Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2014, 6(9):10-15

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Determination of the trace element contents of *Althaea rosea* seeds by Atomic Absorption Spectrometry and evaluation using multivariate analysis

Weijun Du[1], Feng Liu[1], Xi Bai[1] and Shuge Tian[1,2*]

[1]*College of Chemistry and Chemical Engineering, Xinjiang Normal University, Urumqi, China*
[2]*Central Laboratory of Xin Jiang Medical University, Urumqi, China*

ABSTRACT

In the present study, trace elements including Na, Mg, K, Ca, Mn, Fe, Cu, Zn, Cd and Pb in Althaea rosea seeds were determined by flame and graphite furnace atomic absorption spectrometry. This method coupled with microwave-assisted acid digestion, based on a mixture of nitric acid and hydrogen peroxide. In order to get a better insight into the elemental patterns, the chemometric techniques of principal component analysis (PCA), and cluster analysis (CA) were used for the classification studies. Three groups classified by PCA and CA were attributed partly to Althaea rosea seeds from ten different production areas of Xinjiang. The average concentrations of the determined elements (expressed as μg/g) were as follows: 124.539 for Na, 2984.901 for Mg, 14689.265 for K, 391.675 for Ca, 35.556 for Mn, 1036.345 for Fe, 58.058 for Cu, 70.264 for Zn, 3.576 for Cd and 0.011 for Pb.

Keywords: *Althaea rosea* seeds, Trace element, Atomic absorption spectrometry, Multivariate analysis

---

INTRODUCTION

Althaea rosea seeds as an herbaceous perennial plant belonging to the Malvaceae, is a well known ornament herb widely distributed in Sichuan, Xinjiang, Shanghai, Jiangsu, and Fujian region of China [1]. The plant commonly known as hollyhock can be found in woodlands, cultivated beds, sunny edges and grows best in medium-fertile, moist, but well-drained soil [2]. The medicinal parts of *A. rosea* include flowers, roots, and seeds. The *A. rosea* seeds has been used not only as an expectorant, coolant, diuretic, anti-inflammatory, febrifuge, demulcent, and astringent agent, but also used to treat kidney and uterus inflammation by Uyghur physicians [3]. A previous study of Dudek et al (2006) investigated that the phenolic acids distributed in the methanolic and methanolic-aqueous extracts of whole flowers, petals and calyxes of *A. rosea* seeds [4]. The study showed that seven phenolic acids including cinnamic (ferulic, p-coumaric, caffeic), benzoic (phydroxybenzoic, vanillic, syringic) acids and p-hydroxyphenylacetic acid. Raknimov and Mezthlumyan found that the sugars isolated from the plant stems and roots included arabinose, galactose, rhamnose , xylose and galacturonic acid [5]. There have seventeen amino acids including valine, threaonine, methionine, isoleucine, leucine, lysine, phenylalanine, histidine and arginine were found in *A. rosea* stems and roots. Li Yinpin et al used by the method of microwave-assisted digestion of *A. rosea* surveyed eight trace elements (Fe, Cu, Zn, Mn, K, Ni, Ca, and Mg) which are 24.38, 0.0165, 0.1795, 0.526, 18.5, 0.0044, 65.84 and 287.17 mg/g, respectively [6]. Raknimov DA and Mezthlumyan LG investigated that seven trace elements (Pb , Cd , Cu, Zn, Sn, Cr, and Fe) in the stems were 30.1 , 0.67 , 30.0, 93.6, 23.1, 196.0 and 106.0 mg/kg, while, in the roots were 26.1, 0.83, 24.2, 37.5 , 18.5 , 200.5 , and 790 mg/kg, respectively [7].

The diverse applications of *A. rosea* in herbal compound prescription have created interest in this topic which it is important to determine whether they are safe for consumption [8]. Determination of trace elements plays a crucial role in the general state of health of a population. The implementation of this measure can provide good quality control of medicinal herbs in order to protect consumers from contamination [9]. The unique climate and soil conditions are important factors for the differences in trace element content from different producing areas.

According to the content of trace elements differences can reflecting environment condition in specific area, and have great significance for soil environmental quality evolution research, and reasonable development and utilization of land resources [10]. Previous researches shown that the fruits grown at the roadsides polluted by vehicle emissions, industrial waste and uncontrolled factory emissions or possible sources of the heavy metal pollution have high content of mineral and heavy metal contents [11]. Therefore, various analytical methods are used to determine trace elements in plant material such as atomic absorption spectrometry (AAS), inductively coupled plasma-mass spectrometry (ICP-MS), inductively coupled plasma-atomic emission spectrometry (ICP-AES), electrochemical methods, neutron activation analysis and total reflection X-ray fluorescence [12,13]. Currently, it is necessary to establish a method which can rapidly and accurately distinguish *A. rosea* seeds from different producing areas for assurance of quality control.

The objective of this study was to determine ten trace elements namely K, Ca, Na, Mg, Ca, Cu, Fe, Mn, Cd, and Pb in *A. rosea* seeds from ten different producing areas by the method of AAS coupled with microwave digestion. AAS method with the advantages of low cost, rapid analysis and wide linear range, low detection limit and simultaneous determination has been widely used. The relationship between the trace elements of *A. rosea* seeds was using the chemometric techniques such as principal component analysis (PCA) and cluster analysis (CA).

EXPERIMENTAL SECTION

Instrumentation

An AAS instrument (Z-2000, Hitachi Co. Ltd., Japan) was used for the determination of K, Ca, Na, Mg, Ca, Cu, Fe, Mn, Cd, and Pb. A Multiwave WX-4000 microwave system (Shanghai EU Analytical Instrument Co Ltd, China) was used for sample preparation. The ultrapure demonized water obtained from a Milli-Q water purification system (Millipore, Bedford, MA, USA) was used for preparing the solutions and for all dilutions.

Reagents and solutions

Standard stock solutions with a concentration of 1000 $mg \cdot L^{-1}$ of individual metal elements were used to prepared by dilution using a 1% (v/v) nitric acid solution supplied by the National Research Centre for Certified Reference Material of China. For sample digestion, analytical reagent grade concentrated nitric acid (70%) and hydrogen peroxide (30-32%) was used. The plastic/glass containers were cleaned by soaking in10% v/v HNO_3 for at least 24 h and rinsing with distilled water before use. All chemicals used were of analytical grade.

Sample digestion

A total of 10 samples of *A. rosea* seeds were obtained from different production areas of Xinjiang (Table 1). All samples were stored in the Traditional Chinese Medicine Ethnical Herbs Specimen Museum of Xinjiang Medical University. The plant materials were identified by Yonghe Li, a chief apothecary of the Chinese Medicine Hospital of Xinjiang. *A. rosea* seeds were dried in an electric blast drying oven under 60 °C, pulverized by a disintegrator, and screened through a 40 mesh sieve. All powder samples were stored at 4 °C until use.

Table1 The list of *A. rosea* seeds samples.

Sample number	Source
A1	Xinjiang Uygur Pharmaceutical Co. Ltd.
A2	Erdaoqiao Uighur Hospital
A3	Xinjiang QiKang Habo Uygur medicine Co. Ltd.
A4	Xinjiang QiKang Habo Uygur medicine Co. Ltd.
A5	Moyu County, Hetian Prefecture
A6	Moyu County, Hetian Prefecture
A7	Moyu County, Hetian Prefecture
A8	Kashi
A9	Jimusaer County, Changji Hui Autonomous Prefecture
A10	Jimusaer County, Changji Hui Autonomous Prefecture

In order to reduce digestion time, a microwave-assisted digestion procedure was used for all *A. rosea* seeds. Accurately weighed 200 mg dried powdered sample in triplicate was added to PTFE digestion vessel containing 10 mL concentrated HNO_3 and 1mL H_2O_2 and was kept at room temperature for 10 min. The microwave digestion vessel was kept in the microwave oven by two-step program and set for the method as follows: (1) 400 W at 90 °C for 2 min, (2) 400 W at 120 °C for 8 min. After cooling, the resulting solutions were separated with centrifugation at 4000 rpm for 5min. The supernatant were transferred into a volumetric flask and diluted to 50 mL with double distilled water for determination of trace elements by AAS. The blank experiments were carried out in the same way. The determination of Na, Mg, K, Ca, Mn, Fe, Cu and Zn was performed with a flame atomic absorption spectrometry (FAAS) and the contents of Cd and Pb was performed with a graphite furnace atomic absorption spectrometry (GFAAS).

RESULTS AND DISCUSSION

The average results (concentration ± RSD%) of trace elements obtained for ten samples using AAS are shown in Table 2. The relative standard deviations (RSD%) was less than 10% for all investigated elements. Results of regression analyses and the calculated correlation coefficients (R^2) are listed in Table 3. Using the data in Table 2, the relationship between the trace elements in *A. rosea* seeds was classified using cluster analysis and principal component analysis.

Table 2 Total element contents of *A. rosea* seeds (μg/g).

No.	Na	Mg	K	Ca	Mn
A1	44.15±3.93	2604.21±0.59	16864.41±0.45	67.46±4.33	26.66±3.50
A2	157.23±0.84	3830.17±1.02	10367.23±0.49	514.94±0.83	81.97±0.67
A3	118.27±0.06	2571.94±1.34	15932.20±0.24	194.11±1.41	22.33±7.58
A4	149.15±2.12	3346.24±1.39	15423.73±0.60	607.82±2.69	25.58±7.34
A5	167.68±1.66	2765.52±2.28	9604.52±0.74	405.18±1.94	76.55±1.06
A6	124.44±1.96	2765.52±0.45	15790.96±0.25	599.38±1.40	19.07±8.17
A7	124.44±1.73	3894.70±1.94	14802.26±0.41	590.93±1.54	21.24±3.71
A8	127.77±0.99	3281.71±1.06	18305.08±0.21	455.84±2.65	16.91±6.16
A9	107.34±1.27	2410.63±2.19	15310.73±0.49	109.68±2.16	28.83±3.48
A10	124.92±1.55	2378.37±2.50	14491.53±0.33	371.41±2.21	36.42±4.00
No.	**Fe**	**Cu**	**Zn**	**Cd**	**Pb**
A1	661.93±0.84	58.51±2.83	52.63±0.10	3.86±2.43	0.01±0.91
A2	2159.59±1.57	58.51±4.42	86.84±0.89	3.23±8.11	0.03±1.42
A3	497.05±2.77	72.01±2.93	80.26±0.69	1.36±2.98	0
A4	806.20±1.52	58.51±4.22	92.11±0.83	5.11±3.34	-
A5	2310.73±0.48	67.51±4.11	41.45±0.80	3.23±2.74	0.03±0.51
A6	552.01±2.02	58.51±6.31	33.55±1.43	3.44±2.23	0.01±2.67
A7	483.31±3.17	54.01±0.41	79.61±0.36	-	-
A8	442.09±1.72	45.00±1.12	91.45±0.27	8.23±1.23	-
A9	977.95±1.18	58.51±1.64	53.95±0.55	3.65±1.40	0.01±1.77
A10	1472.59±1.32	49.50±10.69	90.79±0.78	3.65±3.37	0.02±0.65

Table 3 Statistical results of linear regression equation analysis in the determination of the ten elements

Element	Linear range (ug/ml)	Calibration curve	Regression coefficient (R^2)
Na	0.05-0.8	Y=0.5262X+0.0061	0.9994
Mg	0.01-0.4	Y=0.5166+0.00628	0.9997
K	0.2-1.0	Y=0.5900X+0.0490	0.9991
Ca	0.2-20	Y=0.0296X+0.0240	0.9991
Mn	0.01-0.6	Y=0.2306X +0.0014	0.9998
Fe	0.4-12	Y=0.0364X +0.0137	0.9996
Cu	0.1-8	Y=0.1111X+0.0090	0.9992
Zn	0.1-0.6	Y=0.3800X+0.0090	0.9997
Cd	0.00125-0.05	Y=0.0012X+0.0025	0.9998
Pb	0.1-80	Y=0.2981X +0.0037	0.9994

Correlation analysis

Correlation coefficient, also known as "r", a measure of the strength and direction of the linear relationship between two variables that is defined as the (sample) covariance of the variables divided by the product of their (sample) standard deviations. The correlation coefficients can range from -1 to +1 and is independent of the units of measurement. If this coefficient is close to 0 indicate that there is a very weak or perhaps even no relation between the two variables; whereas a value near +1 or -1 indicates a high level of correlation. The correlation analysis of ten element contents is presented in Table 4. Correlation analysis of total trace elements in *A. rosea* seeds shows moderate to strong correlations in ten production areas. According to Table 4, sodium is positively correlated with all elements except potassium. Potassium, zinc and cadmium are moderately correlated with each other whereas there is not a significant correlation for these analytes with other metals. The correlation coefficient values of trace element contents higher than 0.5 were used for the interpretation of the correlation analysis. Interpretation of correlation analyses enabled the groupings below to be obtained:

Group 1: Na, Mn, Fe, Cu, Pb
Group 2: Mg, Ca, Zn
Group 3: K, Zn, Cd

The first group including Na, Mn, Fe, Cu, Pb have a very high correlations coefficient between these two trace element, such as 0.965 for Mn and Fe; 0.908 for Mn and Pb and 0.943 for Fe and Pb.

Table 4 Correlation matrix for the element concentrations in plants

Element	Na	Mg	K	Ca	Mn	Fe	Cu	Zn	Cd	Pb
Na	1									
Mg	0.411	1								
K	-0.63	-0.21	1							
Ca	0.68	0.689	-0.22	1						
Mn	0.539	0.198	-0.94	0.093	1					
Fe	0.554	0.041	-0.92	0.072	0.965	1				
Cu	0.108	-0.26	-0.4	-0.29	0.3	0.219	1			
Zn	0.226	0.442	0.16	0.24	-0.08	-0.08	-0.4	1		
Cd	0.025	-0.07	0.352	0.042	-0.11	-0.06	-0.53	0.185	1	
Pb	0.357	-0.08	-0.85	-0.01	0.908	0.943	0.166	-0.27	-0.07	1

Principal component analysis

Principal component analysis (PCA) provides information on the most meaningful parameters that uses orthogonal transformation to convert a set of observations of possibly correlated variables into a set of values of linearly uncorrelated variables called principal components (PCs). Therefore, PC1 usually accounts for as much of the variability in the data as possible from the original data and each succeeding component account for as much of the remaining variability as possible. As mentioned previously, the first principal component has the largest possible variance, and each succeeding component in turn has the highest variance possible under the constraint that it is orthogonal to the preceding components. Thus, if the data is plotted into the different PC spaces, then the data structure which encloses different clusters may simply be detected or verified.

The loadings				The scores			
Element	**PC1**	**PC2**	**PC3**	**Plant**	**PC1**	**PC2**	**PC3**
Na	0.671	0.529	-0.119	**A1**	-0.867	-1.385	0.572
Mg	0.203	0.79	-0.327	**A2**	1.716	0.788	0.170
K	-0.989	0.044	0.105	**A3**	-0.515	-0.887	-1.336
Ca	0.244	0.811	-0.253	**A4**	-0.275	1.128	-0.283
Mn	0.964	-0.041	0.151	**A5**	1.861	-0.732	0.121
Fe	0.951	-0.071	0.271	**A6**	-0.318	-0.210	-0.448
Cu	0.351	-0.591	-0.544	**A7**	-0.339	0.961	-1.769
Zn	-0.133	0.679	0.074	**A8**	-1.074	1.335	1.427
Cd	-0.232	0.309	0.808	**A9**	-0.330	-1.055	0.453
Pb	0.882	-0.22	0.337	**A10**	0.141	0.057	1.093

The results for the ten elements in the ten samples analyzed were evaluated by PCA using the SPSS 19.0 programmer. The principal components with eigenvalues higher than 1 were extracted. The loadings of the original variables on the first three principal components and the variances explained by each principal component are shown in Figure 1. The first three components account for 82.211% of variances for all of the data including the first component accounted for 43.358%, the second for 25.243%, the third for 13.610%. Table 3 also gives the score values for each principal component for *A. rosea* seeds from ten different production areas of Xinjiang. From the scores on the first principal component it can be interpreted that the concentrations of Na, K, Mn, Fe and Pb on the first principal component loadings are higher for A2 and A5 than the other plants and are lower for A1, A3 and A8 than the other plants. When the second principal component is interpreted, Mg, Ca and Zn concentrations are higher for A2, A4, A7 and A8 and are lower for A1, A3, A5 and A9 than for the other plants investigated. On the third principal component, Cd concentrations are higher for A1, A8 and A10 and are lower for A3 and A7 than for the other plants. The classification of the herbal teas from the view point of trace element contents can be made using three way PC score graphs. The herbal samples can be classified into three groups. These groups include:

Group 1: A2, A5.
Group 2: A4, A7, A8.
Group 3: A1, A3, A6, A9, A10.

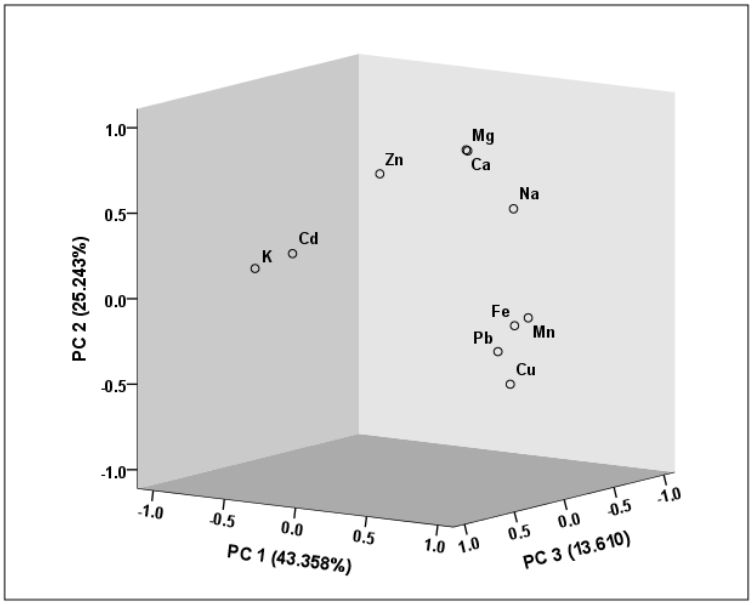

Fig.1 Score plot by PCA analysis on the calibration set

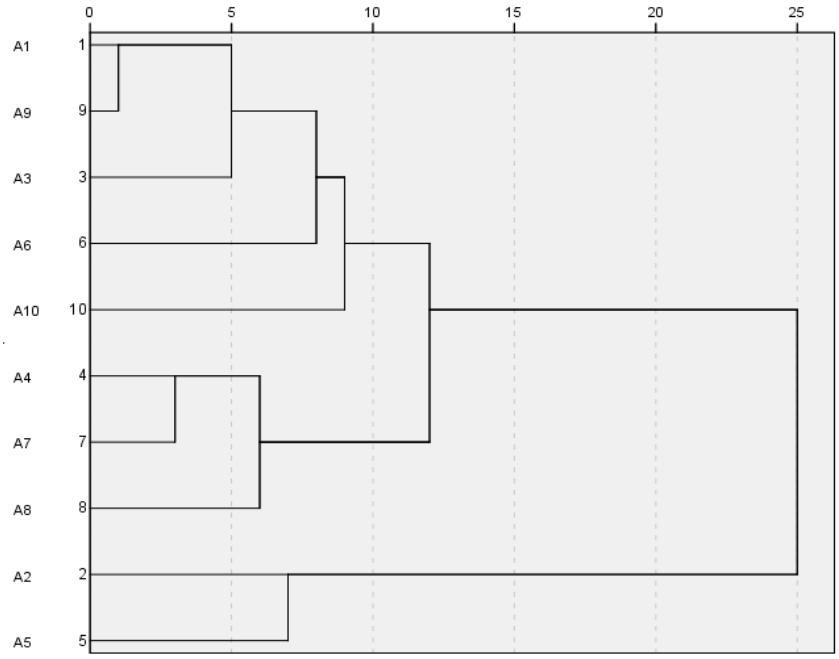

Fig. 2 Dendrogram by CA analysis on the calibeation set

Cluster analysis

Cluster analysis is the most widely used unsupervised pattern recognition technique in chemometrics that involves a measurement of the similarity between objects to be clustered. The objects in the same group are more similar to each other than to those in other groups. The cluster analysis was applied using the SPSS package. The Ward's method of clustering was applied with the squared Euclidean distance for *A. rosea* seeds from ten different production areas of Xinjiang.

Similar groupings to those found above were obtained from cluster analysis as shown in the dendrogram (Fig. 2). In this study, three groupings were obtained from cluster analysis.

These groups were:

Group 1: A1, A3, A6, A9, A10.
Group 2: A4, A7, A8.

Group 3: A2, A5.

Cluster analyses shown in Fig. 3 indicated that Group 1 (A1, A3, A6, A9 and A10) and Group 2 (A4, A7 and A8) samples are close to each other.

CONCLUSION

This study provides a comprehensive survey of the contents of 10 trace elements in *A. rosea* seeds samples from ten different production areas of Xinjiang. The micro-wave assisted digestion coupled with flame and graphite furnace atomic absorption spectrometry was used to determination trace elements in *A. rosea* seeds. The results demonstrated that *A. rosea* seeds had a high content of Magnesium, potassium and iron. The contents of the toxic trace elements Cd and Pb were very low and could not pose any threat to the consuming population. In order to recognize patterns, the chemometric techniques of principal component analysis (PCA), and cluster analysis (CA) were used for data evaluation. The first three components account for 82.211% of variances for all of the data including the first component accounted for 43.358%, the second for 25.243%, the third for 13.610%. PCA and CA revealed three groups of *A. rosea* seeds samples based on trace element concentrations.

REFERENCES

[1] Ali Esmail Al-Snafi, *Int. J. PharmTech Res.,* **2013**, 5, 1378-1385.
[2] Benbassat Niko; Yoncheva Krassimira; Hadjimitova Vera., *Cent. Eur. J. Biol.,* **2014**, 9, 182-188.
[3] Aziguli Yiming; Ainiwaer Aikemu; Song F F., J. *Xinjiang Med. Univ.,* **2013**, 36, 1275-1277.
[4] Dudek M; Awska IM; Szudlarek M., *Acta Poloniae Pharmaceutica-Drug Res.,* **2006**, 3, 207-211.
[5] Maria Kwiatkowska; Dariusz Stępinski; Katarzyna Poplonska; Agnieszka Wojtczak; Justyna Teresa Polit., *Acta Societatis Botanicorum Poloniae*., **2011**, 80, 211-219.
[6] Parisa Sadighara; Soraya Gharibi; Amir Moghadam Jafari; Golamreza Jahed Khaniki; Samira Salari., *Avicenna J. Phytomed*., **2012**, 2, 113-117.
[7] Li Yin-ping; Gao Li; LI Jin; Zhou Xiao-yin., *Spectrosc. Spect. Anal.*, **2009**, 2, 3147-3149.
[8] Cennet Karadas; Derya Kara., *Food Chem.,* **2012**, 130, 196–202.
[9] Naeem Khan; Ji Yeon Choi; Eun Yeong Nho; Nargis Jamila; Girum Habte; Joon Ho Hong; In Min Hwang; Kyong Su Kim, *Food Chem.*, **2014**, 158, 200-206.
[10] Adriana Caires Oliveira; Vitor Silva dos Santos; Debora Correia dos Santos; Rosemary Duarte Sales Carvalho; Anderson Santos Souza; Sergio Luis Costa Ferreira, *Food Chem.*, **2014**, 152, 619-223.
[11] C. Rubio; J.R.D. Lucas; A.J. Gutiérrez; D. Glez-Weller; B. Pérez Marrero; J.M. Caballero; C. Revert; A. Hardisson, *J. Pharmaceut. Biomed. Anal.*, **2012**, 71, 11-17.
[12] Hong-wu Wang; Yan-qing Liu, *Food Chem.,* **2010**, 121, 887-892.
[13] Derya Kara, *Food Chem.,* **2009**, 114, 347-354.