Inorganic Elements in Tuber of Chinese Herbal Medicine Radix Pseudostellariae from Guizhou Province

Shao Daixing[1], Liu Hong[2], Lin Changhu[3], Lin Li[4], Zhou Kaifang[1]
(1Soil and Fertilizer Station of Zunyi City, Zunyi Guizhou 563000;
2Guizhou Industrial Technology Development Research Institute, Guiyang 550025;
3Guizhou Academy of Sciences, Guiyang 550001;
4Agriculture and Animal Husbandry Bureau of Fenggang County, Zunyi Guizhou 564200)

Abstract: By analyzing the content of inorganic elements in radix pseudostellariae from Guizhou, we aim to assess the present quality of radix pseudostellariae, set a limited standard of heavy metals, establish an element finger-print, and find out the characteristic elements. The content of inorganic elements was measured and an element finger- print was drawn by the ICP- MS. Additionally, WM/T_2-2004 was applied to evaluating the quality of heavy metal elements, and the characteristic elements were determined by principal component analysis. The results showed that the content of inorganic elements in radix pseudostellariae was between 0.057 and 959 mg/kg, the coefficient of variation was 0.134 -1.478, and the content of Cd, As, Pb, and Hg was below the Standard of WM/T_2- 2004 in 90% radix pseudostellariae. The limited standard of heavy metals in radix pseudostellariae was Cr≤6.5 mg/kg, Cu≤10 mg/kg, As≤2.0 mg/kg, Cd≤0.3 mg/kg, Hg≤0.15 mg/kg, Pb≤4.0 mg/kg. The features of the inorganic elements finger- print could provide theoretical basis in identifying the quality of radix pseudostellariae and distinguish the radix pseudostellariae from other Chinese herbal medicines. The characteristic inorganic elements of radix pseudostellariae were found to be Cd, Cu, Co, Zn, Fe, Ca, Mg, and Al. Radix pseudostellariae from Guizhou contained abundant inorganic elements, the content of heavy metals was below the evaluation criterion, the limited standard of heavy metals was proposed, the element finger-print was drawn, and the characteristic elements were determined eventually.

Keywords: Guizhou; Radix pseudostellariae; Inorganic element; Principal component analysis; Element finger print
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About the first author: shaodaixing, male, born in 1981 in Tongzi, Guizhou Province, senior agronomist, master's degree, engaged in soil and fertilizer research. Mailing address: No. 154, Fenghuang North Road, Huichuan District, Zunyi City, Guizhou Province, 563000, tel:0851-23139663, e-mal: 65487525@qq.com 。
Corresponding author: Liu Hong, female, born in 1990 in Jining, Shandong Province, intermediate engineer, postgraduate, engaged in environmental analysis and evaluation research. Mailing address: No. 15, Changling South Road, guanshanhu District, Guiyang City, Guizhou Province, 550081, tel:0851-87992953, e-mal: 624188083@qq.com 。
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0 Introduction

Pseudostellariaheterophylla (miq.)Pax ex Pax et Hoffm is also called Baby ginseng (Yinpian Xinshen). Four leaf ginseng or rice ginseng (Annals of traditional Chinese Medicine). Tongshen (commonly used Chinese herbal medicine in Shanghai) is the dry root of heterophyllous Pseudostellaria of Caryophyllaceae [1], mainly distributed in Jiangsu Province. Fujian. Anhui and other provinces, Guizhou. Zhejiang. Jiangxi and other provinces also have cultivation [2]. Radix Pseudostellariae is a commonly used traditional Chinese medicine contained in the Chinese Pharmacopoeia, which has the function of Supplementing Qi and strengthening the spleen. Invigorate the body fluid and nourish the lungs. Anti fatigue. Oxidation resistance. Reduce blood sugar. Reduce blood lipids. The effect of enhancing immunity is often used in clinic for loss of appetite. Deficiency of Qi and Yin. Weakness after illness. Spontaneous sweating and thirst. Lung dryness, dry cough and other symptoms [3-5]. Studies have shown that in addition to organic compounds, Fe, Mg, Ca, Zn, Mn, K, Cu and other inorganic elements also play a very important role in the efficacy of the medicinal components of Rhizoma Radix, and are also an important basis for evaluating the quality[6-8]. There is a certain correlation between the contents of a large number of inorganic elements in Radix Pseudostellariae [7]. Only by finding out the key elements that affect Radix Pseudostellariae with certain methods can we grasp the focus of the research. At the same time, Radix Pseudostellariae also contains some inorganic elements such as arsenic. Mercury. Lead. Cadmium. Chromium and other elements, which are also indispensable to traditional Chinese medicine, will seriously affect the quality and medicinal value of Radix Pseudostellariae if the content exceeds a certain amount, and will also damage human health [9], but there is no standard value for the reasonable content of heavy metals in Radix Pseudostellariae at present. For the evaluation of the quality of Radix Pseudostellariae, Huang Xiuping [10] preliminarily formulated the limit values of some inorganic elements through research, but there is no systematic evaluation standard system, and other countries have only formulated the corresponding limit standards for the content of harmful elements such as heavy metals in traditional Chinese medicine [11-12]. The safety evaluation of inorganic elements and heavy metals in Radix Pseudostellariae in China relies on <<Green industry standard for the import of medicinal plants and preparations (WM/T2-2004)>>[12-15], However, the standard does not specify the limit standard of Cr content; with the continuous progress and development of instruments and technology, fingerprints have been applied to the quality evaluation of traditional Chinese medicine, but there are more studies on the fingerprints of organic substances in Radix Pseudostellariae [16-17], and there are few reports on the fingerprints of inorganic elements. Through the analysis and Research on the inorganic elements of Radix Pseudostellariae, the content law of inorganic elements is explored, and a scientific and reasonable quality evaluation system and standard are formulated to provide a reference basis for the development and utilization of Radix Pseudostellariae.

Through the detection, research and analysis of inorganic elements in the authentic Chinese medicinal material Pseudostellaria heterophylla produced in Guizhou, this study understands the basic law of the content of inorganic elements and adopts the current standards to evaluate the quality. Combined

with the "green industry standard for the import of medicinal plants and preparations (WM/T2-2004)" for statistical analysis, it preliminarily formulates the limit values of Cr and other heavy metal elements in Pseudostellaria heterophylla, in order to know more about the content law of inorganic elements in Pseudostellaria heterophylla. To provide basis for evaluating the quality of Radix Pseudostellariae. By analyzing the fingerprint of inorganic elements of Radix Pseudostellariae, we can provide technical reference for identifying the quality of Radix Pseudostellariae and distinguishing Radix Pseudostellariae from other traditional Chinese medicines; with the help of principal component analysis, a large number of inorganic elements in Radix Pseudostellariae were statistically analyzed to determine the inorganic elements that have a major impact on Radix Pseudostellariae.

1 Materials and methods

1.1 Drugs and reagents

Nitric acid, GR grade, Merck company of Germany; shrub branches and leaves reference material, No. GBW07603 (GSV-2), Institute of geophysical and geochemical exploration, Ministry of Geology and mineral resources.

Mixed Standard Solution of Heavy Metals and Trace Elements: Agilent part # 5183-4688. Elements Ca, Mg, Fe 1000 mg/L, Mn, Ni, Zn, Cu, Co, Pb, Al, As, Cd, Cr, Se, V, Th 10 mg/L. Hg is a single standard solution with concentrations of 0.0, 1.0, 2.0, 4.0, 6.0, and 8.0 ng/mL.

Internal standard solution: Agilent part #5188-6525, 100 mg/L Li6, Sc, Ge, Lu, Bi, Rn, In, Tb in 10% nitric acid; Tuning solution: Agilent part #5184-3566, with 2 % Nitric acid as medium 100 mg/L Li, Ge, Y, Co, Ti 100 mg/L.

1.2 Sample processing and digestion

The samples are from the radix Pseudostellariae base in niudachang Town, Shibing County, Qiandongnan Miao and Dong Autonomous Prefecture, Guizhou Province. In July, 2013, 100 representative Radix Pseudostellariae root samples from different plots were collected. After the samples are washed with deionized water, they are naturally dried for 2 hours, and dried in an 80℃ electric constant temperature blast drying oven for standby.

Sample processing: accurately weigh a certain quality of Radix Pseudostellariae root sample with an electronic balance of one ten thousandth, put it into a polytetrafluoroethylene nitrate boiling tank, add 5mLnitric acid, heat it in a constant temperature drying oven at 170℃ for 3 hours, cool it, fix the volume to a glass colorimetric tube of 50 mL, shake it well, and get the solution to be tested for the sample. Set 2 blanks for each batch of samples.

1.3 Optimization of instrument conditions

Inductively coupled plasma mass spectrometer (ICP-MS, Agilent 7500a) was used for determination. The repetition number of the analyzed samples was 10% - 15%. See Table 1 [18] for the condition parameters.

Table 1 Operation and data acquisition parameters of inductively coupled plasma mass spectrometer

Project	Working conditions	Project	Working conditions
RF power/w	1300	Atomization chamber temperature/℃	2.0
Carrier gas flow rate/(l/min)	0.80	Sampling speed of peristaltic pump/(r/s)	0.1
Auxiliary gas flow rate/(l/min)	0.35	Integration time/s	2

Sampling depth/(mm)	8.0	Repetition times/time	3

1.4 Data processing

The 15 inorganic elements in Radix Pseudostellariae were sorted by Excel, and the copper in Radix Pseudostellariae was analyzed by software origin 7.5. Arsenic. Cadmium. Mercury. The measured values of lead and heavy metals were compared and analyzed with the green industry standard for the import of medicinal plants and preparations. The software unscrambler 9.1 was used to fit and analyze the map of inorganic elements in Radix Pseudostellariae, and SPSS 19.0 was used to analyze the principal components of Radix Pseudostellariae samples.

2 Results and analysis

2.1 Analysis of inorganic elements in Radix Pseudostellariae

The statistical analysis of the content of 15 inorganic elements in Radix Pseudostellariae samples is shown in Table 2. The content of various elements in Radix Pseudostellariae is different. Generally, the content of inorganic elements is higher than that of heavy metals. The highest content of elements is mg: 959 mg/kg, and the lowest content is hg: 0.057 mg/kg. The difference between the maximum and minimum values of different elements is different. Se is the most obvious, and the highest content is 70.4 times of the lowest content; the difference of aluminum is the smallest, and the highest content is 1.98 times of the lowest content. In terms of coefficient of variation, the average value is 0.49. Except that the coefficient of variation of Hg is greater than 1, the coefficient of variation of other elements is less than 1, and the smallest coefficient of variation is Fe.

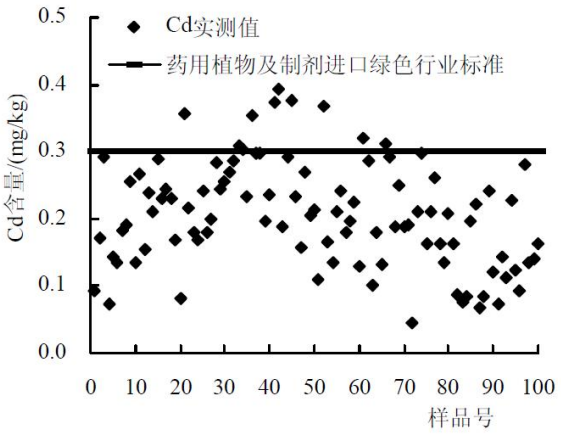
Table 2 Inorganic element content of Radix Pseudostellariae mg/kg

Project	Se	Cd	As	Pb	Cu	Co	Ni	Zn	Mn	Fe	Ca	Mg	Al	Cr	Hg
Maximum value	0.352	0.392	2.72	5.98	14.23	3.75	33.48	36.99	734	734	1093	1638	399	8.83	0.360
Minimum value	0.005	0.044	0.20	0.95	2.43	0.10	7.46	5.60	104	342	501	502	202	1.05	0.000
Average value	0.120	0.205	1.15	2.65	7.55	1.30	15.94	16.64	256	558	728	959	310	4.00	0.057
Standard deviation	0.093	0.079	0.71	1.41	3.00	0.85	4.82	8.73	111	75	127	178	43	2.38	0.084
Coefficient of variation	0.777	0.384	0.617	0.533	0.397	0.653	0.302	0.525	0.432	0.134	0.175	0.186	0.139	0.596	1.478

2.2 Comparison and analysis of heavy metal content in Radix Pseudostellariae with wm/t_2-2004

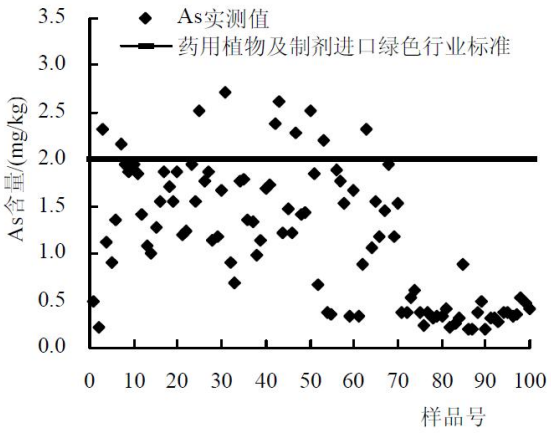
By determining CD in the sample. As. Pb. Cu. For the content of five heavy metals such as Hg, the comparison diagram between the measured value of heavy metals and the green industry standard for the import of medicinal plants and preparations (WM/T2- 2004)(Figure 1-5) was drawn by using origin 7.5 data processing software. Because the green

industry standard for the import of medicinal plants and preparations did not specify the standard value of Cr, the comparison diagram between chromium and the green industry standard for the import of medicinal plants and preparations was not drawn. From Figure 1, 2, 3, 5 it can be seen that 90% of Radix Pseudostellariae contains CD. As. Pb. The Hg content is lower than the corresponding element value in the green industry standard for the import of medicinal plants and preparations. It can be seen from Figure 4 that the measured content of Cu is lower than the value of 20 mg/kg of Cu in the green industry standard for the import of medicinal plants and preparations, indicating that the heavy metal pollution of Guizhou Prince Taizi is relatively light.



Sample No
CD content
Measured value of CD
Green industry standard for the import of medicinal plants and preparations

Figure 1 Comparison between measured CD value and WM/T2-2004 Standard



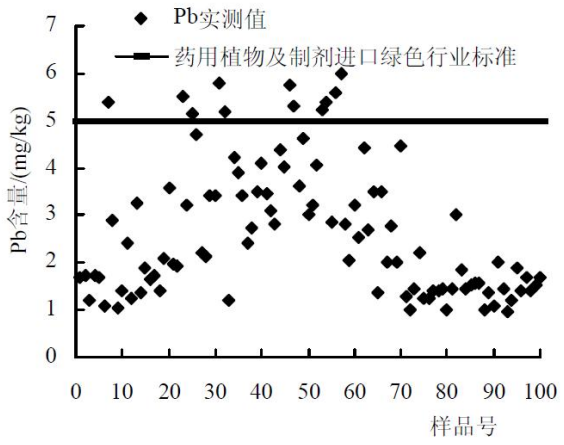
Sample No
As content
As measured value
Green industry standard for the import of medicinal plants and preparations

Figure 2 Comparison between as measured value and WM/T2-2004 Standard

2.3 Formulation of standard values of heavy metals in Radix Pseudostellariae

Through the detection and analysis of heavy metals in Radix Pseudostellariae samples, and the correction and statistical analysis of the detection data, the abnormal data are eliminated, so that the confidence interval is within 90%. In order to ensure that more than 80% of the data meet the requirements, the preliminary limit value of heavy metals in radix Pseudostellariae is: Cr ≤ 6.5 mg/kg; cu≤10 mg/kg; as≤2.0 mg/kg; cd≤0.3 mg/kg; hg≤0.15 mg/kg; pb ≤ 4.0 mg/kg, which provides a reference basis for formulating the heavy metal limit standard of Radix Pseudostellariae in the future. The proposed standard as. The value of CD is the same as the green industry standard for the import of medicinal plants and preparations (WM/T2-2004), Cu, Hg, Pb is slightly lower than the green industry standard for the import of medicinal plants and preparations (WM/T2-2004). Origin 7.5 was used to plot heavy metal Cd, As, Pb, Cu, Cr, Hg measured

value and proposed standard diagram (Figure 6-11).



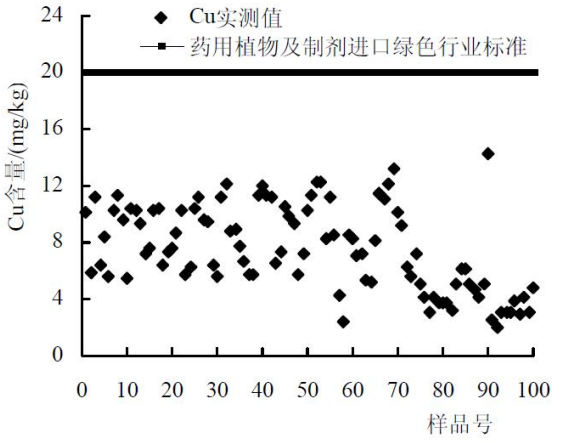
Sample No

Pb content

Pb measured value

Green industry standard for the import of medicinal plants and preparations

Figure 3 Comparison between Pb measured value and WM/T2-2004 Standard



Sample No

Cu content

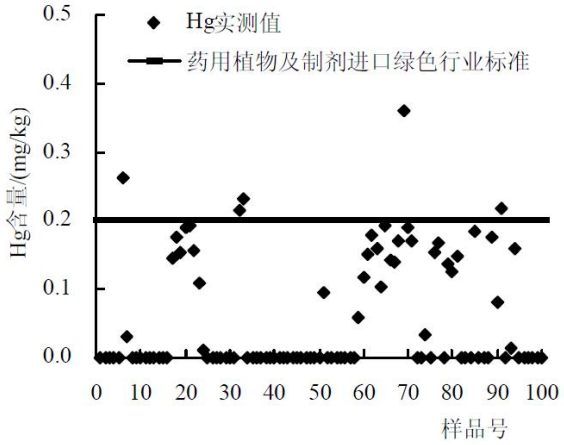
Measured value of Cu

Green industry standard for the import of medicinal plants and preparations

Figure 4 Comparison between measured Cu value and WM/T2-2004 Standard

2.4 Establishment of inorganic element Atlas of Radix Pseudostellariae

According to the test data, the content distribution curves of 14 elements in 33 Radix Pseudostellariae samples were drawn, and the element distribution maps were fitted together, as shown in Figure 12. Figure 12 shows that 14 inorganic elements in 33 samples of Radix Pseudostellariae have similar peak shapes. This commonness is used to distinguish the inorganic element Atlas of Radix Pseudostellariae from that of other traditional Chinese medicinal materials, providing a reference for the identification of Radix Pseudostellariae. Due to the different content of inorganic elements in different samples, there will be some differences in the peak shape of the spectrum. With this feature, it can be used to establish the genuine pattern map of inorganic elements of Radix Pseudostellariae, and can also be used as the basis for identifying the quality of Radix Pseudostellariae. The average values of 14 inorganic elements in 33 samples of Radix Pseudostellariae were used to draw the distribution map of inorganic elements, which provided a reference for establishing the standard map of inorganic elements of Radix Pseudostellariae.



Sample No

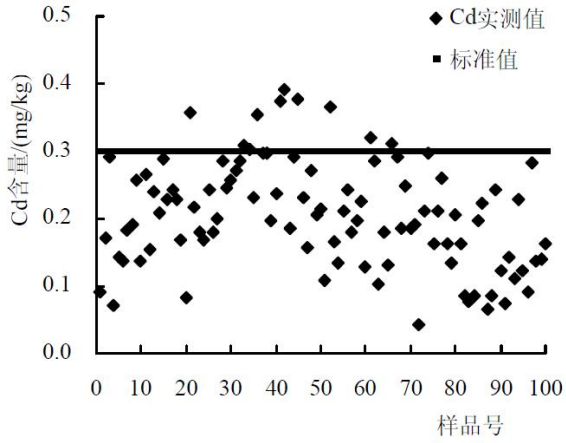
Hg content

Hg measured value

Green industry standard for the import of medicinal plants and preparations

Figure 5 Comparison between Hg measured value and

WM/T2-2004 Standard



Sample No

CD content

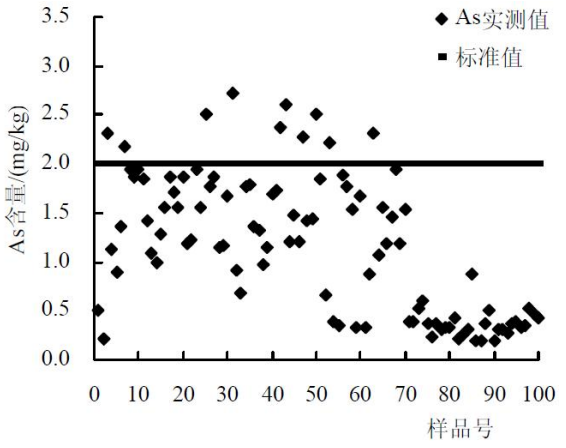
◆ Measured value of CD

▪ Standard value

Figure 6 Proposed standard value of CD

2.5 Factor analysis of different Radix Pseudostellariae samples

The principal component comprehensive evaluation method uses the sample covariance matrix to calculate the principal component after preprocessing the element data [19]. The eigenvalue and contribution rate in the principal component analysis are the basis for selecting the principal component, as shown in Table 3. It can be seen from Table 3 that the cumulative contribution rate of the first five principal components is 74.524%, that is, the five principal component models explain 74.524% of the test data.



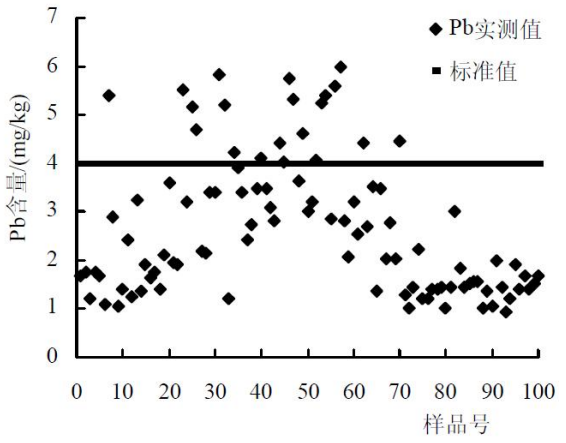
Sample No

As content

◆ As measured value

▪ Standard value

Figure 7 Proposed standard value of as



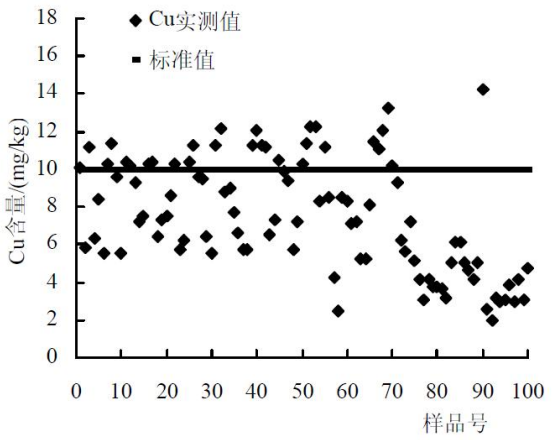
Sample No

Pb content

◆ Pb measured value

▪ Standard value

Figure 8 Proposed standard value of Pb



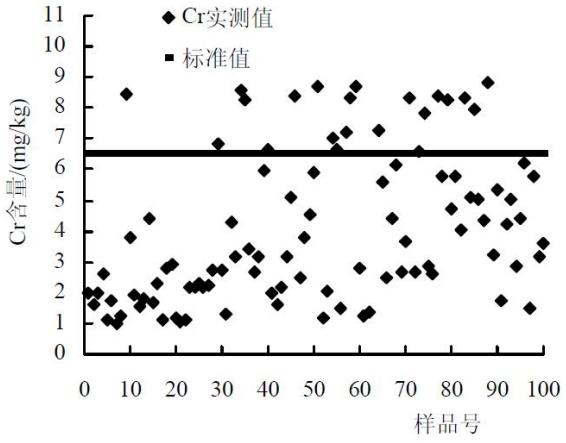
Sample No

Cu content

◆ Measured value of Cu

▪ Standard value

Figure 9 Proposed standard value of Cu



Sample No

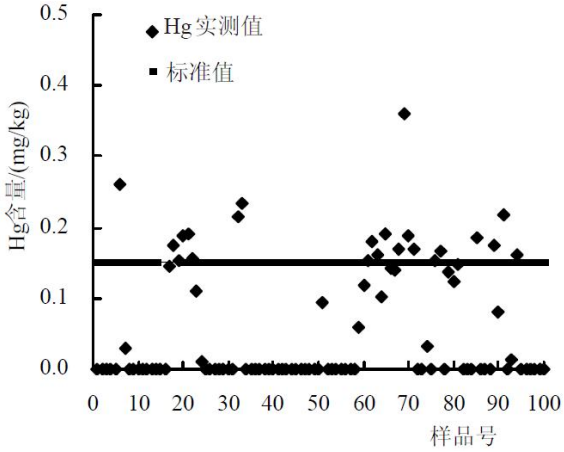
Cr content

◆ Measured value of Cr

▪ Standard value

Figure 10 Proposed standard value of Cr

In order to better see the contribution rate of each element in the principal component loading matrix to the principal component, the maximum variance method is used to perform rotation analysis on the initial loading matrix, and the rotated factor loading matrix is shown in Table 4. It can be seen from Table 4 that the elements that make a large contribution to the first main component are Cd, Cu, Co, and Zn; the elements that make a large contribution to the second main component are Fe, Ca; the elements that make a large contribution to the third main component are Zn, Mg , Al; the elements that contribute greatly to the fourth principal component are As, Pb; the elements that contribute greatly to the fifth principal component are Se, Cr, and the contribution of more than 55% of the total variance comes from the first, second, and third principal components, thus It is inferred that Cd, Cu, Co, Zn, Fe, Ca, Mg, and Al are the characteristic inorganic elements of Radix ginseng.



Sample No

Hg content

◆ Hg measured value

▪ Standard value

Figure 11 Proposed standard value of Hg

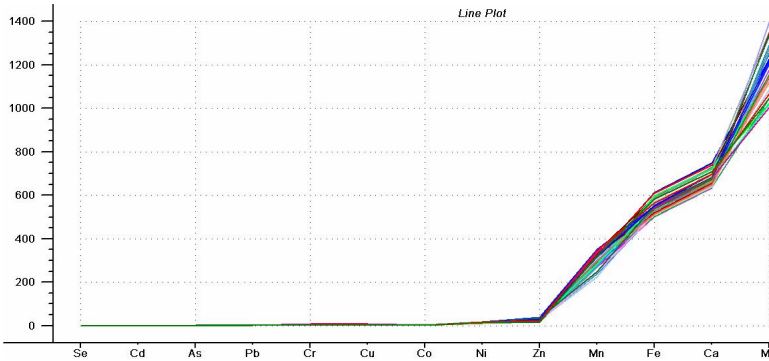


Figure 12 Fitting diagram of inorganic element Atlas of 33 Samples of Pseudostellaria heterophylla

Table 3 Initial statistical values of principal component analysis

Initial eigenvalue

Principal component	Characteristic value	Contribution rate%	Cumulative contribution rate%
1	3.430	24.502	24.502
2	2.669	19.066	43.567
3	1.719	12.279	55.846
4	1.456	10.401	66.247
5	1.159	8.277	74.524
6	0.964	6.886	81.411
7	0.642	4.584	85.995
8	0.484	3.457	89.452
9	0.472	3.375	92.826
10	0.400	2.860	95.687
11	0.319	2.276	97.963
12	0.193	1.381	99.344
13	0.083	0.595	99.939
14	0.009	0.061	100.000

Table 4 Results of inorganic elements after rotating the principal component load matrix

Element	Principal component				
	1	2	3	4	5
Se	0.037	0.089	-0.148	-0.035	0.859
Cd	0.851	0.159	-0.138	0.279	-0.030
As	0.213	0.266	-0.013	0.815	0.185
Pb	0.382	-0.392	0.069	0.560	0.111
Cr	0.042	-0.079	0.323	0.279	0.748
Cu	0.840	0.021	0.140	0.170	0.076
Co	0.819	-0.235	0.179	-0.158	0.036
Ni	0.240	0.428	-0.061	-0.439	0.305
Zn	0.728	0.147	-0.526	0.177	0.041
Mn	0.455	-0.067	0.437	-0.043	0.092
Fe	-0.025	0.958	0.128	0.057	0.024
Ca	-0.043	0.935	0.133	0.039	0.007
Mg	0.084	-0.102	-0.799	-0.199	-0.039
Al	0.197	0.405	0.711	-0.181	-0.043

3 Discussion and conclusion

(1)The content of 15 inorganic elements in Radix Pseudostellariae samples varies greatly, the highest is Mg (959 mg/kg), the lowest is Hg (0.057 mg/kg); the difference between the maximum and minimum content of different elements is also different. The maximum content of se is 70.4 times of the minimum content, and that of aluminum is only 1.98 times; the coefficient of variation Hg is greater than 1, the other elements are less than 1, and the smallest is iron. The content of inorganic elements in Radix Pseudostellariae may be related to the content of elements in soil and environmental conditions [20]. Through the analysis of the content of inorganic elements in Radix Pseudostellariae, we can objectively understand the basic law of the content of inorganic elements in Radix Pseudostellariae produced in Guizhou, and provide reference data for the development and utilization of Radix Pseudostellariae.

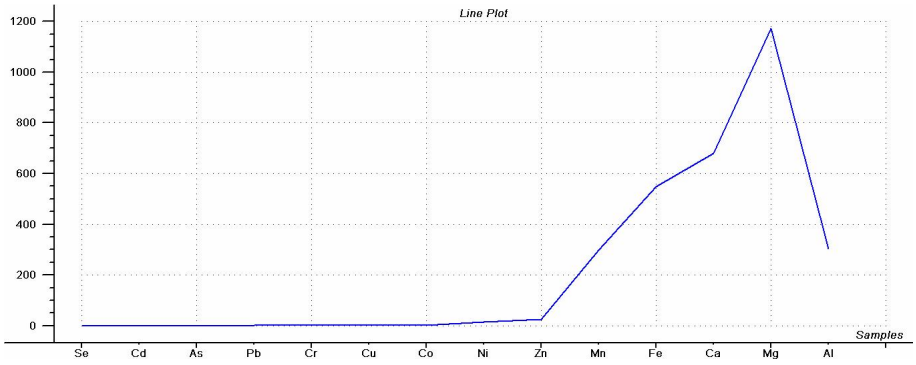


Figure 13 33 Average values of inorganic elements of Radix Pseudostellariae samples

(2)The content of heavy metals in Radix Pseudostellariae was compared with the standard value of green industry for the import of medicinal plants and preparations. The

content of Cu was lower than the limit value, and CD, As, Pb, Hg were found lower than the limit value in 90% of the samples. It shows that Guizhou Radix Pseudostellariae is basically not polluted by heavy metals, and its quality is good, which is consistent with the research conclusions of Wu Qing et al. [21]. Through the investigation and analysis of the samples of heavy metal elements exceeding the standard, it can provide quality control reference for the production process of Radix Pseudostellariae.

(3)Through statistical analysis of the test data, the limit value of heavy metals in Radix Pseudostellariae was established: Cd ≤ 0.3 mg/kg; As≤2.0 mg/kg; Pb≤4.0 mg/kg; Cu≤10 mg/kg; Cr≤6.5 mg/kg; Hg≤0.15 mg/kg. As. The value of CD is the same as the green industry standard for the import of medicinal plants and preparations (WM/T2-2004), Cu, Hg, Pb is slightly lower. At present, the standards of heavy metals in Chinese medicinal materials such as Radix Pseudostellariae are all based on the green industry standard for the import of medicinal plants and preparations (WM/T2-2004), and there is no limit standard for Radix Pseudostellariae, which can provide a reference method and basis for formulating the limit value of heavy metals in Chinese medicinal materials such as Radix Pseudostellariae in the future.

(4)Fingerprint is the key technology to study and control the internal quality of natural products, and has become an effective means of quality evaluation of traditional Chinese medicine. Through the establishment and analysis of 14 kinds of inorganic element fingerprints of Radix Pseudostellariae, this study shows that the characteristics of the fingerprints can be used to distinguish the inorganic element fingerprints of Radix Pseudostellariae from those of other traditional Chinese medicinal materials and provide a basis for identifying the quality of Radix Pseudostellariae. The average value of inorganic elements is used to draw the distribution map of inorganic elements, which provides a reference for establishing the standard map of inorganic elements of Radix Pseudostellariae.

(5)Principal component analysis shows that, first. II. The three principal components account for more than 55% of the total variance, reflecting more than 55% of the information in Radix Pseudostellariae. The main contribution element of the three principal components is CD, Cu, Co, Zn, Fe, Ca, Mg, Al can be considered as the main inorganic element for the reuse of Radix Pseudostellariae.

There are a lot of inorganic elements in Radix Radix, and the content of various elements is different. In-depth research and generalization of the rules of the content of Radix Rhizoma Rhizoma Polygoni can provide a basis for the development and utilization of Radix Rhizoma Radix. Inorganic elements such as Mg, Ca, Fe, Mn, Zn, etc. are beneficial to human health, but some inorganic elements are heavy metal elements that are harmful to human body, such as As, Cd, Hg, Pb, etc. Residues have been found, and a small number of samples have exceeded current evaluation criteria. The safety evaluation of the heavy metal content of Radix Rhizoma Rhizoma Radix is currently carried out in China with reference to the "Green Industry Standard for the Import of Medicinal Plants and Preparations (WM/T2-2004)", and there is no scientific system for the quality evaluation of Radix Rhizoma Radix. The analysis and research of heavy metal elements were carried out, and the limit values of heavy metals and inorganic elements in Radix Radix were proposed, which can provide a reference for formulating the limit values of heavy metals in

Radix Radix Radix et Rhizoma in the future. derived on the basis of function and toxicology. Due to the high content of inorganic elements in Radix Radix et Rhizoma, the relationship between them is also complicated. Through principal component analysis, it is found that the elements that play a decisive role in Radix Rhizoma Radix are Cd, Cu, Co, Zn, Fe, Ca, Mg, Al, It can be used as the characteristic inorganic element of Taizi ginseng to reduce the research object. Fingerprint is one of the advanced methods to identify the basic properties of Chinese medicinal materials and evaluate the quality. By establishing the inorganic element map of Rhizoma Polygoni, it can be used to establish the standard spectrum of inorganic elements of Rhizoma Rhizoma Polygoni. Identification of the quality of ginseng. Provide a reference for distinguishing the prince's participation in other Chinese herbal medicines.

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