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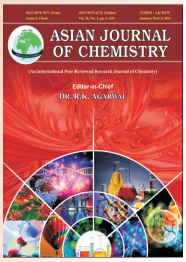

Multielemental Analysis of Important Tibetan Medicine *Gentiana straminea* from Qinghai-Tibet Plateau

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Here, we identified 17 elements in samples of the important Tibetan medicinal herb *Gentiana straminea*, which were collected from 28 different locations throughout the Qinghai Plateau of China. The samples were dissolved in HNO_3 and H_2O_2 for inductively coupled plasma-atomic emission spectrometry (ICP-AES) analysis. For each element, the analysis lines with the highest sensitivity and lowest interference spectrums were chosen. Recovery of samples ranged from 93 to 106 %, indicating the accuracy of the analysis. The mean concentrations of the 17 elements were as follows: Ca > K > Mg > Ba > Fe > Na > Al > Zn > Cu >Mn> V > Ni > Ti > As > Sn > Cd > Co. Quantitative analysis of the identified elements was also performed to elucidate the elemental characteristics and influences of geographical distribution. The results indicated that K, Zn, Mg and Ca were the main characteristic elements of this herbal medicine and each analyzed element showed significant variations among the different geographical locations. Our results provide a scientific foundation for the utilization and further research of *G. straminea*.

Keywords: Trace element, Tibetan medicine, Quantitative analysis, *Gentiana straminea.*

INTRODUCTION

Gentiana straminea Maxim., a member of the family Gentianaceae, is a perennial herb that plays an important role in traditional Chinese and Tibetan folk medicine and is distributed in alpine environments in the high mountain ranges of the Qinghai-Tibet Plateau, a region in East Asia covering most of the Tibet Autonomous Region, and most of its medicinal value is derived from the roots[1]. *G. straminea* is one of the four "Qing jiao" medicines listed in the 2010 version of the Chinese Pharmacopoeia[2] and is also recorded as an important Tibetan medicine in the classic work entitled "Jing zhu ben cao. " Generally, this herb is widely applied for the treatment of various ailments, especially for rheumatism, anaesthetization and the alleviation of inflammation[3]. Most current studies regarding *G. straminea* have focused on the bioactive constituents of extracts, especially fatty acids and amino acids, whereas systematic determination of trace elements is rarely reported[4].

Most current studies on *G. straminea* have focused on the organic compounds gentiopicroside and loganic acid, which are generally considered to be the main active ingredients. However, chemical elements are also important plant constituents with various biological activities that make significant physiological contributions to the pharmaceutical efficacies of herbal medicines[5]. Studies referring to the inorganic elements in herbal medicines have attracted considerable attention[6,7] and have demonstrated a close relationship between the concentrations of trace elements and the curative effects of extracts that contain them[8]. Therefore, we used inductively coupled plasma-atomic emission spectrometry (ICP-AES) to quantify levels of 17 elements derived from *G. straminea* distributed through different locations of the Qinghai Plateau.

In recent years, quantitative analysis has attracted increasing attention as a method to interpret the mass quantity of data and is now widely applied to evaluate societal, economic and administrative challenges. Quantitative analysis can also be employed to discern information and has been demonstrated to be a convenient and effective interpretive statistical tool[9]. Grey relation analysis (GRA) and principle component analysis (PCA) are two common and useful analytical methods that we employed in the present study to analyze the elemental characteristics and the geographical distribution patterns of *G. straminea*. In the present report, we discuss the value of grey relation analysis and principle component analysis for the evaluation of medicinal resources, especially its potential as an emergent evaluation method to provide clear scientific evidence on which to base quality control of *G. straminea*-derived medicinal and their elemental characteristics.

EXPERIMENTAL

Concentrations of trace elements in *G. straminea* root extracts were measured using full spectrum ICP-AES (IRIS 1000 ER/S, Thermo Jerrell Ash Co., Franklin, MA, USA). The spectrophotometer was composed of the following components: (a) anaxial double observation plasma system, (b) an echelle grating double light two-dimensional dispersion optical system, (c) a working method of full spectrum sequence determination of multi-elements and (d) an orthogonal nebulizer. Working parameters of the instrument were as follows: high-frequency power of 1150 W, nebulizer pressure of 30.06 psi, auxiliary gas-flow rate of 0.5 L/min, turbo pump rate of 100 rpm, uptake and wash time of 45 s and an injection volume of 1.85 mL/min.

Working standard solutions of 17 *G. straminea*-derived elements were purchased from the National Center of Standard Reference Materials (Beijing, China) and prepared from standard solutions (1,000 mg/mL). Five concentrations of each working standard solution were in the linear range of the calibration curve for each element analyzed in the plant samples. Reagent-grade HNO_3 and H_2O_2 were purchased from the Chemical Reagents Co. (Shanghai, China). All solutions were purified using a Milli-Q system (Millipore, Bedford, MA, USA).

We collected *G. straminea* roots from the Qinghai Plateau at altitudes ranging from 2,100 to 4,200 m. Samples were collected from 28 locations along two lines, one of which ranfrom East to West, whereas the other from south to north (Table-1, Fig. 1). Voucher specimens were authenticated by Dr. Xuefeng Lu and deposited at the Herbarium Department of the Northwest Institute of Plateau Biology (Chinese Academy of Sciences, Xining, China). For each sample, at least 20

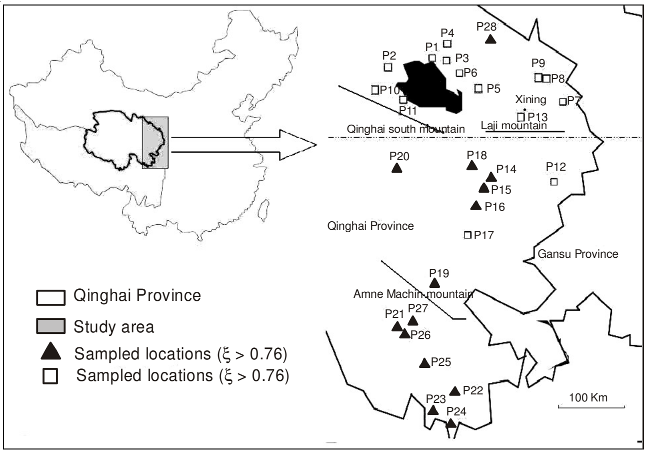

Fig. 1. Sampling locations for the comprehensive evaluation of the elemental composition of *G. straminea* root extracts

TABLE-1
COLLECTION SITES OF *Gentiana straminea* ROOT SAMPLES

Sampling number	Longitude and latitude	Localites	Altitude (m)	Sampling number	Longitude and latitude	Localites	Altitude (m)
1	N 37º15.363′E 100º14.496′	Gangcha county	3220	15	N 35º38.127′E 101º05.581′	Senduo, Guinan county	3690
2	N 37º08.297′E 99º 30.872′	Buhahe, Gangcha county	3250	16	N 35º24.238′E 100º57.666′	Wangjia, Zeku county	3470
3	N 37º13.258′E 100º28.488′	Haergai, Gangcha county	3120	17	N 35º02.311′E 100º49.489′	Lingxiu, Zeku county	3680
4	N 37º26.112′E 100º29.402′	Reshui county	3480	18	N 35º54.516′E 100º53.058′	Tongde county	3880
5	N 36º52.423′E 101º00.155′	Jintan, Haiyan county	3020	19	N 34º26.509′E 100º16.116′	Shanggongma, Dawu county	3750
6	N 37º03.977′E 100º41.504′	Ganzihe, Haiyan county	3400	20	N 35º52.291′E 99º 39.894′	Shanggongma, Gande county	4030
7	N 36º42.438′E 102º23.626′	Ledu county	2990	21	N 33º53.088′E 99º 40.348′	Gande county	4050
8	N 36º29.820′E 102º07.500′	Ping'an county	2118	22	N 33º05.281′E 100º36.081′	Duogongma, Banma county	3680
9	N 37º00.629′E 101º59.328′	Huzhu county	3010	23	N 32º50.206′E 100º15.266′	Jika, Banma county	3880
10	N 36º51.420′E 99º18.060′	Dashuiqiao, Gonghe county	3280	24	N 32º40.556′E 100º32.889′	Zhiqin, Banma county	3830
11	N 36º43.980′E 99º46.020′	Heimahe, Gonghe county	3193	25	N 33º25.632′E 100º06.307′	Mazhang, Dari county	4170
12	N 35º42.170′E 102º14.920′	Tongren county	3200	26	N 33º48.229′E 99º47.636′	Dari county	3980
13	N 36º30.983′E 101º42.103′	Huangzhong county	3750	27	N 33º57.987′E 99º55.700′	Gande county	4020
14	N 35º46.265′E 101º12.209′	Guomaying, Guinan county	3310	28	N37º29.450′E10 1º12.230′	Menyuan county	3200

All the samples were collected in Qinghai Province randomly selected individual plants from different positions separated by more than 1 m were pooled. The roots were lightly washed in the field to remove as much excess soil as possible and then taken to the laboratory, where they were cut, mixed and rinsed lightly again with tap water while avoiding damage of the fragile root surfaces. After rinsing with demineralized water, the roots were dried at 50 °C to a constant moisture content. All plant samples were ground using a pestle and mortar. When not in use, all samples were storedat 4 °C.

Sample preparation: Samples of ground root material were each accurately weighed to 1 g and then dissolved in 10 mL of HNO_3. After 1 h, 2 mL of H_2O_2 was added to the samples and then stored at 25 ºC for 12 h before heating on a hot-plate at 150-200 ºC for 15 min. All samples were cooled and then transferred to 50 mL volumetric flasks for analysis.

Statistical analysis: Data were analyzed by the principle component analysis method using SPSS version 13 statistical software for Windows (SPSS, Inc., Chicago, IL, USA) and the grey relation analysis method was performed using a data processing system (DPS 9.50)[10]. The grey relation analysis method was developed in accordance with the grey theory of Julong Deng[11]. All 28 sampling locations were considered as a gray system and each referenced locality was included in this system as a parameter. The referring data rank was designated as X_0 and the comparative data rank as $X_i = \{X_i(k) \mid k = 1, 2, \ldots, n\}$, $i = 1, 2, \ldots, m$, where $X_0 = \{X_0(k) \mid k = 1, 2, ..., n\}$, m is the number of the locality and n is the number of elements.

RESULTS AND DISCUSSION

Selection of analyzed lines: Whether the selection of the analyzed line was correct directly affected the accuracy and reliability of the determined method. Therefore, selection of the wavelength for the measured element was an important link in the analysis. When the analyzed line of the determined element was chosen, it was usually confirmed according to the sensitivity of the spectral line and the interference, as far as possible, to choose a spectrum with high sensitivity and less interference as the analyzed line. Further, the mutual interference of each analyzed line was also considered.

In our study, when the elements were determined by the ICP-AES method, each element was characterized by a series of spectral lines and the spectrometer maintained the function and automatically amended the synchronous background. Considering the mutual interference of coexisting elements, we selected 2-3 spectral lines for each identified element. The main reason of some elements not using the most sensitive line depended on the spectral interference. For example, the most and least sensitive lines for the element Zn were 213.856 and 202.551 nm, respectively, which were commonly interfered by spectra for the element Cu at 213.851 and 202.548 nm, respectively. Since it was common to encounter false-positive errors, the element Zn was analyzed using 206.191 nm as the spectral line and the wavelength of each element was selected while considering the interference andthe analysis strength and stability (Table-2).

Recovery test: To evaluate the accuracy and reliability of the analytical methods, a multi-element standard solution at concentrations of 0.1 mg/L and 1.0 mg/L was added to known amounts of the samples, respectively. All spectrophotometric analyses were performed in triplicate. The results indicated that the recovery rate of each analyzed element ranged from 93 to 106 %, thus good accuracy of the method was achieved.

TABLE-2
ANALITCAL WAVELENGTHS OF EACH IDENTIFIED ELEMENT

Element	Wavelength (nm)	Element	Wavelength (nm)
Al	396.152	Mg	285.213
Ba	233.527	Mn	257.610
Ca	317.933	Na	589.592
Zn	206.191	Ni	341.477
Fe	259.940	V	290.880
Cu	327.396	K	766.490
Ti	324.754	Sn	189.989
Co	228.616	Cd	228.802
As	197.197	–	–

Sample analysis: Elemental concentrations of different *G. straminea* populations were determined in triplicate and were presented in Table-3. The mean concentrations of the 17 elements analyzed were ranked (highest to lowest concentration) in the order Ca > K > Mg > Ba > Fe > Na > Al > Zn > Cu > Mn > V > Ni > Ti > As > Sn > Cd > Co. Ni was detected at trace levels (< 2.0 mg/g) and found the residues was safe, whereas the concentrations of the other elements were much higher, especially Ca, K, Mg and Fe. Similar results were also found for the Kuding tea plant (*Aquifoliaceae Ilex latifolia*), which reportedly have the same *qing re* (antiinflammatory) effect associated with *G. straminea* extracts and similarly high Ca, K and Mg concentrations[12]. It had been reported that when sprayed moderate concentrations of trace elements, such as Fe, the concentration of gentiopicro side also increased[13]. So, the higher concentrations of Fe, Mg may be related with the active compounds in *G. straminea*.

A comparison of the variation coefficients for each element indicated that levels of elemental Ni and Al varied the most (with variation coefficients > 1), whereas levels of Ca, K and Mg varied the least. Thus, the greater extent of changes inelement concentrations was attributed to the geographical differentiation of the sampling locations.

Elemental characteristic analysis: principle component analysis is an effective method for analysis of chemical constituents in herbal plants and has been widely applied for statistical analysis of mass chemical experimental data[14,15] because it can reduce mass data to lower dimensions and use fewer factors to describe the tested variables. Therefore, the principle component analysis method can be used to describe the elemental characteristics of *G. straminea*. The eigen values, percentages and variable scores of the elements are presented in Table-4. As shown, the cumulative percentage of the first three former components reached 91.067 % and represented over 91 % of all of the original variables, which basically reflected the distribution of elementsin *G. straminea*. Thus, the first three principal components were extracted and then analyzed.

It can be seen that in the component 1, K and Zn, accounted for more positive load capacity; *i.e.*, these elements had more consistent accumulation trends in the roots of

TABLE-3
CONCENTRATIONS OF 17 ELEMENTS IN *Gentiana straminea* ROOTS (µg/g, n = 3)

Number of locality	Elements																
	Al	As	Ba	Ca	Cd	Co	Cu	Fe	K	Mg	Mn	Na	Ni	Sn	Ti	V	Zn
P1	136.6	1.673	345.6	1291	0.1665	0.3080	18.79	226.4	1996	791.3	21.04	123.2	0.097	1.6890	2.870	0.9827	40.91
P2	44.60	1.603	336.3	1461	0.2276	0.1020	12.30	102.8	1344	665.4	9.181	77.09	0.087	0.6552	1.189	0.6329	33.92
P3	48.97	0.968	368.0	1391	0.1762	0.0960	15.04	87.72	1205	596.7	13.79	77.11	0.096	0.8971	1.497	0.5428	30.60
P4	38.59	1.075	294.1	1530	0.1133	0.1355	11.86	86.93	1327	639.0	12.66	110.8	0.079	0.8983	1.258	0.6576	27.81
P5	23.09	1.497	367.5	1414	0.0837	0.0827	15.10	73.29	1241	729.4	20.96	96.79	0.101	0.4716	0.7255	0.8635	31.78
P6	30.34	0.933	315.3	1665	0.1309	0.1390	13.85	83.76	1112	760.0	18.52	80.68	0.113	0.6842	0.9768	0.6524	30.36
P7	70.45	1.109	355.8	1504	0.0837	0.2481	19.97	144.8	1133	587.0	14.82	103.5	0.097	0.8063	2.617	0.5789	29.97
P8	47.95	0.335	489.5	1556	0.1053	0.1883	15.11	118.6	1087	562.5	17.10	86.44	0.123	0.8364	1.796	0.7568	26.63
P9	178.7	1.074	448.1	1800	0.0600	0.2340	19.33	296.0	1194	659.0	23.57	62.98	0.118	0.8465	4.065	0.2853	32.38
P10	30.57	1.498	360.1	1608	0.2178	0.2024	15.00	88.83	1369	721.0	13.27	104.0	0.109	0.0464	1.228	0.9325	25.78
P11	24.50	0.828	364.3	1343	0.2158	0.1250	18.15	97.75	1251	542.8	11.65	129.1	0.095	0.6246	0.8401	0.9457	46.24
P12	123.6	0.863	453.0	1643	0.2256	0.3257	13.83	238.2	1240	677.0	19.96	59.19	0.087	0.2593	3.407	0.8652	24.40
P13	34.26	1.304	430.2	2442	0.3486	0.2640	22.30	77.72	1379	695	11.05	117.9	0.089	0.7149	1.88	0.9168	37.6
P14	7.602	0.519	403.5	1730	0.0860	0.0210	13.34	39.62	1052	763.6	10.72	77.52	1.694	0.8305	0.4334	3.286	18.06
P15	9.752	0.427	354.1	1746	0.1759	0.0070	13.53	63.47	975.1	515.0	16.33	59.87	1.911	0.5148	0.5883	2.434	25.85
P16	18.62	0.595	525.5	1544	0.1350	0.1367	11.98	59.79	1331	615.2	14.15	79.82	2.423	0.4125	0.4334	2.776	25.70
P17	116.6	0.634	511.5	1353	0.1682	0.0210	16.19	212.0	1404	804.0	16.83	47.38	3.552	0.2115	2.239	3.418	32.12
P18	10.13	0.498	454.7	1393	0.1935	0.0596	14.18	60.25	1210	673.6	9.846	76.44	3.131	0.4428	0.5469	2.666	23.77
P19	19.34	0.531	396.7	1575	0.2249	0.0631	20.68	83.79	1332	643.8	14.75	85.04	6.830	0.3478	0.6704	2.919	24.19
P20	19.62	0.527	436.7	1348	0.0586	0.1612	11.06	80.92	1530	787.1	10.59	78.27	2.202	0.4846	0.8668	3.296	30.02
P21	24.11	0.532	493.3	1455	0.1662	0.0429	15.04	111.8	1461	842.8	11.71	78.88	3.187	0.2786	0.5063	3.422	35.12
P22	7.295	0.599	391.1	1494	0.1036	0.0210	14.12	62.98	926.3	624.8	10.02	71.12	2.459	0.1816	0.3302	2.732	29.89
P23	17.62	0.479	418.0	1595	0.3129	0.0175	13.96	150.8	1075	742.8	18.37	72.93	4.244	0.3024	0.5057	3.208	27.74
P24	21.24	0.316	496.5	1545	0.1055	0.1402	15.09	74.53	1442	817.2	27.72	69.77	2.730	0.5128	0.3298	3.459	31.85
P25	12.40	0.035	444.4	1489	0.2463	0.0841	15.89	58.02	1305	900.1	14.04	68.87	9.249	0.4377	0.3199	3.551	25.95
P26	15.51	0.493	760.0	1482	0.2112	0.1612	16.11	85.96	1219	794.5	19.07	83.19	3.169	0.4955	0.7224	3.440	22.73
P27	8.283	0.523	613.9	1517	0.0978	0.1718	12.03	47.66	1101	869.2	13.98	49.25	2.641	0.3789	0.3300	3.560	21.83
P28	23.38	0.486	631.1	1517	0.1095	0.0456	15.82	59.88	1404	745.6	13.59	42.79	2.532	0.1812	0.6708	3.142	26.13
Mean	41.56	0.784	437.8	1551	0.1625	0.1287	15.34	106.2	1273	705.9	15.33	81.07	1.902	0.5515	1.2090	2.033	29.26
Variation coefficient	1.058	0.547	0.237	0.139	0.454	0.700	0.184	0.598	0.162	0.143	0.295	0.271	1.179	0.5950	0.8300	0.620	0.203
Referring rank (X_0)	7.295	0.035	294.1	2442	0.0586	0.3257	22.30	296.0	1996	900.1	27.72	129.1	9.249	1.6890	0.3199	3.560	46.24

TABLE-4
EIGEN VALUES, PERCENTAGES, AND VARIABLE SCORES OF ELEMENTS IN *Gentiana straminea*

	Component 1	Component 2	Component 3
Eigen values	37382.803	22835.131	43885.294
Percentage	32.702	19.976	38.390
Cumulative	32.702	52.677	91.067
Variable			
Al	0.458	-0.027	0.082
As	0.579	-0.267	0.161
Ba	-0.454	0.737	0.008
Ca	-0.166	-0.162	0.973
Cd	0.101	0.050	0.384
Co	0.409	0.083	0.327
Cu	0.290	-0.065	0.420
Fe	0.380	-0.002	0.031
K	0.888	0.455	-0.012
Mg	0.012	0.876	0.030
Mn	0.088	0.209	0.032
Na	0.522	-0.236	0.142
Ni	-0.248	0.430	-0.138
Sn	0.498	-0.132	0.071
Ti	0.404	-0.167	0.220
V	-0.371	0.613	-0.158
Zn	0.613	-0.131	0.011

G. straminea. When concentrations of K and Zn increased, their load capacities increased accordingly and *vice versa*. These two elements have been associated with enzymatic activity in plants and their enrichment in *G. straminea* roots favored synthesis and activity enhancement of nucleic acids and proteins. The elements Mg, Ba and V were the second most common components in *G. straminea* and had relatively high load capacities and Mg accounted for the largest load capacity, which can be explained by its well-known roles in the activation of many enzymes and promotion of respiration and carbohydrate metabolism in plants. Among the third most common components, Ca had a greater index load capacity, which was significantly higher than those of other elements. Ca plays roles in many plant functions, such as promotion of root and leaf growth as well as consolidation of plant structure, among others.Therefore, K, Zn, Mg and Ca could be considered as the main characteristic elements of *G. straminea*, which are in accordance with the antipyrotic effect of this herbal medicine[12,13].

Evaluation of grey relation analysis: When we used the grey relation analysis method to analyze the data, we found relatively lower concentrations of harmful elements (*e.g.*, Al, As, Ba, Cd and Ti) and higher concentrations of beneficial and pharmaceutical elements (*e.g.* Ca, K, Mg and Zn)[16-18] (Table-3). The relation coefficient was calculated using the DPS data system[10] with the Δmin parameter and the distinguishing coefficient set at 0 and 0.1, respectively. The grey relation analysis results of 17 elements from 28 different locations are listed in Table-5.

As shown in Table-5, when a relation coefficient (ξ) of 0.7600 was used as the selection criterion for plants within a study area bounded by the southern Qinghai mountain range and the Laji mountain range, the results indicated that the 28 samples could be divided into two equal groups of 14 samples each. When the evaluation results were evaluated in the context of the sampling locations (Fig. 1), two groups emerged: one mostly located in the central and southern regions of the Qinghai Plateau and the second mostly localized to the northern regions of the Qinghai plateau.

TABLE-5
GRAY RELATION ANALYSIS RESULTS

Number of locality	Relation coefficient (ζ)	Number of locality	Relation coefficient (ζ)
P1	0.7409	P15	0.7943
P2	0.7464	P16	0.7694
P3	0.7456	P17	0.7414
P4	0.7479	P18	0.7953
P5	0.7586	P19	0.7645
P6	0.7515	P20	0.7651
P7	0.7435	P21	0.7618
P8	0.7473	P22	0.8032
P9	0.7399	P23	0.7699
P10	0.7525	P24	0.7660
P11	0.7583	P25	0.7937
P12	0.7409	P26	0.7800
P13	0.7530	P27	0.7919
P14	0.8098	P28	0.7643

Geographical distribution pattern of *G. straminea*: Generally, the geographical and spatial distributions of the plants varied widely among different species, but not randomly. Areas that have conditions that inhibit growth can be considered as distribution boundaries[19]. When the conditions of geological and geomorphological areas varied drastically, many plant species often adapted their distribution boundaries. Our study area was just located at the eastern edge of the Qinghai-Tibet plateau mountain range in China, which has provided unique habitats for many plant species[20].

Reportedly, the common alpine *G. stramineais* dependent on insects for pollination[21] and in recent years, this medically important species has been continuously collected, thus the size of natural populations has decreased drastically[1]. Sexual reproduction is the only mechanism for *G. straminea* to maintain its population and increase its distribution area[21]. However, the height of the mountain ranges obstructs its pollination and may therefore deteriorate restoration and conservation efforts for this species[22]. Therefore, the distinct effects of the environments of the Laji and southern Qinghai mountain ranges on *G. straminea* populations likely limit pollination of this species and may protect germplasm resources. Despite the effects of these two high mountain ranges, variations in the concentrations of elements in *G. straminea* roots throughout the Qinghai plateau is likely due to the obvious geographical component in our study. Furthermore, these mountain ranges may act as boundaries of separated distribution areas of *G. straminea* populations in the Qinghai plateau, thus giving rise tounique geographical distribution areas. Although the grey relation analysis method based on the determination of elements showed varied geographical distributions of elements in *G. straminea*, changes of other chemical components from different geographical locations in this species should be further elucidated in future studies.

Conclusion

Seventeen trace elements in root extracts from the important traditional Chinese and Tibetan medicinal plant *G. straminea* were identified *via* ICP-AES. The principle component analysis results indicated that K, Ca, Mg and Zn were the main characteristic elements in *G. straminea*. grey relation analysis was further used to evaluate the multi elemental distribution patterns and the effects of geographical location with in the Qinghai plateau on levels of trace elements in *G. straminea*. Our results showed that the concentration of each element in *G. straminea* was dependent on geographical distribution. Our preliminary results indicated an obvious effect of geographical location throughout the Qinghai Plateau on the composition of elements with in the root extracts of *G. straminea* and emphasized the role of different environments on the chemical compositions of plant extracts used in traditional medicine. Obstruction of pollination caused by the height of the mountain ranges likely induced the varied geographical distribution of *G. straminea* in this study.

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