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Characterization of Codonopsis Radix with Agro-product Geographical Indication products "Tanchang Dangshen" according to the analyses of elements and bioactive compounds combined with ecological factors

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Abstract

"Tanchang Dangshen" codonopsis radix is a high-quality protected agro-product geographical indication (AGI), mainly grown in Gansu Province, China. In this study, combining multivariate analysis techniques with bioactive constituents and mineral elements, the differences between AGI-labeled "Tanchang Dangshen" codonopsis radix and adulterations from other geographical origins were investigated. Furthermore, the relationship between the chemical or mineral element characteristics of "Tanchang Dangshen" and its soil and climatic factors of geographical origin area was also explored. Using partial least square discriminant analysis, "Tanchang Dangshen" and the other codonopsis radix, with a similar botanical origin, were grouped according to mineral elements, especially the contents of potassium, selenium, and nickel. The environment with low temperatures was more conducive to the accumulation of potassium, which is the most abundant element of codonopsis radix. Soil factors, such as soil pH, soil organic matter, and soil nutrients, had an impact on the contents of trace elements it contains, i.e., nickel and selenium, which helped elucidate the relationship between the characteristics of high-quality AGI-labeled "Tangchang Danshen" and its geographical origin.

Keywords: codonopsis radix; mineral content; bioactive compounds; partial least squares analysis; agro-product geographical indication.

Practical Application: This work helps to identify AGI-labeled "Tanchang Dangshen" codonopsis radix and adulterations from other geographical origins and effectively reduces the risk of the mixing of adulterations or imitation products in the market.

1 INTRODUCTION

Codonopsis radix (roots of *Codonopsis*; Chinese name Dangshen) is one of the most commonly used traditional Chinese medicines that has been used for hundreds of years for replenishing the energy and invigorating the spleen (Chou et al., 2006). It is also a food medicine homology plant and used as food materials in China and Southeast Asia, such as tea, wine, soup, plaster, and porridge (Gao et al., 2018). The quality of codonopsis radix is highly dependent on the territory because each geographical area has particularities of climate, soil, and other ecological factors (Wan et al., 2021). Agro-product geographical indication (AGI) is a useful tool for the protection of the quality of codonopsis radix products (Katerinopoulou et al., 2020; Zhang et al., 2016). Gansu Province is the main geographical origin-producing region of codonopsis radix in China, with an annual output of more than 40,000 tons in 2021. "Tanchang Dangshen" codonopsis radix is one of three codonopsis radix products certified with AGI in Gansu Province and has valuable nutritional and medicinal qualities. Unfortunately, due to the incomplete quality evaluation methods of the AGI-labeled "Tanchang Dangshen" codonopsis radix products, their quality evaluation is usually based on macroscopic characteristics and other subjective features. There are risks such as mislabeling of origin, the substitution of products from non-AGI areas, and the mixing of adulterations or imitation products in the market. For the sake of brand protection and traceability following adulterations or imitation products offered to consumers, effective methods for authenticating "Tanchang Dangshen" codonopsis radix are therefore urgently needed.

In recent years, bioactive constituents and mineral elements have been mainly proposed to distinguish the geographical origins and varieties of codonopsis radix (Bai et al., 2021; Li et al., 2012; Wan et al., 2021). Meanwhile, the application of multivariate analysis has proved as a useful tool in the characterization of complex foods and medicinal plants, such as honey, ephedra, and codonopsis radix (Bai et al., 2021; Ma et al., 2018; Rodríguez-Flores et al., 2019). Among the methods most applied in distinguishing the geographical origins and

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varieties are principal component analysis (PCA), partial least squares analysis (PLS), and discriminant analysis (DA) (De Andrade et al., 2023; Nardecchia et al., 2020). In this study, combinations of these techniques with bioactive constituents and mineral elements were developed to detect the differences between AGI-labeled “Tanchang Dangshen” codonopsis radix and adulterations from other geographical origins. In addition to that, because AGI-labeled “Tanchang Dangshen” codonopsis radix characteristics depend on the planting environment, the climate, and the soil in which it is grown, these ecological factors can achieve more reliable geographical authentication of “Tanchang Dangshen” codonopsis radix product (Katerinopoulou et al., 2020). Accordingly, this study further investigated the relationship between the chemical or mineral element characteristics of AGI-labeled “Tanchang Dangshen” codonopsis radix and its soil and climatic factors of geographical origin area, using multivariate analysis techniques.

2 MATERIALS AND METHODS

2.1 Plant samples

A total of 33 batches of dried *Codonopsis pilosula* samples were mainly collected from Gansu Province, which is the main geographical origin-producing area of *C. pilosula* in China. A total of 20 batches of dried *C. pilosula* samples were collected in Tanchang district, and the others were obtained from different geographical origin-producing areas, mainly Dingxi district. The details of the samples are shown in Supplementary Table S1.

2.2 Reagents

Lobetyolin which was used as a standard was purchased from Forever-biotech Ltd. (Shanghai, China), and the purity was above 98% according to HPLC/UV. D-anhydrous glucose (No. 110833-201506, 99.9%) was purchased from National Institutes for Food and Drug Control. The methanol and acetonitrile (Shandong Yuwang Industrial Co., Shandong, China) used in our study were of HPLC grade. All other reagents and chemicals were of analytical grade.

Mono-elemental, high-purity grade 1 mg mL^{-1} standard stock solutions of Co, Cr, Cu, Fe, K, Mg, Mn, Mo, Na, Ni, Se, V, and Zn were purchased from the National Institution Metrology of China, which were further prepared in a multi-elemental solution containing 2% v v^{-1} HNO_3 (~65% w w^{-1}, Merck, Germany). Li, Sc, Ge, Rh, In, and Bi at 10 mg L^{-1}, internal standards, were purchased from Agilent (Part # 5188-6525). The element determinations were performed by an inductively coupled plasma mass spectrometer (ICP-MS, Agilent 7700e, USA).

2.3 Determination of bioactive constituents

All codonopsis radix extractives were determined by the hot extraction method, using 45% ethanol as solvent (Commission, 2020). The anthrone-sulfuric acid colorimetry method was used to determine the total polysaccharides content (Commission, 2020). Codonopsis radix samples (0.1 g) were added with 100 mL of 80% ethanol and 60 mL water were extracted by reflux for 1 and 2 h, respectively, and the absorbance at 581 nm (by a UV-vis spectrophotometer) was measured. The calibration curve was obtained with glucose solutions as the reference standard (0.04–0.24 mg). The linearity of the curve was 0.9981 (R), and the results were expressed as total polysaccharides equivalents in g/100 g. Lobetyolin in codonopsis radix was determined by HPLC (Chou et al., 2006), using a Waters 2659 HPLC system (Waters, USA) with a diode array detector and the analytical column, Capcell Pak C18-MGIIreversed phase column (250 mm × 4.6 mm i.d. 5 μm particle diameter). The samples of codonopsis radix (2.0 g) added 50 mL methanol were extracted by ultrasonic for 30 min and filtered. A volume of 25 mL of the subsequent filtrate was evaporated to dryness under reduced pressure, and the residue was diluted to 5 mL methanol. The sample solution of codonopsis radix was separated with a gradient of two mobile phases (A and B). Phase A involved ultrapure water, while phase B involved acetonitrile (HPLC grade, Merck). The calibration curve was obtained with lobetyolin as the reference standard (0.33–6.55 mg). The linearity of the curve was 0.9970 (R), and the results were expressed as lobetyolin in g/100 g.

2.4 Determination of mineral elements

All codonopsis radix samples were digested before the determination of mineral elements. About 0.3 g of the samples were accurately weighed into the digestion tank; 10 mL of nitric acid and 3 mL of hydrogen peroxide were added and soaked for 12 h (Commission, 2020). The microwave digestion system (Milestone Microwave Labstation ETHOSD) was used for the digestion of all codonopsis radix samples. After digestion, the contents of the tubes were transferred to a volumetric flask and made up to 25 mL with 2% nitric acid. Blank experiments (n = 3) were carried out in the same way. For analytical quality assurance, the performance of the method to analyze the contents of the mineral elements in codonopsis radix samples was evaluated by method detection limit, instrument detection limit, and linearity. The results are shown in Supplementary Table S2.

2.5 Data on climate factors

The annual mean Earth Skin Temperature (TS), January mean Earth skin temperature (JAN), July mean Earth Skin Temperature (JUL), Surface Soil Wetness (GWETTOP), Root Zone Soil Wetness (GWETROOT), Frost Days (FROST_DAYS), Precipitation Corrected (mm/day) (PRECTOT), and All Sky Surface Photosynthetically Active Radiation Total (W/m^2) (ALLSKY_SFC_PAR_TOT) were obtained from the NASA 30-year Meteorological and Solar Monthly and Annual Climatologies data (from January 1990 to December 2019).

2.6 Data on soil factors

The soil acidity (pH), the contents of organic matter (OM), alkali-hydrolyzable nitrogen (S-N), available phosphorus (S-P) and potassium (S-K) in soil, and the contents of soil cation exchange capacity (CEC) were determined by the Agricultural Testing Center of Gansu Academy of Agricultural Sciences. The results are shown in Supplementary Table S3.

2.7 Statistical analysis

One-way multivariate analysis of variance (MANOVA) was used to analyze multiple-sample comparison for bioactive constituents and mineral elements in codonopsis radix between the two main geographical origin-producing areas and was performed with IBM SPSS Statistics 18 (SPSS Inc., Chicago, IL, USA). The PCA, PLS, and partial least square discriminant analysis (PLS-DA) were performed by using Unscrambler X version 10.3 (Camo Software, Oslo, Norway).

3 RESULTS AND DISCUSSION

3.1 Analysis of bioactive constituents in Codonopsis Radix

The geographical origin-producing areas of codonopsis radix have a statistically significant effect on bioactive constituents, polysaccharides, lobetyolin, and ethanol-soluble extractives as determined by one-way MANOVA, F (3, 29) = 3.95, $p < 0.05$, Wilk's $\Lambda = 0.710$, and partial $\eta^2 = 0.29$. Furthermore, codonopsis radix ethanol-soluble extractives had statistically significantly higher mean values in samples from the Tanchang district (74.96 ± 3.92%, $p < 0.005$) than from other geographical origin-producing areas (69.02 ± 6.41%) as well as one-way MANOVA revealed that the lobetyolin content was statistically significantly lower in samples from Tanchang district (0.088 ± 0.035%, $p < 0.05$) compared to other geographical origin-producing areas (0.123 ± 0.05%) (Table 1). There were no statistically significant polysaccharides content differences between Tanchang district and other geographical origin-producing areas ($p = 0.224$) (Table 1).

3.2 Analysis of mineral content in Codonopsis Radix

Multiple-sample comparisons for the mineral content (mg/kg) in codonopsis radix from the two main geographical origin-producing areas by one-way MANOVA are also shown in Table 1. The geographical origin-producing areas of codonopsis radix had a statistically significant effect on macro-mineral contents, i.e., K, Mg, Fe, Na, Mn, Zn, and Cu as determined by one-way MANOVA, F (7, 25) = 3.44, $p < 0.05$, Wilk's Λ = 0.509, and partial $\eta^2 = 0.49$. Also, one-way MANOVA showed that the K, Mg, Fe, Mn, and Zn contents in codonopsis radix were statistically different between Tanchang district and other geographical origin-producing areas ($p < 0.05$), but not for the Na and Cu contents ($p > 0.05$). Potassium (K) was the most abundant metal in codonopsis radix, with an average mean content of 9114.665 mg/kg in all samples, which coincides with the results mentioned by other authors (Bai et al., 2021).

Likewise, the geographical origin-producing areas of codonopsis radix had a statistically significant effect on micro-mineral contents, i.e., Se, Ni, V, Cr, Mo, and Co determined by one-way MANOVA, F (6, 26) = 2.64, $p < 0.05$, Wilk's Λ = 0.621, and partial $\eta^2 = 0.38$. Also, one-way MANOVA showed that the V, Cr, and Co contents in codonopsis radix were statistically significantly different between Tanchang district and other geographical origin-producing areas ($p < 0.05$), but not for the Se, Ni, and Mo contents ($p > 0.05$).

3.3 Classification of codonopsis radix by multivariate techniques

The above results indicated that the chemical and mineral characteristics of codonopsis radix produced in the two main geographical origin-producing areas were highly similar. How to detect the differences between AGI-labeled "Tanchang Dangshen" codonopsis radix and adulterations from other geographical origins? First, a PCA was applied because it is convenient to reduce the data dimension and visualize the similarities among samples of codonopsis radix produced in different geographical

Table 1. Multiple-sample comparison for bioactive constituents and mineral elements in codonopsis radix between the two main geographical origin-producing areas by one-way MANOVA.

Cultivation region	Tanchang district (TC)	Other geographical origin-producing areas (GS)	MANOVA	
	Mean ± SD	Mean ± SD	F-ratio	*p*-Value
Polysaccharides %	13.34 ± 2.53	12.18 ± 2.79	1.54	0.22
Lobetyolin %	0.088 ± 0.03	0.123 ± 0.05*	5.17	0.03
Ethanol-soluble extractives %	74.96 ± 3.92**	69.02 ± 6.41	10.97	0.00
K	8207.22 ± 1096.78	10022.11 ± 2353.90*	9.00	0.01
Mg	1269.13 ± 125.34	1478.07 ± 316.23*	7.12	0.01
Fe	388.31 ± 111.51	575.79 ± 265.78*	7.92	0.01
Na	113.26 ± 73.61	123.21 ± 95.98	0.11	0.74
Mn	25.56 ± 3.38	31.81 ± 9.21*	7.72	0.01
Zn	17.08 ± 3.40	27.97 ± 16.77*	8.05	0.01
Cu	6.07 ± 0.97	5.83 ± 1.15	0.39	0.53
Se	4.12 ± 0.76	4.22 ± 0.82	0.12	0.73
Ni	1.70 ± 0.83	1.57 ± 0.44	0.27	0.61
V	0.89 ± 0.26	1.34 ± 0.63*	8.32	0.01
Cr	0.79 ± 0.30	1.23 ± 0.54*	9.06	0.01
Mo	0.26 ± 0.11	0.28 ± 0.17	0.10	0.76
Co	0.20 ± 0.04	0.27 ± 0.10*	8.38	0.01

*Significant differences between the two main geographical origin-producing areas by one-way MANOVA ($p < 0.05$); **Highly significant differences between the two main geographical origin-producing areas by one-way MANOVA ($p < 0.005$).

origins. Upon applying PCA to the bioactive constituents and mineral elements content for all codonopsis radix samples, the first three PCs collectively explained 68% of the total data variability. The first two PCs were used to generate score plots in which the 33 codonopsis radix samples were represented using different shapes and colors indicating their geographical origins, while a strong overlap between "Tanchang Dangshen" and the other codonopsis radix samples remained (Fig. 1A). This was expected because despite having different geographical origins, the flora of the two studied regions is similar. The loading plot (Fig. 1B) indicated that the content of ethanol-soluble extractive contributed most to PC1, and the contents of Se and K contributed most to PC2. These results obtained with PCA were limited and unsatisfactory. Therefore, further supervised classification techniques such as PLS-DA were used to classify codonopsis radix samples based on their geographical origin-producing areas.

A PLS-DA, which is a widely used classification method, was used to further increase the separation of codonopsis radix samples with different geographical origins. In this study, 33 samples that were divided into 20 from Tanchang district (class TC) and 13 from other geographical origin-producing areas (class GS) were used for building a discriminant model. In the score plot of the PLS-DA model, the samples of codonopsis radix projected to components 1 and 2 were divided into two clustering regions (Figure 2A). Codonopsis radix samples originating from Tanchang district were separated from samples from other geographical origins, indicating that these two groups were well separated on the two first factors by PLS-DA. From the scores plot (Figure 2A), the combination of two main factors, factor 1 and factor 2, reflects the variations in the chemical and mineral data of codonopsis radix, which indicated that 70% (X1 53, X2 17) of the chemical and mineral data variance explains 97% (Y1 90, Y2 7) of the response discrimination between the two main geographical origin-producing areas of codonopsis radix.

The classification cross-validation error, root-mean-square error of cross-validation, was 0.1709; the R^2 value was 0.9712 (Supplementary Figure S1). The results demonstrated the model's robustness and accuracy. Two variables (the contents of K and Se) were found to be highly important (with close to TC and highly positive correlation) in the discrimination model for codonopsis radix produced from Tanchang district (TC) and other geographical origin-producing areas (GS) (Figure 2B).

To further analyze the cumulative importance of each chemical and mineral variable in classing radix codonopsis produced from the two main geographical origin-producing areas, the regression coefficients are used to calculate the response value of discrimination between TC and GS areas of codonopsis radix from the chemical and mineral variables. The size of the weighted regression coefficients (BW) indicates which chemical and mineral variables have a major impact on the classing codonopsis radix produced from the two main geographical origin-producing areas. From the weighted regression coefficients plot (Figure 3), K, Se, and Ni (B8, B12, and B13) were statistically significant in discriminating between TC and GS areas of codonopsis radix (Supplementary Table S4), which indicated that they were the most important variables that separated the two groups, along with ethanol-soluble extractive (B3), although with a slightly lower discrimination ability (Supplementary Table S4). This result further clarified that the main variables in the model for the discrimination of codonopsis radix produced from TC and GS.

3.4 Correlation analysis between ecological factors and the contents of K, Se, Ni, and ethanol-soluble extractive

The study further examined how geographical origin influenced the contents of K, Se, Ni, and ethanol-soluble extractive of "Tanchang Dangshen" codonopsis radix, to elucidate the significant ecological factors for the formation of "Tanchang Dangshen" codonopsis radix AGI product.

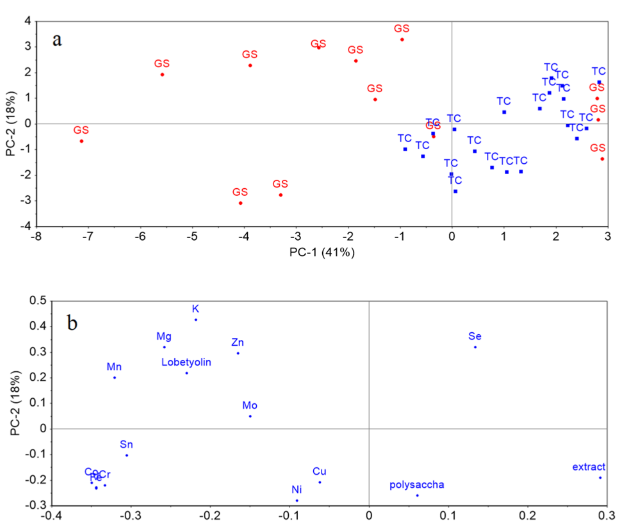


Figure 1. (A) PCA scores and (B) loading plots obtained from the bioactive constituents and mineral elements content for all codonopsis radix samples: Tanchang District (▪) and other geographical origin-producing areas (●).

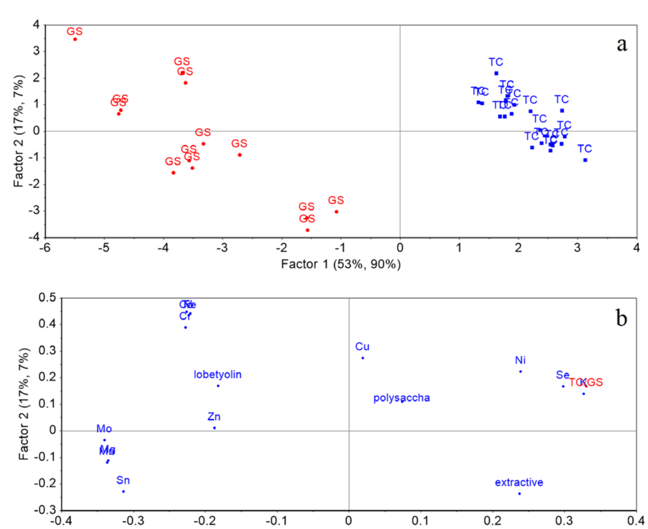


Figure 2. (A) PLS-DA score and (B) loading plots based on the chemical and mineral data of codonopsis radix produced from Tanchang district (TC) and other geographical origin-producing areas (GS).

"Tanchang Dangshen" codonopsis radix is produced in Tanchang district with subtropical, temperate, and frigid transitional climate areas; other geographical origin belongs to a typical temperate continental climate zone (Wan et al., 2021). We analyzed the interpretation of correlations between the contents of K, Se, Ni, and ethanol-soluble extractive in "Tanchang Dangshen" codonopsis radix and climate and soil factors of the cultivation areas – Tanchang district – using a PLS analysis. In the PLS plot of X- and Y-correlation loading weights (Figure 4), PLS factor 1 described the climatic factors (Elevation, All Sky Surface PAR Total, Latitude, Frost Days, JAN, JUL, TS, and Longitude) of Tanchang district and PLS factor 2 described the soil factors (pH, S-K, S-P, S-N, and OM) of Tanchang district. The plot also showed that these climatic factors, Elevation, All Sky Surface PAR Total, Latitude, and Frost Days, had an extreme position to the right of the plot, and they were close to each other (i.e., they were highly positively correlated); these four variables were opposite to the variable temperature and longitude which had lower values for this PLS. Moreover, these four variables were far from the center and very close to the K, which also means that they may be positively correlated with K content. Similarly, ethanol-soluble extractives may be strongly positively correlated with soil factors, i.e., S-K, S-P, S-N, and OM.

To further determine which specific climate and soil factors variables have a significant effect on the contents of K, Se, Ni, and ethanol-soluble extractive in "Tanchang Dangshen" codonopsis radix, the sizes of the weighted regression coefficients (BW) will indicate the cumulative importance of each of the climatic and soil variables to the contents of K, Se, Ni, and ethanol-soluble extractive of "Tanchang Dangshen."

In the weighted regression coefficient plot of K content (Figure 5A), Frost days (BW3), Sky Surface PAR Total (BW5), and

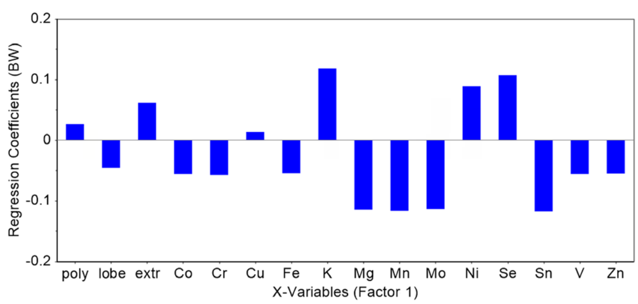


Figure 3. The weighted regression coefficients plot of PLS-DA (poly, polysaccharides; lobe, lobetyolin; extr, ethanol-soluble extractives).

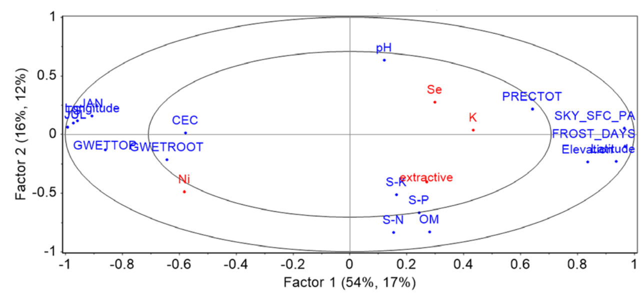
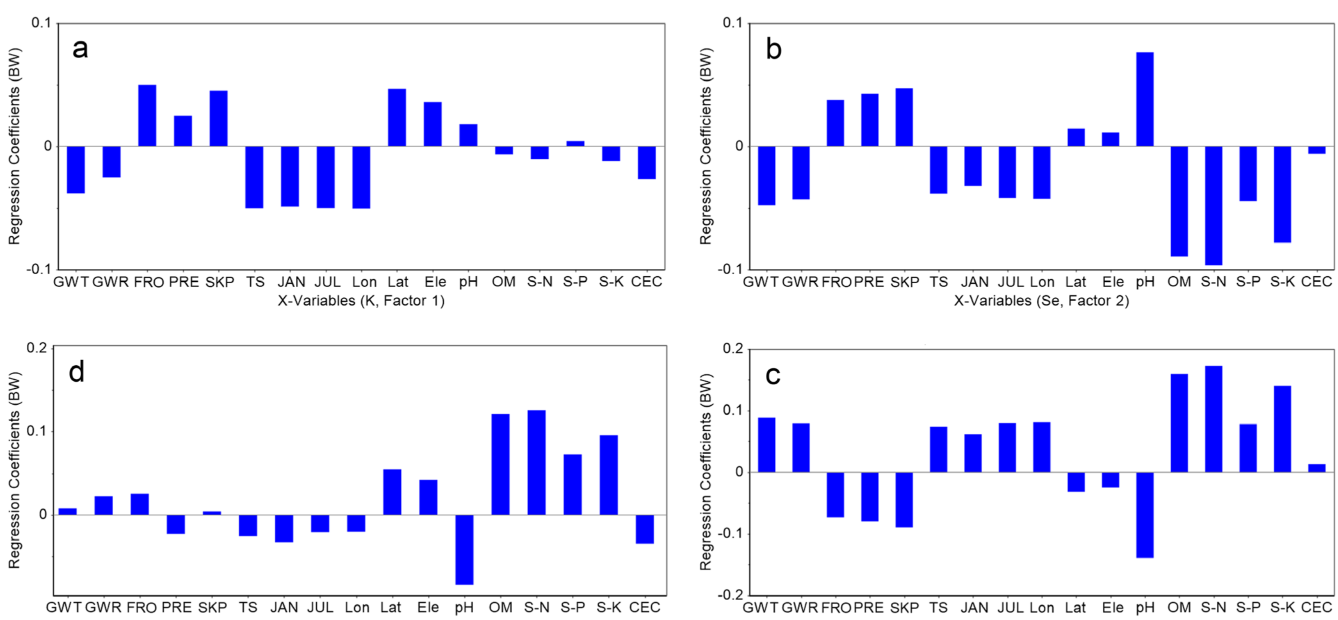


Figure 4. PLS plot of X- and Y-correlation loading weights based on the chemical, mineral data, and ecological factors of codonopsis radix produced from Tanchang district.



GWT: Surface Soil Wetness; GWR: Root Zone Soil Wetness; FRO: Frost Days; PRE: Precipitation Corrected; SKP: All Sky Surface Photosynthetically Active Radiation Total; TS: Annual mean Earth Skin Temperature; JAN: January mean Earth Skin Temperature; JUL: July mean Earth Skin Temperature; Lon: longitude; Lat: latitude; Ele: elevation; pH: soil acidity; OM: organic matter in soil; S-N: alkali-hydrolyzable nitrogen in soil; S-P: available phosphorus in soil; S-K: available potassium in soil; CEC: soil cation exchange capacity.

Figure 5. The weighted regression coefficients plot of (A) K, (B) Se, (C) Ni, and (D) ethanol-soluble extractive content of codonopsis radix produced from Tanchang district.

Latitude (BW10) had a more impact on the K content of radix codonopsis produced from Tanchang district (Supplementary Table S5). TS, JAN, JUL, and Longitude (BW6, BW7, BW8, and BW9) were also significant but contributed negatively to the K content (Supplementary Table S5). The growing area of "Tanchang Dangshen" codonopsis radix is an alpine and damp zone, with an average annual temperature of 6.5°C. The result may be related to the previous studies: Potassium assists plants against abiotic stress conditions in the environment, such as low temperature and chilling (Johnson et al., 2022).

In the weighted regression coefficient plot of the Se content (Figure 5B), soil pH (BW12) had an impact on the Se content of codonopsis radix produced from Tanchang district, as well as Frost days (BW3), Precipitation (BW4), and Sky Surface PAR Total (BW5) although with a slightly lower impact ability (Supplementary Table S5). The reason for this result is that some soil properties will affect the bioavailability of Se in soils and consequently influence the efficiency of Se uptake in plants, such as pH in soils (Feng et al., 2021).

In the weighted regression coefficient plot of the Ni content (Figure 5C), soil organic matter (BW13) and soil nutrients such as nitrogen (BW14) and potassium (BW16) played an important role in the Ni content of "Tanchang Dangshen" codonopsis radix, as well as Surface Soil Wetness (BW1), Root Zone Soil Wetness (BW2), JUL, and Longitude (BW8 and BW9) although with a slightly lower impact ability (Supplementary Table S5). The reason for this result is that the phytoavailability of Ni has been correlated with free nickel ion activity in soil solution. Hence, plant uptake is also dependent on soil pH and organic matter content (Kashem & Singh, 2001).

In the weighted regression coefficient plot of ethanol-soluble extractive content (Figure 5D), soil organic matter (BW13) and soil nutrients (e.g., nitrogen, phosphorus, and potassium (BW14, BW15, and BW16)) had a much impact on the ethanol-soluble extractive content of codonopsis radix produced from Tanchang district (Supplementary Table S5). Soil pH (BW12) was also significant but contributed negatively to the extractive content (Supplementary Table S5). This suggested that soil factors may have a greater effect on the content of ethanol-soluble extractives in codonopsis radix than climatic factors.

4 CONCLUSIONS

The application of mineral elements and bioactive constituents combined with multivariate techniques such as PLS-DA analysis and MANOVA confirmed the discrimination and classification of "Tanchang Dangshen" codonopsis radix concerning the other codonopsis radix from the geographical origin-producing areas, mainly from Dingxi district in Gansu Province. Using PLS-DA, "Tanchang Dangshen" and the other codonopsis radix, with a similar botanical origin, were grouped based on mineral elements, especially the contents of potassium, selenium, and nickel. The environment with low temperatures was more conducive to the accumulation of potassium, which is the most abundant element of codonopsis radix. Soil factors, such as soil pH, soil organic matter, and soil nutrients, had an impact on the contents of trace elements it contains, i.e., nickel and selenium, which helped elucidate the relationship between the characteristics of high-quality AGI-labeled "Tangchang Danshen" and its geographical origin.

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