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Research Article

Quality Survey on Medicinal Herbs of *Adenophora Stenanthina (Ledeb.) Kitag*

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Abstract

Adenophora stenanthina (Ledeb.) Kitag is a raw material for mongolian medicine and is cultured in different fields across China. To ensure the quality of the *Adenophora stenanthina (Ledeb.) Kitag*, we determined the concentration of heavy metals (copper, lead and cadmium), moisture, general ash and acid-insoluble ash content in *Adenophora stenanthina (Ledeb.) Kitag* in eight samples from different geographical regions in China, according to the WHO guidelines on the good agricutural practices (GAPS). *Adenophora stenanthina (Ledeb.) Kitag* was prepared by nitric acid: hydrochloric acid (4: 1) solution. The cadmium and lead content were determined by graphite furnace atomic absorption spectrometry; copper content was determined by flame atomic absorption method. Pb concentrations in the samples from Nai Man and Chen Ba Er Hu were higher than GAPS guidelines. Cd concentrations in the samples from Ku Lun, A Ba Ga and Chen Ba Er Hu were higher than GAPS guidelines; concentrations of acid-insoluble ash in samples from E Wen Ke and A Ba Ga were higher than GAPS guidelines; moisture, general ash content of the eight samples are all in the normal range. To sum, the results indicate that, for the safety of clinical usage of *Adenophora stenanthina (Ledeb.) Kitag*, it is not recommended to collect medicinal herbs from areas in Nai Man, Chen Ba Er Hu, Ku Lun, E Wen Ke and A Ba Ga.

Key words: *Adenophora Stenanthina (Ledeb.) Kitag,* Copper, Lead, Cadmium.

Introduction

The *Adenophora stenanthina (Ledeb.) Kitag*, recorded in the Chinese Pharmacopoeia, is a family of Campanulaceae and is used as a raw material for Mongolian medicine as a substitute for Lance Asiabell Root. According to traditional medical books, the *Adenophora stenanthina (Ledeb.) Kitag* contains high levels of glycosides, sugars and almonds. Taking *Adenophora stenanthina (Ledeb.) Kitag could induce* sweetness, increase glucose levels in the serum, ameliorate fatigue and reduce tumors and so on [1]. Additionally, it is widely accepted by the traditional doctors that *Adenophora stenanthina (Ledeb.) Kitag* may assist the treatment of blame, gout, rheumatism, jaundice, bacterial disorders, cancer and leprosy [2]. For the safety of the raw material of *Adenophora stenanthina (Ledeb), Kitag*, in this study, we determined heavy metals: copper (Cu), lead (Pb) and cadmium (Cd), moisture, general ash and acid-insoluble ash content of eight samples from different geographical regions.

1. Materials and methods

1.1 Materials

1.1.1 Main instruments

The eight samples of *Adenophora stenanthiana (Ledeb. Kitag)* were collected from different geographical regions of China (Table 1.). Cu, Pb and Cd determined by atomic absorption spectrophotometer (American Thermo Fisher Scientific Co., Ltd.). Samples were weighed by electronic balance (Shanghai Precision Scientific Instrument Co., Ltd.).

Table 1. 8 different geographical locations.

Sample	Geographic area	Longitude	Latitude	Altitude (m)	Collection time
1	Ba Lin	42˚12` 0` `	118˚43` 8` `	1214	2018.7.10
2	Ku Lun	42˚76` 05` `	121˚76` 17` `	289	2018.8.10
3	Hai La Er	49˚20` 77` `	119˚44` 46` `	654	2018.8.19
4	E Wen Ke	48˚44` 21` `	119˚34` 13` `	645	2018.7.20
5	A Ba Ga	43˚27` 11` `	116˚7` 9` `	1140	2018.7.18
6	Nai Man	42˚18` 41` `	121˚2` 14` `	412	2018.8.13
7	Xi Wu Zhu Mu Qin	44˚28` 55` `	117˚39` 53` `	1030	2018.7.19
8	Chen Ba Er Hu	49˚29` 24` `	120˚22` 39` `	600	2018.8.21

1.1.2 Main reagent

Copper Cu standard solution (1000 μg/mL, batch number GSB04-1742-2018), lead Pb single element standard solution (1000 μg/mL, national standard solution, batch number GSB04-1742-2018) and cadmium Cd single element standard solution (1000 μg/mL, batch number GBW04-1721-2018) were used as standards for the heavy metal content determination. Nitric acid and perchloric acid used are all excellent grades. The remaining reagents used are analytical grade. The test water used was robust purified water.

1.2 Methods

According to the Chinese pharmacopeia [3], heavy metals (Cu, Pb, and Cd) in the samples were determined by standard atomic absorption spectrometric method.

1.2.1 Preparation of test solution

First, the medicinal materials are selected for impurity removal, smashed by a stainless steel pulverizer, and passed through a 60 mesh nylon sieve to make medicinal powders for future use. Test powder of 1g was accurately weighed and put in a tall beaker; 10 mL of nitric acid-perchloric

acid (4:1, V/V) mixed solution was added and mixed well and soak overnight. The mixture was heated and digested on an electric hot plate; keep boiling the mixture until it turns brown and black; appropriate amount of nitric acid-perchloric acid (4:1, V/V) mixed solution was added to the mixture and continue heating the mixture until the solution is clear. The lid was opened to remove the acid. The mixture was heated to produce thick smoke. Water (20 ml) was added 4 times until the white smoke disappears. The digestion solution is transparent and colorless or slightly yellow. After cooling down, the solution was transferred to a 50 mL measuring flask. The previous container was washed with 2% nitric acid solution and this washing liquid was combined with the digestion solution in the measuring flask. Corresponding masking agent was added. 2% nitric acid solution was added to the mark to dilute the solution to the desired concentration. The solution was shaken well, stand still until testing. Meanwhile, the blank solution was prepared following the same protocol.

1.2.2 Preparation of heavy metal standard solution

Copper (10 μg/mL), cadmium (1 μg/mL) and lead (1 μg/mL) stock solutions (Cu 0, 1, 2, 4, 6, 8 mL; Cd 0, 0.1, 0.2, 0.4, 0.6, 0.8 mL; Pb 0, 1, 2, 3, 4, 6, 8 mL) was placed in a 100 mL volumetric flask and the volume was adjusted with 2% nitric acid to prepare a standard series solution of appropriate mass concentration. Among them, the standard mass concentrations of the heavy metals are shown in Table 2.

1.2.3 Working parameters of the instruments

Optimal working parameters of the apparatus are shown in Table 3 and 4.

Table 2. Heavy metal element standard solution.

Elemental	stock solutions	Volume of stock solutions used to dilute to 100 mL	mass concentrations of the standards
Cu	1 μg/mL	0, 1, 2, 3, 4, 6, 8 mL	0, 0.1, 0.2, 0.4, 0.6, 0.8 μg/mL
Pb	10 μg/mL	0, 1, 2, 4, 6, 8 mL	0, 10.0, 20.0, 40.0, 60.0, 80.0 ng/mL
Cd	1 μg/mL	0, 0.1, 0.2, 0.4, 0.6, 0.8 mL	0, 1.0, 2.0, 4.0, 6.0, 8.0 ng/mL.

Table 3. Graphite furnace atomic absorption spectrometry

Element	wavelength (nm)	Lamp current (%)	pass band (m)	Carrier gas flow rate (L•min－1)	Graphitefurnace temperature (℃)
Pb	283. 3	6.0	1.3	0.2	2000
Cd	228.7	5.0	1.3	0.2	1900

Table 4. Flame atomic absorption spectrometry.

Element	wavelength (nm)	Lamp current (%)	pass band (m)	Gas flow rate (L•min－1)
Cu	324. 7	7. 5	1. 3	20

1.2.4 Investigation of the linear relationship

An appropriate amount of Cu, Pb and Cd standard series solutions were pippetted, appropriate amounts of corresponding masking agents were added and the absorbance values were measured according to the working conditions in item1.2.4. The standard series mass concentration (x) was used as the

abscissa and the measured absorbance value (y) as the ordinate to obtain a linear regression equation. Correlation coefficient of the three elements is 0.996 ＜ r ≤ 1. 000 0, indicating a good linear relationship with the absorbance value (Table 5.).

Table 5. Equation for heavy metals detection.

Element	Regression equation	r
Cu	Y=0.2157x+0.0055	0.9992
Pb	Y=0.0026+0.0762	0.996
Cd	Y=0.0847x+0.0379	0.996

1.2.5 Determination of heavy metals (Cu, Pb, and Cd) in the samples

According to the 2010 Chinese Pharmacopoeia, standard solutions were prepared for each heavy metal. According to item 2.3, the absorbance and mass fraction of the heavy metals in the samples were measured and calculated according to formula $m = (C_{sample}-C_{blank}) \times 50/ M_{sample}$ (5).

2. Results and discussion

Pb content of sample 6 and the sample 8 are relatively high in concentrations. Cd content of sample 2、5、8 are relatively high in concentrations. Moisture, general ash content of the eight samples is in the normal range. Acid-insoluble ash concentrations in sample 4 and the sample 5 were relatively high in concentrations (Table 6 and 7).

The results of the current study showed that medicinal herbs of *Adenophora stenanthina (Ledeb.) Kitag* from different regions contain different levels of heavy metals, some are higher than the standards of the guidelines. The differences in the contents of heavy metals may be caused by different geographical soils, growth environment, especially environmental pollution derived from chemical plants or mining. Governing or improving the environmental pollution in Nai Man, Chen Ba Er Hu, Ku Lun, E Wen Ke and A Ba Ga is recommended, until then, it is not recommended to collect medicinal herbs from areas in Nai Man, Chen Ba Er Hu, Ku Lun, E Wen Ke and A Ba Ga.

Table 6. The Cu, Pb and Cd content of the eight samples (μg / g).

Sample	1	2	3	4	5	6	7	8
Cu	0.06± 0.02	0.05± 0.02	0.12± 0.01	0.12± 0.02	0.05 ±0.01	0.05± 0.02	0.13± 0.02	0.11 ±0.02
Pb	-0.64± 0.09	-1.35± 0.14	0.79± 0.01	3.55± 0.18	3.54± 0.25	18.55± 0.27	-1.22± 0.06	41.57.± 0.30
Cd	0.34± 0.02	1.33± 0.12	0.12± 0.01	0.02± 0.03	0.53± 0.02	0.24± 0.02	-0.01± 0.02	0.37± 0.17

"Medicinal Plasma Standard" (GAPS) (Cu ≤ 20.0 μg / g, Pb ≤ 5,0 μg / g, Cd ≤ 0.3 μg / g).

Table 7. Moisture, General Ash and Acid-insoluble ash content of the eight samples (%).

Sample	1	2	3	4	5	6	7	8
Moisture	5.93± 1.92	5.64± 1.68	5.56± 2.36	5.42± 1.10	5.72± 0.87	5.71± 1.10	5.70± 1.38	5.40± 1.47
General Ash	4.50±	4.22±	4.69±	5.50±	5.76±	4.67±	3.98±	4.42±

	1.27	0.71	1.29	0.21	1.62	0.59	0.85	1.16
Acid-insoluble ash	1.44± 2.31	1.15± 1.94	1.59± 3.91	2.89± 1.47	2.83± 0.99	1.27± 2.50	1.26± 1.70	1.33± 2.84

"Medicinal Plasma Standard" (GAPS): Moisture ≤ 15%, General Ash ≤ 6%, Acid-insoluble ash ≤ 2%.

Declarations

1) Consent to publication

We declare that all authors agreed to publish the manuscript at this journal based on the signed Copyright Transfer Agreement and followed publication ethics.

2) Ethical approval and consent to participants

Not applicable.

3) *Disclosure of conflict of interests*

We declare that no conflict of interest exists.

4) Funding

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5) Availability of data and material

We declare that the data supporting the results reports in the article are available in the published article.

6) Authors' contribution

HD: study design, literature search and data extraction. LL, BR, XLW: obtaining the grant. DD, literature search and data extraction. DE, supervising the project, drafting the manuscript.

7) Acknowledgement

None.

8) Authors' biography

None.

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