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Evaluation of Mineral Elements in *Gentiana rigescens* in China

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Gentiana rigescens is an important traditional Chinese herbal medicine. Mineral elements play an important role in the formation of active components in medicinal plants. This study aimed to determine nine mineral elements in *G. rigescens* collected from 41 different sites in Yunnan and Guizhou provinces of China by atomic absorption spectroscopy. The average concentrations of mineral elements were in the order of K > Ca > Mg > Fe > Na > Zn > Cu > Cr > Se. According to correlation analysis, Zn-Cu had the highest positive correlation ($r = 0.834$), while Fe-Mg were the strongest negatively correlated ($r = -0.649$) ($p < 0.01$). Three significant groups classified by principal component analysis contained more than 71.39 % of all the important influential sources. This study provides a comprehensive survey of the concentrations of 9 mineral elements in wild *G. rigescens* samples collected from different sites in Yunnan and Guizhou provinces of China. Magnesium, Fe, Zn and Cu, were viewed as the characteristic mineral elements of *G. rigescens* according to principal component analysis.

Keywords: *Gentiana rigescens*, Mineral element, Principal component analysis, Correlation analysis.

INTRODUCTION

Gentiana rigescens is an important traditional Chinese herbal medicine, which belongs to Gentianaceae and distributes in Yunnan, Guizhou, Sichuan and Guangxi provinces of China. In Yunnan, it mainly grows under the pine trees at the altitude of 1,100-3,000 m[1]. *G. rigescens* is well known for broad bioactivities such as liver protection, antiinflammatory and cholagogue[2]. Importantly, it has been officially recorded in the Chinese pharmacopoeia[3]. Medicinal plants can synthesis a great number of important chemical components with physiological action. Not exceptional, the primary active compositions in *G. rigescens* were gentiopicroside, swertiamarin and sweroside[4]. Essential mineral elements play an important role in the formation of these active constituents and also are significant roles as functional and structural components of metalloproteins and enzymes in living cells[5-7].

Mineral elements not only affect our growth, development and reproductive functions, but also are the key links of prevention and cure of many diseases. For human beings, mineral elements are essential nutrients with a gamut of functions[8]. Calcium plays an important role in the formation of teeth and bones and the function of central nervous system. Copper takes an important part in cell functions and structural stability of chromosomes and energy transfer[9]. Chromium, Fe, Cu and Zn are known to be the contributors in maintenance of normoglycemia by activating β-cells of pancreas[10]. The essential trace mineral selenium is the fundamental element to human health[11,12].

Many processes of plant physiology are affected or regulated through minerals[13]. It is reported that abiotic factors such as heavy metals may alter the production of bioactive compounds by changing secondary metabolism process in plants[14]. The concentrations of mineral elements in the important medicinal herbs, such as the species of *Paris*, *Panax*, *Gentiana*, *Cordyceps* and *Murraya* have been investigated[15-19]. However, the report about the mineral elements in *G. rigescens* is rare. In this study, we assessed concentrations of mineral elements (K, Ca, Na, Mg, Fe, Zn, Cu, Se and Cr) in *G. rigescens* samples collected from 41 sites in Yunnan and Guizhou provinces of China. In order to provide a better insight into the patterns of mineral elements, common chemometrics methods including correlation analysis (CA) and principal component analysis (PCA) were used for analysis relationships among mineral elements in *G. rigescens*.

EXPERIMENTAL

G. rigescens samples were collected from 41 sites in Yunnan and Guizhou provinces of China (Fig. 1). All of the

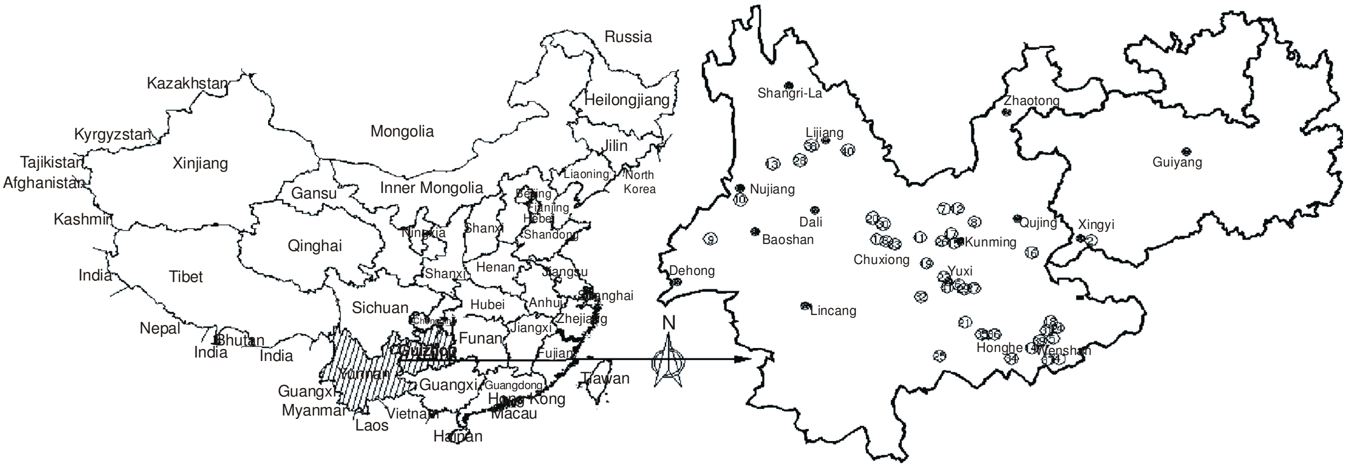

Fig. 1. Collection sites of the *G. rigescens* in Yunan and Guizhou provinces in China, 1. Nanhua, Chuxiong 2. Anlong, Xingyi 3. Nanhua, Chuxiong 4. Maguan, Wenshan 5. Yan shan, Wenshan 6. Jiangchuan, Yuxi 7. Luquan, Kunming 8. Songming, Kunming 9. Tengchong, Baoshan 10. Lushui, Nujiang 11. Lufeng, Chuxiong 12. Luquan, Kunming 13. Lanping, Nujiang 14. Pingba, Wenshan 15. Qiongzhusi, Kunming 16. Shizong, Qujing 17. Xiaoshao, Kunming 18. Yanshan, Wenshan 19. Yimen, Yuxi 20. Yao'an, Chuxiong 21. Jianshui, Honghe 22. Mangshi, Dehong 23. Beicheng, Yuxi 24. Yanshan, Wenshan 25. Lvchun, Honghe 26. Xishan, Kunming 27. Qinglongshan, Yuxi 28. Shibaoshan, Dali 29. Jiangchuan, Yuxi 30. Yao'an, Chuxiong 31. Yanshan, Wenshan 32. Xinping, Yuxi 33. Nanhua, Chuxiong 34. Pingbian, Honghe 35. Gejiu, Honghe 36. Mengzi, Honghe 37. Maguan,Wenshan 38. Yulong, Lijiang 39. Tangzi, Wenshan 40. Yongsheng, Lijiang 41. Jiangchuan, Yuxi

samples were identified by Dr. Jinyu Zhang. A voucher specimen was deposited at Institute of Medicinal Plants, Yunnan Academy of Agricultural Sciences. The reference substances (standard solutions of metal elements 1,000 μg mL^{-1}) were provided by the National Research Center for Certified Reference Materials (NRCCRMS). The analytical reagents (nitric acid and muriatic acid) were from Beijing Beihua Fine Chemical Co., Ltd. The water was deionized water (18.25 MΩ cm).

Sample preparation: The whole plants of samples were dried at 60 °C until constant weight. Powdered samples 0.5000 ± 0.0005 g were weighed accurately and dissolved in moderate concentrated nitric acid for 12 h. The mixture was heated until the reddish brown fumes disappear. The residue was heated again at 550 °C for 4 h and then was dissolved in 1 mol L^{-1} muriatic acid up to 25 mL for determination. A blank control group was carried out in the same way. All the determinations were performed in quintuplicate[20].

All mineral elements were determined by atomic absorption spectrometer (SOLAAR, Thermo Elemental, USA). Detection limit value (mg mL^{-1}), operating condition and standard curve of nine elements were shown in Table-1. R^2 represents the square of correlation coefficient. Statistical analysis was carried out by SPSS 17.0 (Chicago, IL, USA).

RESULTS AND DISCUSSION

Determination of mineral elements: The mean, range and relative standard deviations (≤ 10 %) of elements were listed (Table-2). A t-test was applied to determine significant difference between mean values ($p < 0.05$).

The average values of elements were found in the order K (2692.253 ± 18.280) > Ca (1143.202 ± 107.176) > Mg (940.142 ± 8.706) > Fe (744.719 ± 42.256) > Na (217.134 ± 9.415) > Zn (187.482 ± 11.675) > Cu (62.689 ± 5.384) > Cr (19.803 ± 1.829) > Se (4.916 ± 0.315 mg kg^{-1}).

TABLE-2
RANGE AND MEAN OF MINERAL ELEMENT CONTENTS OF SAMPLES

Element	Mean ± SD (mg kg^{-1})	Range (mg kg^{-1})
Ca	1143.202 ± 107.176	37.046 ± 0.579 – 4127.475 ± 208.904
Cr	19.803 ± 1.829	0.047 ± 0.009 – 68.265 ± 6.477
Cu	62.689 ± 5.384	2.123 ± 0.173 – 293.737 ± 2.821
Fe	744.719 ± 42.256	92.706 ± 4.185 – 181.039 ± 39.423
K	2692.253 ± 18.280	263.412 ± 26.411 – 3047.602 ± 24.2922
Mg	940.142 ± 8.706	747.721 ± 1.079 – 1180.626 ± 21.305
Na	217.134 ± 9.415	43.801 ± 4.382 – 441.328 ± 6.393
Se	4.916 ± 0.315	0.043 ± 0.028 – 102.671 ± 1.278
Zn	187.482 ± 11.675	1.569 ± 0.071 – 459.532 ± 1.966

TABLE-1
MINERAL ELEMENTS DETECTION LIMIT, OPERATING CONDITION AND STANDARD CURVE

Element	Detection limit (μg mL^{-1})	Operating condition		Standard curve	R^2
		Detection wavelength (nm)	Pass band (nm)		
K	0.247	776.5	0.5	A = 0.0279X + 0.0049	0.9951
Ca	0.096	422.7	0.5	A = 0.006X-0.001	0.9959
Na	0.172	589.0	0.2	A = 0.2456X + 0.0313	0.9936
Mg	0.165	285.2	0.5	A = 0.072X + 0.0264	0.9953
Fe	0.081	248.3	0.2	A = 0.0187X-0.005	0.9966
Cu	0.063	324.8	0.5	A = 0.0622X-0.0005	0.9996
Zn	0.026	213.9	0.5	A = 0.1305X + 0.0003	0.9971
Se	0.043	196.0	0.5	A = 0.0018X + 0.0027	0.9965
Cr	0.047	357.9	0.5	A = 0.0005X-0.0001	0.9979

TABLE-3
CORRELATION OF THE MINERAL ELEMENT IN SAMPLES

	K	Ca	Na	Mg	Fe	Zn	Cu	Se	Cr
K	1.000								
Ca	0.070	1.000							
Na	0.046	-0.088	1.000						
Mg	-0.324**	0.000	0.198*	1.000					
Fe	0.540**	0.356**	-0.060	-0.649**	1.000				
Zn	0.451**	0.128	-0.282**	-0.626**	0.632**	1.000			
Cu	0.407**	0.019	-0.133	-0.582**	0.579**	0.834**	1.000		
Se	0.108	-0.333**	0.196*	-0.264**	0.159	0.124	0.275**	1.000	
Cr	0.412**	0.127	-0.203*	-0.416**	0.476**	0.560**	0.557**	-0.061	1.000

Correlation is significant at the* 0.05 level and ** 0.01 level, two-tailed

Correlation analysis: The results of the correlation analysis between elements were presented (Table-3). Zn-Cu had the highest significantly positive correlation (r = 0.834). Whereas, Fe-Mg were the highest negative correlated (r = -0.649) ($p < 0.01$). Fe was significantly positive correlated with K, Ca, Zn, Cu and Cr ($p < 0.01$). Mg was significantly negative correlated with the elements K, Fe, Zn, Cu, Se and Cr ($p < 0.01$).

Principal component analysis (PCA): The loading plot indicated the similarity and correlation among mineral elements (Fig. 2). The principal components with eigenvalues higher than 1 were extracted and gathered into three different groups, which explained more than 71.39 % of the total variance of initial data set. The principal component was the result of the final structure of the data, which was extracted by varimax orthogonal rotation of correlation matrix. The mineral elements with small loadings located near the origin indicating they had only little influence on the data structure. On the contrary, those with high loadings represented they had greater influence on the grouping and separation of the samples. A close relation (covariation) was observed: (a) the first new principal component (PC1) correlated well with K, Mg, Fe, Zn, Cu and Cr and explained 42.537 % of the total variance. Magnesium was negatively correlated with the new PC1. (b) The second new principal component (PC2) accounted for 16.764 % of the total variance and correlated well with Ca and Se. More specifically, Se was positively correlated with the new PC2, while Ca was negatively correlated with it. (c) sodium was contained in PC3 and explained 12.091 % of the total variance.

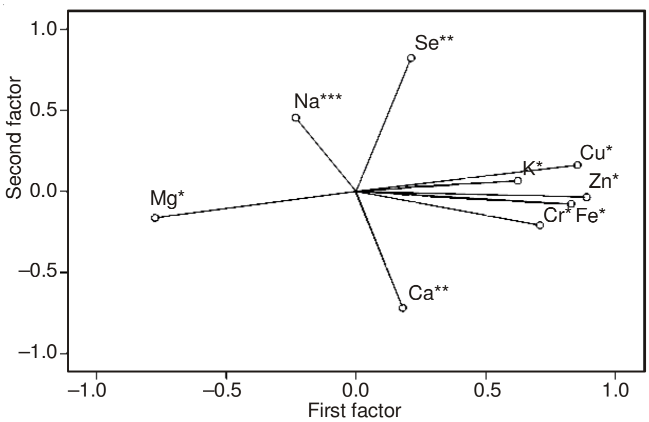

Fig. 2. Loading plots of mineral elements in *G. rigescens*. The loadings of K (0.624), Mg (-0.775), Fe (0.829), Zn (0.889), Cu (0.854), Cr (0.709) in the factor 1 marked in asterisk (*); Ca (-0.719), Se (0.823) in the factor 2 marked in double asterisks (**) and Na (0.752) in the factor 3 marked in three asterisks (***). The explained variance and proportion of total variance for three factors were 3.828, 42.537 %; 1.509, 16.764 % and 1.088, 12.091 %, respectively

As we know, mineral elements play an important role in organisms[21]. The concentrations of these mineral elements have been investigated in the herbal medicines, respectively[22-26]. The order K > Ca > Fe > Na > Zn > Cu was in agreement with those reported in *Rheum emodi*[24]. Except Se, the determined mineral contents were all higher than in *Paris polyphylla*[18]. Compared with *Murraya koenigii*, contents of Ca, Mg and K were lower, while Cr, Cu, Fe, Na, Se, Zn were higher in the *G. rigescens*[10]. For Cu and Zn in the flour of barley, wheat, corn and rice, they were all much less than them in the *G. rigescens*[27].

All the samples showed the high concentration of K, which coincided with the literatures on the medicinal herbs in the genus *Gentiana*[25-26]. The concentrations of Ca varied in a wide range, the highest and the lowest were 4-fold and one tenth of the former's results, respectively[6]. The content of Zn was no more than 3.7 mg kg^{-1} in food samples, while in agricultural products should be 15-200 mg kg^{-1} [23,27]. However, Zn was much higher in most sites in this study than it in agricultural products. The level of Cu in many samples were higher than the value (10.27 mg kg^{-1}) in the genus *Gentiana*[6]. According to World Health Organization (WHO), the permissible limit of Cu is 150 mg kg^{-1} in the medicinal plants in Singapore[28]. The mean contents of Cu in sites of 7, 12, 22, 24 and 28 were above it. It is known that the level of Se in the soil of the most areas in China is very low, particularly in Yunnan province. In our study, the average concentration of Se in *G. rigescens* was higher than it in the soil reported previously, but was lower than in Flos Chrysanthemi indici (19.205 mg kg^{-1})[29].

Chromium plays an important role in the human body, such as regulation of blood sugar level and lipid metabolism[30]. In this study, the highest Cr was 68.265 ± 6.478 mg kg^{-1}, while it in some of the sites was not detected. In medicinal plants, permissible limits for Cr set by Canada were 2 mg kg^{-1} in raw medicinal plant[28]. The average concentration of Cr in *G. rigescens* was higher than in the order Gentianales[6-7]. The significant differences were found between the concentrations of mineral elements in the samples from different sites, which would be related to the geographical environment of *G. rigescens* growth.

Conclusion

This study provides a comprehensive survey of the concentrations of 9 mineral elements in wild *G. rigescens* samples collected from different sites in Yunnan and Guizhou provinces of China. In order to recognize mineral element patterns, chemometric approaches including CA and PCA were used for data evaluation. The results of CA indicated that there were significantly positive correlation between Zn-Cu and negative correlations between Fe-Mg ($p < 0.01$). A three-factor model was established, which could explain more than 71.39 % of all data information. Magnesium, Fe, Zn and Cu, were viewed as the characteristic mineral elements of *G. rigescens* according to principal component analysis.

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