could be divided into low- (group A), middle- (group B) and high- (groups C and D) quality production regions according to the content of four marker compounds during the harvest season. Interestingly, of the 26 populations, four populations contained superior constituent content to the others and these populations were located in the eastern region of the Qinghai province (Fig. 4). This suggests that there are several "geo-authentic" production regions of "Mahua Jiao". Similar results have also been observed in "Qinjiao" [22] and "Huang Qi" [10]. *G. straminea* was characterised by a combination of dichogamy and herkogamy [23] and had high population genetic diversity [13]. Our results suggest that the populations of this species also have significant chemical variation among different populations on the QTP. Chinese medicinal material cultured in different localities is believed to differ in therapeutic potency [10]. Therefore, the constituent variation observed in four of the *G. straminea* populations is likely to relate to the therapeutic potency of *G. straminea*. In addition, although genetic difference was suggested to guide the exploration and conservation of *G. straminea* [6, 13], bioactive constituent

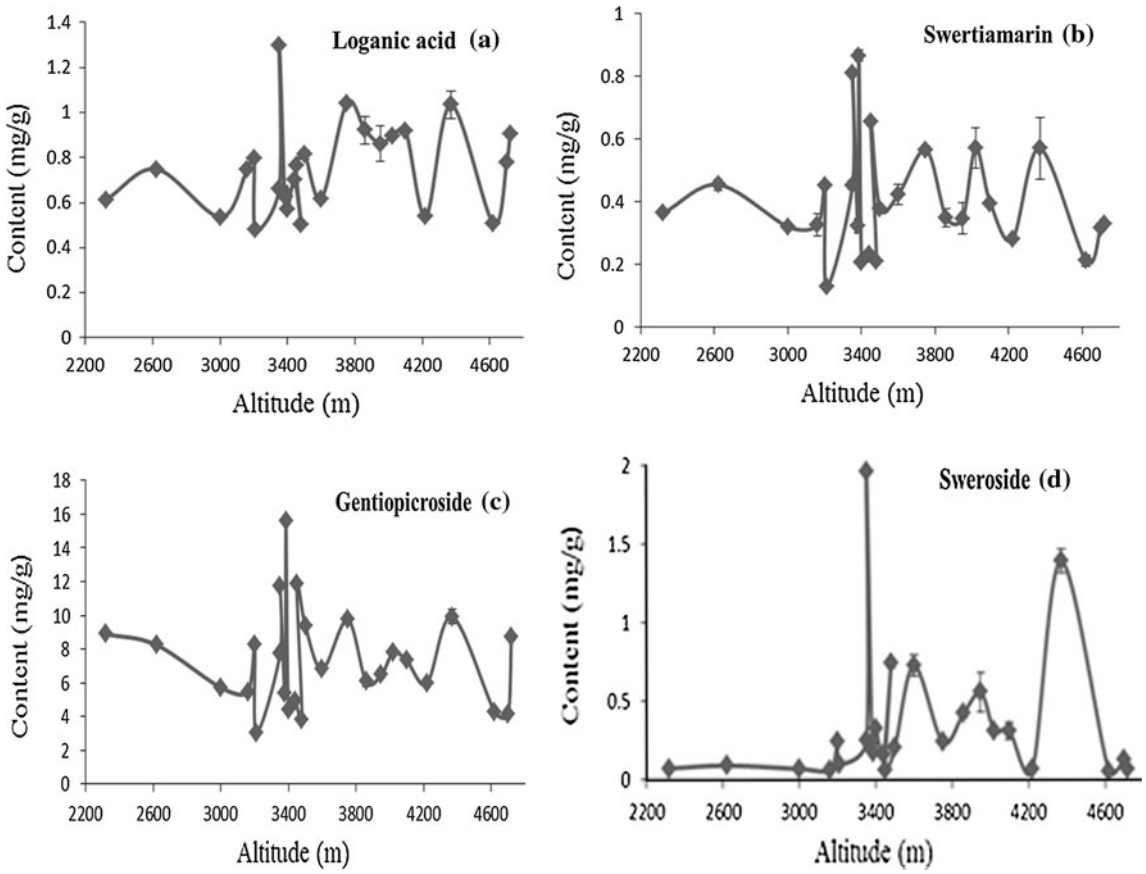


Fig. 3 Variations in the mean content of the four active constituents (**a**) loganic acid, (**b**) swertiamarin, (**c**) gentiopicroside and (**d**) sweroside, over the altitude gradient. The scale of the contents for gentiopicroside was increased by 10-fold

concentrations should also be considered, as the genetic proof was quite limited. Because this region is highly vulnerable to climate change and exploration by humans, the overharvest of species from the wild could make the population scarce [18]. The policy of harvest and conservation of species from the wild must be implemented with caution in the bioactive constituent concentrations, especially in the high-quality regions. Therefore, the results here provide chemical information for species conservation and development.

Altitude, longitude and latitude have been recognised as the three important factors that affected plant growth, distribution and metabolism. Caroteroid, flavonoid, sucrose, fructose, glucose and total soluble sugar content increased with altitude; altitude is thought to be the main reason for chemical variation [14]. Some chemical constituents also have significant latitudinal and longitudinal correlation [23]. However, our results showed that there was no significant correlation between the four active constituent concentrations and any of these three factors on the QTP. This result is consistent with the findings in *Swertia franchetiana* [24] and *Maytenus ilicifolia* [25]. The climate and environment on the QTP are both extremely harsh and many environmental factors such as drought and high UV radiation affect the secondary metabolite [26, 27]. In addition, low temperature and soil type affect phytochemical diversity [28]. Therefore, it seems likely that the phytochemical diversity in *G. straminea* could be influenced by either a single environmental factor or a combination of factors. Genetic mutation or differentiation also affects the secondary metabolites of plants [29, 30]. The rich genetic diversity of this species [13] suggests that genetic factors also contribute to the variation profiling. The chemical variation might be caused by genetic diversity and mini-climate; therefore, these relationships should be further tested.

Conclusions

Active constituents analysis of *Gentiana straminea* showed that rich chemical diversity exists among the geo-authentic producing populations. The gentiopicroside was the most abundant constituent and all of the populations have gentiopicroside contents that are far higher than the national standard level. Although there was no significant

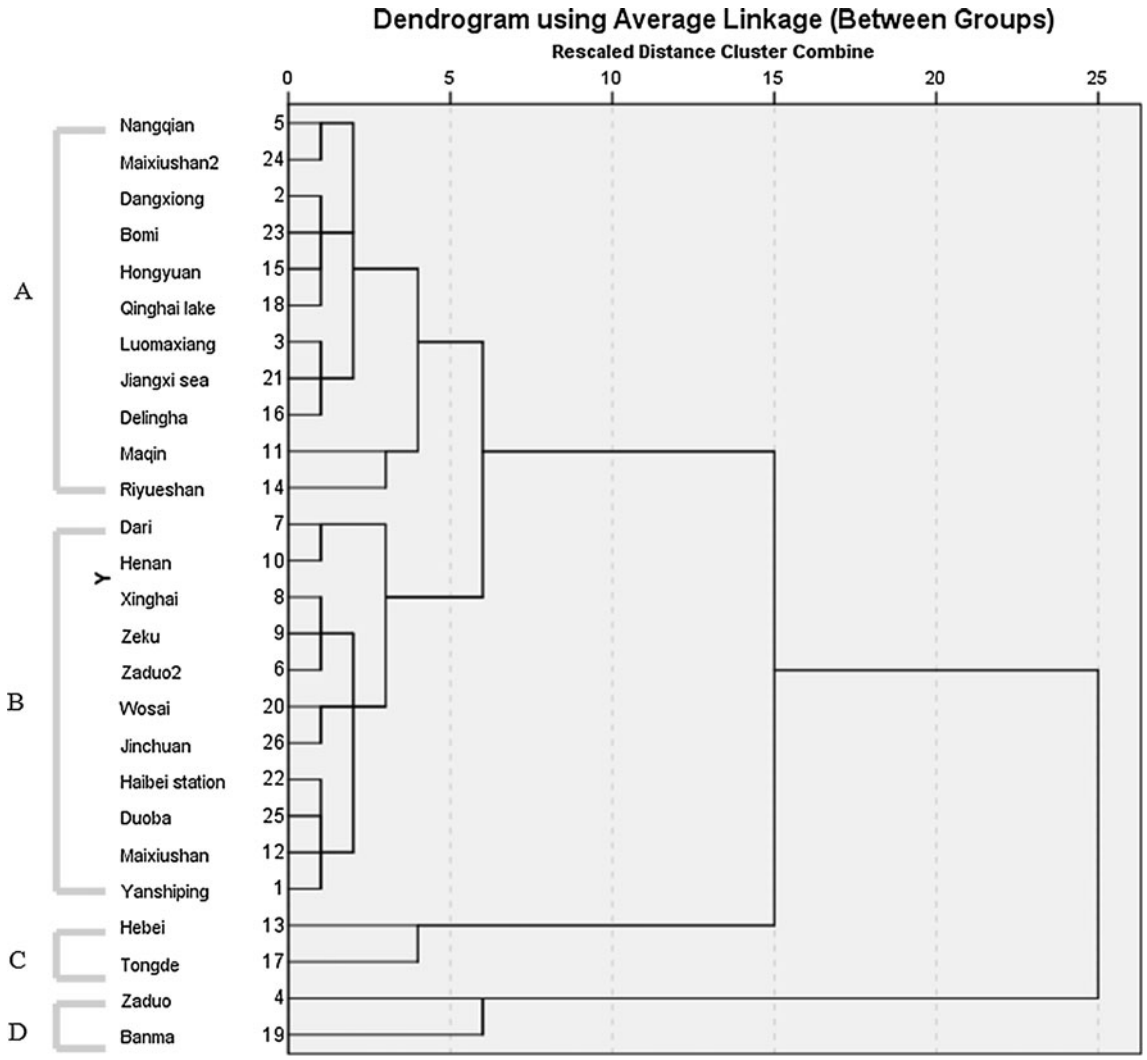


Fig. 4 Dendrogram of clustering analysis for all the samples by using the peak areas of four constituents as input data

Table 3 Comparison of the content of the four constituents and the total among all groups

Group	Loganic acid (% ± SD)	Swertiamarin (% ± SD)	Gentiopicroside (% ± SD)	Sweroside (% ± SD)	Total (% ± SD)
A	0.603 ± 0.101a	0.271 ± 0.080a	4.903 ± 1.057a	0.245 ± 0.250a	6.022 ± 1.214a
B	0.834 ± 0.123b	0.423 ± 0.085b	8.049 ± 1.109b	0.225 ± 0.149a	9.561 ± 1.214b
C	0.697 ± 0.077a	0.761 ± 0.114c	13.725 ± 2.051c	0.122 ± 0.060a	15.304 ± 2.149c
D	1.166 ± 0.150c	0.405 ± 0.179c	10.847 ± 1.044d	1.682 ± 0.316b	14.384 ± 1.650c

The different letters represent significant differences between groups ($p < 0.01$)

correlational trend between any of the four constituents and the altitude, latitude or longitude, there are four populations with significantly high concentrations of active constituents. Hierarchical clustering analysis suggests that the population could be divided into low-, middle- and high-content groups according to the content of marker compounds. Our results provide definite phytochemical evidence for resource conservation and more careful utilisation of this traditional Tibetan herb. Based on analysis of the four constituents, it also seems reasonable to set up quality standards within the authentic medicinal cultural regions on the Qinghai–Tibetan Plateau (QTP).

Table 4 Matrix correlation of altitude, latitude, longitude and analysed constituents

	1	2	3	4	5
Altitude	0.243	−0.087	−0.135	0.089	−0.096
Longitude	−0.018	0.247	0.250	0.049	0.237
Latitude	−0.199	−0.032	−0.015	−0.048	−0.007
1		0.585**	0.489*	0.597**	–
2			0.920**	0.428*	–
3				0.258	–

1 loganic acid, *2* swertiamarin, *3* gentiopicroside, *4* sweroside, *5* total content of the four constituents

* Correlation is significant at the 0.05 level (two-tailed)

** Correlation is significant at the 0.01 level (two-tailed)

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References

1. Yang YC, Ho TN, Lu SL, Huang RF, Wang ZX (1991) Tibetan medicines. Qinghai People's Press, Xining
2. Tan RX, Wolfender JL, Zhang LX, Ma WG, Fuzzati N, Marston A, Hostettmann K (1996) Acyl secoiridoids and antifungal constituents from *Gentiana macrophylla*. Phytochemistry 42:1305–1313
3. Ji LJ, Sun HF, Ding JY (1992) Study on chemical compositions of four *Gentiana* plants from Qinghai–Xizang Plateau. Acta Biol Plateau Sin 11:113–118
4. Ji LJ, Liao ZX, Sun HF (2002) A survey of Gentian plants of the Tibetan traditional herb medicines. Acta Biol Plateau Sin 15:243–250
5. Chen G, Wei SH, Yu CY (2009) Secoiridoids from the roots of *Gentiana straminea*. Biochem Syst Ecol 37:766–771
6. Zhang X-L, Wang Y-J, Ge X-J, Yuan Y-M, Yang H-L, Liu J-Q (2009) Molecular phylogeny and biogeography of *Gentiana* sect. *Cruciata* (Gentianaceae) based on four chloroplast DNA datasets. Taxon 58:862–870
7. Zhou DW, Hou QZ, Si QW, Yang HL (2010) Concentrations of the active constituents of the Tibetan folk medicine Qinjiao (*Gentiana* sect. *Cruciata*) within and between taxonomic species across the Qinghai–Tibetan Plateau. Chem Biodivers 7:2088–2094
8. Committee for the Pharmacopoeia of the People's Republic of China (2005) Pharmacopoeia of the People's Republic of China, part I. Chemical Industry Publishing House, Beijing
9. Ho T-N, Liu S-W (2001) A worldwide monograph of *Gentiana*. Science Press, Beijing
10. Liu J, Chen HB, Guo BL, Zhao ZZ, Liang ZT, Yi T (2011) Study of the relationship between genetics and geography in determining the quality of Astragali Radix. Biol Pharm Bull 34:1404–1412
11. Zheng D (1996) The system of physic-geographical regions of the Qinghai–Xizang (Tibet) Plateau. Sci China Ser D 39:410–417
12. Pluess AR, Stöcklin J (2004) Genetic diversity and fitness in *Scabiosa columbaria* in the Swiss Jura in relation to population size. Conserv Genet 5:145–156
13. Li XJ, Yang HL, Liu JQ (2008) Genetic variation within and between populations of the Qinghai–Tibetan Plateau endemic *Gentiana straminea* (Gentianaceae) revealed by RAPD markers. Belg J Bot 141:95–102
14. Han F, Ben GY, Shi SB (1998) A comparative study on the resistance of *Kobresia humilis* grown at different altitudes in Qinghai–Tibet Plateau. Acta Ecol Sin 18:654–659
15. Körner C (1999) Alpine plant life: functional plant ecology of high mountain ecosystems. Springer, Berlin
16. Hu FZ, Song YL, Liu M, Shi ZX (2003) Analysis of medicinal bioactive composition of Gentianaceae in Qinghai–Tibet Plateau by high performance liquid chromatography. Chin J Chromatogr 21:63–65
17. Nie Y, Lin PC (2010) Determination of five active constituents in aerial part of Tibetan medicine *Gentiana straminea* by HPLC. Zhongguo Zhong Yao Za Zhi 35:1276–1279
18. Guo WN, Xiong WY, Wei SN (2009) The distribution of *Gentiana macrophylla* Pall. and its relative species in China. Chin Wild Plant Resour 28:21–28
19. Chen Y, Qiu DY, Guo FX, Wang EJ, Liu FZ (2007) Investigation on explovitage of *Gentiana straminea*. Zhong Yao Cai 30:1214–1216
20. Yang HL, Si QW, Hou QZ, Liu JQ, Zhou DW (2010) Analysis chemical constituents content of loganic acid, swertiamarin, gentiopicroside and sweroside in *Gentiana waltonii* Burk. growing at different altitudes with HPLC method. Chin Trad Herb Drugs 41:28–31
21. Lin PC, Ye Z, Lu Y, Zhao S, Hu FZ, Ji LJ (2004) Content determination of loganic acid and gentiopicroside in Tebitan herbal medicines *Gentiana macrophylla* and *G. straminea* by HPLC. Zhong Yao Cai 27:819–821
22. Cao XY, Wang ZZ (2010) Simultaneous determination of four iridoid and secoiridoid glycosides and comparative analysis of Radix *Gentianae macrophyllae* and their related substitutes by HPLC. Phytochem Anal 21:348–354
23. Duan Y-W, He Y-P, Liu J-Q (2005) Reproductive ecology of the Qinghai–Tibet Plateau endemic *Gentiana straminea* (Gentianaceae), a hermaphrodite perennial characterized by herkogamy and dichogamy. Acta Oecologica 27:225–232
24. Yang HL, Duan YW, Hu FZ, Liu JQ (2004) Lack of altitudinal trends in phytochemical constituents of *Swertia franchetiana* (Gentianaceae). Biochem Syst Ecol 32:861–866
25. Mossi AJ, Mazutti M, Paroul N, Corazza ML, Dariva C, Cansian RL, Oliveira JV (2009) Chemical variation of tannins and triterpenes in Brazilian populations of *Maytenus ilicifolia* Mart. Ex Reiss. Braz J Biol 69:339–345
26. Mewis I, Schreiner M, Nguyen CN, Krumbein A, Ulrichs C, Lohse M, Zrenner R (2012) UV-B irradiation changes specifically the secondary metabolite profile in broccoli sprouts: induced signaling overlaps with defense response to biotic stressors. Plant Cell Physiol 53:1546–1560
27. Silvente S, Sobolev AP, Lara M (2012) Metabolite adjustments in drought tolerant and sensitive soybean genotypes in response to water stress. PLoS One 7:e38554
28. Nobel PS (1999) Physicochemical and environmental plant physiology. Academic Press, San Diego
29. Hashimoto T, Yamada Y (1994) Alkaloid biogenesis: molecular aspects. Annu Rev Plant Physiol Plant Mol Biol 45:257–285
30. Vogel BS, Wildung MR, Vogel G, Croteau R (1996) Abietadiene synthase from grand fir (*Abies grandis*). cDNA isolation, characterization, and bacterial expression of a bifunctional diterpene cyclase involved in resin acid biosynthesis. J Biol Chem 271:23262–23268