Multivariate Statistical Analysis of Multiple-Elements in *Anisodus Tanguticus* (Maxim.) Pascher from Different Regions to Determine Geographical Origins

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Research

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Abstract

Background: *Anisodus tanguticus* (Maxim.) Pascher, which is an important species used in traditional Tibetan medicine, is grown in the Qinghai -Tibet Plateau. Because the quality of *A. tanguticus* varies depending on where it is grown, a method for determining the geographical origin of this species is needed.

Methods: A quantitative analysis of 18 elements (Al, Ba, Be, Ca, Co, Cu, Fe, K, Li, Mg, Mn, Mo, Na, Ni, P, Sr, V, and Zn) in *A. tanguticus* and the soil collected from different locations was conducted using an inductively coupled plasma optical emission spectrometer. The elemental contents underwent a principal component analysis (PCA), and an orthogonal partial least-squares discriminant analysis (OPLS-DA). Furthermore, elemental accumulation and elemental transfer coefficients were calculated according to the element concentration gradients.

Results: Distinct element fingerprints were detected for the *A. tanguticus* collected from different regions. Additionally, the PCA and OPLS-DA results indicated *A. tanguticus* plants can be distinguished on the basis of their geographical origin. The Tibet samples were easily separated from the other samples. Elemental accumulation and transfer patterns differed significantly among the analyzed elements and plant parts. Ca and P were identified as the elements with the highest in elemental accumulation and elemental transfer patterns of *A. tanguticus*.

Conclusions: Our approach can be used to efficiently and accurately distinguish herbs according to their varietal characteristics and geographical origins.

Introduction

Anisodus tanguticus (Maxim.) Pascher, which belongs to the family *Solanaceae*, is an herb that is mainly grown in the Qinghai -Tibet Plateau, China [1]. The name for this species when used in traditional Tibetan medicine is “Tang Chun Na Bao”. It has been used to treat septic shock, analgesia, and motion sickness, as well as an anesthesia [2]. *A. tanguticus* is a natural source of tropane alkaloids with pharmacological effects, including atropine, anisodamine, anisodine, and scopolamine. These compounds are used as narcotics, sedatives, analgesics, and antispasmodics as well as for treating Parkinson’s disease, motion sickness and asthma [3–5]. Sales of tropane alkaloid-based drugs, which are mostly derived from *A. tanguticus* in China, can be as high as about 200 million. However, the quality and efficacy of *A. tanguticus* vary among samples. Thus, determining the geographical origin of plants is important for maintaining the high quality of commercially produced herbs.

Geographical origin is one of the most important parameters for many herbs, because the diversity in climate and soil conditions can influence the quality of herbs. Various techniques, including high-performance liquid chromatography (HPLC), gas chromatography (GC), HPLC coupled with mass spectrometry (HPLC-MS), near infrared reflectance (NIR) spectroscopy, inductively coupled plasma optical emission spectrometer (ICP-OES), and inductively coupled plasma mass spectrometry (ICP-MS),

combined with chemometric methods have recently been used to discriminate the geographical origin of herbs and other food and agricultural products [6–10].

Elemental compositions in plants can reflect the environmental elemental compositions in different geographical locations. Thus, ICP-MS/OES may be appropriate for determining the geographical origin of foods and herbs. Unlike the chemical composition, the elemental composition of plants is an inherent property that is relatively stable. Elemental contents in plants are influenced by plant characteristics as well as environmental factors, including soil and climate conditions. For example, the geographical origins of three *Ephedra* species were revealed by ICP-MS. Similar strategies have been used to discriminate various regions in which herbs and food crops originated as well as to identify the variables important for authentication [11–13]. To date, there have been no studies that systematically distinguished geographical origins of *A. tanguticus* samples on the basis of multiple-elements. In this study, we combined multi-element fingerprinting and a multivariate statistical analysis to elucidate the geographical origins of *A. tanguticus*.

Soil is one of the main factors influencing the element fingerprints of plants. Regarding the dynamics of the soil -plant element system, plants absorb elements from the soil through their roots, after which the elements are transported to different tissues. This absorption and transport can vary depending on the plant species and the type of element [14–16]. Therefore, it is necessary to study the relationships between different plant tissues and the soil.

The objectives of this study were as follows: (1) establish the element fingerprints of different parts of *A. tanguticus* collected from the Qinghai -Tibet Plateau by ICP-OES; (2) develop a method for a multivariate statistical analysis that classifies *A. tanguticus* according to its geographic origin. (3) clarify the relationship between the elements in *A. tanguticus* and soil.

Results

General features of the analyzed elements

The contents of the 18 analyzed elements (Al, Ba, Be, Ca, Co, Cu, Fe, Li, K, Mg, Mn, Mo, Na, Ni, P, Sr, V, and Zn) in the AAT, RAT and soil samples are listed in supplementary material, and the data are presented as mean $\pm$ standard deviation. The elemental contents in different parts of *A. tanguticus* plants and in the soil differed significantly among the sampling locations.

Several of the examined elements (Ca, Na, K, P, Mg, and Fe) are important for multiple physiological processes. The mean Ca, Na, K, P, Mg and Fe contents at the different locations were respectively 13085.2 mg/kg, 360.2 mg/kg, 14005.0 mg/kg, 1915.6 mg/kg, 5491.2 mg/kg, and 936.2 mg/kg in AAT, and 10744.8 mg/kg, 327.2 mg/kg, 4016.1 mg/kg, 2019.3 mg/kg, 2775.1 mg/kg, and 747.1 mg/kg in RAT. Calcium is important for preventing of dyslipidemia, arterial hypertension, and osteoporosis [17]. Potassium has important cellular functions, especially in the nervous system [18]. Both Mg and Fe are essential micronutrients, with Mg is required for nucleic acid in protein synthesis and Fe mainly

contributing to the synthesis of hemoglobin [19, 20]. Some studies have indicated that people exposed to high levels of aluminum may develop Alzheimer's disease, but other studies were unable to verify this relationship. The Ca, Na, K, P, Mg, and Fe contents in the AAT and RAT samples determined in this study suggest *A. tanguticus* is an important herb for mineral supplementation, especially for people with deficiencies in these minerals.

The mean value of Ba, Be, Cu, Li, Mn, Sr, V, and Zn elements at different locations were respectively 27.7 mg/kg, 11.3 mg/kg, 12.2 mg/kg, 35.4 mg/kg, 68.8 mg/kg, 44.7 mg/kg, 29.2 mg/kg, 23.9 mg/kg in AAT, and 35.8 mg/kg, 16.4 mg/kg, 19.2 mg/kg, 75.8 mg/kg, 36.2 mg/kg, 60.1 mg/kg, 19.1 mg/kg, and 36.1 mg/kg in RAT. The essential metals Cu and Zn serve as enzymatic cofactors that are crucial for the immune, central nervous, and reproductive systems [21]. There are several adverse effects associated with a lack of V, including anemia and abnormal iron metabolism [22].

The mean value of Co, Mo, and Ni at the different locations were respectively 2.2 mg/kg, 4.8 mg/kg, 16.3 mg/kg in AAT, and 7.9 mg/kg, 0.8 mg/kg, and 11.2 mg/kg in RAT. An analysis of the mean contents in the AAT and RAT samples revealed that the most abundant element was Ca, with concentrations exceeding 10000 mg/kg. The K content in AAT was also greater than 10000 mg/kg. The P, Mg, Al, Fe, and Na contents in the AAT and RAT samples were greater than 1000 mg/kg. The concentrations of the remaining elements exceeded 10 mg/kg.

Regarding the soil elemental abundance, the mean value of 18 elements in soil were 59657.9 mg/kg, 370.7 mg/kg, 7.8 mg/kg, 13099.7 mg/kg, 32.7 mg/kg, 73.9 mg/kg, 32549.4 mg/kg, 66.7 mg/kg, 10124.9 mg/kg, 8747.6 mg/kg, 1268.8 mg/kg, 92.7 mg/kg, 11747.3 mg/kg, 107.5 mg/kg, 948.4 mg/kg, 155.5 mg/kg, 110.3 mg/kg, and 121.1 mg/kg, respectively.

The multi-element fingerprint map of *A. tanguticus* at different locations was established and is presented in Fig. 1. The lowest and highest elemental contents varied among the locations. For example, QH22 had the highest Ca, Mn, and Ni contents in the AAT samples and QH2 had the lowest Ba and Mg contents in the AAT samples. Additionally, XZ22 had the highest Mg and V contents in the RAT samples and GS3 had the lowest Cu, K, Mn, and Zn contents in the RAT samples. Thus, the multi-element fingerprint of *A. tanguticus* at different locations revealed elemental variations. The general features of the elements in *A. tanguticus* indicated that plants originating from specific regions have characteristic elemental contents. Furthermore, the element fingerprints varied substantially, reflecting the considerable diversity among the examined regions.

Principal component analysis

A PCA of the elemental contents of the AAT, RAT, and soil samples was conducted as an exploratory data analysis before the classification modeling. The auto-scaled data matrix was applied for the PCA, to facilitate a data structure study in a reduced dimension. Four extracted principal components (PCs) with eigenvalues explained 53% of the total variance. More specifically, PC1, PC2 and PC3 explained 28%, 11% and 8% of the total variance, respectively. Additionally, PC1 was associated with the most variables (K,

Mg, Mo, Fe, and Al) among the soil samples, whereas PC2 was associated with the most variables (Cu, Ni, Zn, and K) among the RAT samples.

The PCA score plot generated for 67 samples (Fig. 2a) clearly divided the samples from the various locations into three groups. The XZ samples could be distinguished from the GS, SC, and QH samples along the PC2 axis. Most of the GS samples could be distinguished from the SC samples along the PC1 axis. However, the QH samples could not be distinguished from the other samples along the PC1 axis. There was a considerable overlap between the QH samples and SC samples as well as between the QH samples and the GS samples. According to Fig. 2b, the XZ samples were positively correlated with PC1; thus, they were characterized on the basis of five elements (K, Mg, Mo, Fe, and Al in soil). The Be, Co, Ni, and Mo contents in the RAT samples and the Be and Cu contents in the AAT samples were negatively associated with PC1. The Cu, Mn, and Zn contents in the RAT samples were highly positively correlated with PC2, which was in contrast to the highly negative correlations between the Ca, Fe, and Sr contents in AAT samples and PC2. Hence, the XZ samples most affected by the soil K, Mg, Mo, Fe, and Al contents whereas the GS, SC, and QH samples were most affected by the RAT Be, Co, Ni, and Mo contents and the AAT Be and Cu contents.

Clearly, the results reflected the utility of the PCA for distinguishing *A. tanguticus* samples from different locations. Next, an OPLS-DA was performed to assess sample authenticity. **Orthogonal partial least squares discriminant analysis**

The OPLS-DA of the AAT, RAT, and soils elemental contents was conducted to further separate the *A. tanguticus* samples according to their geographical origins. The OPLS-DA model presented the sample classification results in a scores chart (Fig. 3a). An R^2Y of 0.903, and a Q^2 of 0.794, suggested the model appropriate for generating accurate predictions [23].

The samples from the different regions were classified into three groups. The XZ samples were clearly separated from the GS, SC, and QH samples. There was some overlap between the GS and SC samples. The loading distribution of the variables indicated that the XZ samples could be identified on the basis of Be, K, Mo, Fe, and Al in soil (Fig. 3b). In contrast, the Be contents in the AAT and RAT as well as the Ni, Co, Mo contents in RAT samples were more useful for identifying the samples from the other locations. The variable importance of projection plot (Fig. 3c) identified the following as the most important discriminant variables: Ni, Cu, Mo, Mn, Be, Co, V, Zn, Al, and Fe in the RAT samples; K, Mo, Be, Al, Fe, Mg, Na, Ba, Li, and Sr in the soil samples, and Li, Be and Cu in the AAT samples.

Elemental relationship

All elements were analyzed by plotting the correlation matrix using Pearson's associations (Fig. 4). Significant correlations were detected between the elemental contents. For example, there were significant correlations between Al in AAT and Fe in RAT ($r > 0.85$), as well as between the Mg in AAT and V in RAT ($r > 0.85$). The Be content in RAT was significantly correlated with the Mo content in RAT ($r >$

0.90). Similarly, Be content in RAT and Ni content in RAT have a significant correlation ($r > 0.90$). The soil Al content was significantly correlated with the RAT Ba, Be Fe, K, and Mo contents ($r > 0.70$).

The AF represents the capacity of plants to accumulate elements from the soil, whereas TF reflects the transport of elements from the roots to aerial parts, which is important for the growth and development. The mean AF and TF value at the different sampling locations are listed in Table 1. The AF values indicated the plants were better able to take up Al, Ca, Fe, K, Mg, Mn, Na, P and V from the soil than the other elements. In terms of plant growth and development, Ca, Fe, K, Mg, Mn, Na, and P are the major required element. Calcium binds to pectin, which strengthens growing plants. Potassium contributes to the maintenance of vigor which also affecting storability. Notably, although Mg is a central atom of the light-absorbing complex in chloroplasts, the TF value of Mg was relatively low [24].

Table 1
The mean AF and TF value of
sampling locations

Element	AF	TF
Al	1.88±1.82	0.01±0.01
Ba	0.94±0.59	0.21±0.76
Be	0.8±0.24	3.55±3.98
Ca	1.52±1.2	1.43±4.05
Co	0.66±0.7	0.36±0.48
Cu	1.10±1.94	0.38±0.37
Fe	2.18±2.72	0.02±0.02
Li	0.59±0.59	1.60±1.60
K	4.01±3.26	0.46±0.22
Mg	2.31±1.37	0.45±0.59
Mn	2.64±2.72	0.04±0.03
Mo	0.80±0.82	0.18±0.21
Na	2.01±2.12	0.03±0.03
Ni	1.00±0.87	0.13±0.13
P	1.06±0.53	2.48±1.27
Sr	0.85±0.47	0.55±0.48
V	2.78±5.11	0.20±0.13
Zn	0.73±0.38	0.36±0.29

Discussion

The elemental contents in *A. tanguticus* were in accordance with the relevant data produced in earlier studies on traditional Tibetan medicine. For example, in previous investigations of *A. tanguticus* cultivated in Qinghai, the Co, Cu, Fe, Mg, Mn, and Zn contents determined by Xu et al., and Li et al. [25–27], were similar to the corresponding concentrations in the current study; however, the Ca, K, and P contents are apparently lower in cultivated *A. tanguticus* than in wild *A. tanguticus*. The contents of 10 elements in Tibetan medicine *Hypecoum leptocarpum* Hook. f. et Thoms. collected from different locations in a previous study were similar to the corresponding contents in *A. tanguticus* determined in our study, with the exception of K, Li, and V, the K content was higher, and the Li and V contents were lower than in the current study [28]. Many factors may contribute to the diversity in the elemental contents among *A. tanguticus* plants with different geographical origins, including plant species characteristics, and soil characteristics (e.g., soil cation exchange capacity, pH) [29].

In this study, we developed a method for determining the geographical origin of *A. tanguticus* on the basis of a PCA and OPLS-DA. This method can efficiently distinguish *A. tanguticus* samples from different locations. The PCA and OPLS-DA results revealed a relationship between the *A. tanguticus* growing regions and the contents of various elements. The selected regions in China were geographically separated, with different climatic, and soil conditions as well as distinct elemental characteristics. Accordingly, our multivariate analysis can rapidly, accurately, and efficiently trace the geographical origins of *A. tanguticus*.

The observed difference in the ability of plants to take up various elements from the soil may reflect the differential importance of the elements for *A. tanguticus* growth, development, and metabolism. The uptake of elements from the soil by the roots is influenced by several factors, including cation exchange, cellular transport, rhizosphere effects and soil characteristics which can be affected by root respiration and reactions involving root secretions [30, 31]. Additionally, Be, Ca, Li, and P can be transported from *A. tanguticus* roots to aerial part relatively easily. The TF values of Be, Ca, Li, and P were greater than 1.0, implying these elements are needed by aerial plant parts. At the same time, the transport of elements in plant tissues and organs involved processes: movement into phloem and xylem, accumulation, storage. Intraspecific differences in the elemental contents of the roots and aerial parts may be associated with the natural ability of plants to select elements from the soil and transport them to the aerial tissues and organs [32].

Conclusions

To the best of our knowledge, this is the first report describing the element fingerprinting and geographical classification and authentication of *A. tanguticus* via a PCA and OPLS-DA as well as the relationship between plant and soil elemental contents. We developed a method involving ICP-OES coupled with microwave-assisted digestion for an analysis of multiple -elements in *A. tanguticus*. The results indicated that *A. tanguticus* from different areas had distinct element fingerprint that could be

used to discriminate samples from different geographical origins. Furthermore, multi-element signatures were used to divide samples with different geographical origins into three groups. The Ni, Cu, Mo, Mn, Be, Co V, Zn, Al, and Fe contents in the RAT samples, the K, Mo, Be, Al Fe Mg, Na Ba Li, and Sr contents in the soil samples, and the Li, Be, and Cu content in the AAT samples were the most important discriminant variables in the OPLS-DA model. The method developed in this study may be useful for identifying various herbs and determining their geographical origins. Furthermore, the multi-element content in *A. tanguticus* depends on the soil conditions in a particular geographical region. Intraspecific differences in AF and TF are associated with the natural ability of plants to select and transport specific elements and may reflect the stress resistance mechanisms that have enabled plants to adapt to different environmental conditions.

Methods

Sample collection

The aerial parts (AAT) and roots (RAT) of *A. tanguticus* were collected in August 2020, in Qinghai (QH), Gansu (GS), Sichuan (SC), and Tibet (XZ) in China. Samples were collected at altitudes ranging from 2700 m to 4100 m above sea level (latitude: 30° -38°; longitude: 95° -102°) (Fig. 5). Three *A. tanguticus* samples and three soil samples were collected at each location. Ultrapure water was obtained from a MilliQ system (Millipore, USA), ultrapure HNO₃ and hydrogen peroxide were purchased from Jingrui (Suzhou, China).

Sample preparation

Briefly, 0.2 g plant sample was mixed with 10 mL HNO₃ and 2 mL hydrogen peroxide in a poly tetra fluoro ethylene digestion vessel and then incubated for 12 h. Additionally, 0.2 g soil sample was mixed with 10 mL HNO₃, 5 mL hydrofluoric acid and 5 mL perchloric acid in a poly tetra fluoro ethylene digestion vessel and then incubated for 12 h. The samples were digested using a microwave assisted digestion system as described by Chen et al [33]. The digestion solution was diluted to 50 mL using ultrapure water and then stored in a tube until analyzed.

The standard solution comprising Al, Ba, Be, Ca, Co, Cu, Fe, K, Li, Mg, Mn, Mo, Na, Ni, P, Sr, V, and Zn (National Research Center for Reference Materials, Beijing, China) was diluted to 0 -200 µg/L using 5% HNO₃. The GBW10052 green tea standard was used to verify the precision and accuracy of the analysis.

Analytical procedure

The elemental contents of AAT, RAT and soil were determined using the Optima 8000 ICP-OES system (PerkinElmer, Shelton, USA), with the following main conditions: RF power, 1300; auxiliary gas, 0.2 L/min; nebulizer gas, 1.2L/min; and sample uptake, 1.0 L/min.

Statistical analysis

Graphs were created using SigmaPlot 12.5 (Systat Software, Inc., Point Richmond, CA, USA). Pearson's correlation analysis, and the principal component analysis (PCA) were performed using the SPSS 18.0 software package (SPSS Inc., USA). The orthogonal partial least-squares discriminant analysis (OPLS-DA) was performed using SIMCA (Umetrics, Sweden). The elemental accumulation coefficient (AF) was calculated using the following formula:

$AF = C_{\text{root}} / C_{\text{soil}}$, where C=concentration of the element (mg/kg)

The elemental transfer coefficient (TF) was calculated using the following formula:

$TF = C_{\text{aerial}} / C_{\text{root}}$, where C=concentration of the element (mg/kg)

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Conflict of interest

All authors declare that they have no conflict of interest.

Availability of data and material

The datasets used and/or analysed during the current study are available from the corresponding author on request.

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Authors' contributions

GZ designed the experiments; CC, JL and BW established and validated the methods. CC and YX were involved in writing of the manuscript. All authors read and approved the final manuscript.

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Figures

Figure 1

The fingerprint of each multi-element distribution of *A. tanguticus*.

Figure 2

a) Score plot with the first two principal components of *A. tanguticus*. b) loading plot of PC1 and PC2 of *A. tanguticus*.

Figure 3

Comparison of OPLS-DA results from multi-elements of *A. tanguticus*. Score plot (a), loading plot from OPLS-DA (b), and VIP plots of variables (c).

Figure 4

Correlation of multi-elements of different parts of *A. tanguticus* and soil.

Figure 5

Geographical distribution of the regions of *A. tanguticus* were collected.

Supplementary Files

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