Study on the Accumulation of Heavy Metals in Alpinia Oxyphylla Miq. at Different Growth Stages

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Abstract. Objective: to compare the contents of heavy metals in Alpinia oxyphylla Miq. at different growth stages. Methods: The content of Cu was determined by atomic absorption spectroscopy. The contents of other four elements, i.e. As, Hg, Pb and Cd, were determined by atomic fluorescence spectroscopy. Result: the contents of heavy metals in fruit and rhizome of Alpinia oxyphylla Miq. were lower than the criteria for the limit content of heavy metals issued by Green Trade Standards of Importing & Exporting Medicinal Plants & Preparations at different growth stages. The content of heavy elements was the lowest at the ripe fruit of the 40th day. Conclusion: The 40th day was the best harvest time for Alpinia oxyphylla Miq.

Introduction

In recent years, whether the contents of heavy metals in Chinese herbal medicine were consistent with The Green Trade Standards or not had become the challenge of Chinese medicinal materials' import and export. Green Trade Standards of Importing & Exporting Medicinal Plants & Preparations, which was awarded by the State Department of Commerce in 2001, had set bounds to the contents of heavy elements in medicinal plants. Therefore, research on the role of heavy metals in Chinese herbaceous plants had become an area of particular concern and high priority in environmental research and protection [1-3].

Alpinia oxyphylla Miq. was a dry ripe fruit of herbaceous perennial, which belongs to Zingiberaceae, and is mainly produced in Hainan, Guangdong, Guangxi and Yunnan provinces[4]. Its capsular fruits were commonly used in traditional East Asian medicine for treating diarrhea[5], intestinal disorders[6],dieresis[7], frequent ruination and urinary incontinence[8].

Modern pharmacological studies had shown that Alpinia oxyphylla Miq. had many pharmacological effects such as anti-inflammatory activities, anti-allergy, anti-ulcer and neuroprotective roles[9,10].

Chemical analysis revealed that Alpinia oxyphylla Miq. contained flavonoids, diarylheptanoids, sesquiterpenes, volatile oil, steroids and their glycosides, etc [11,12]. Our lab also reported the content levels of nine representative compounds occurring in the fruits of Alpinia oxyphylla Miq. harvested at different stages [10]. However, the major constituents are only emphasized aiming at current drug safety of Alpinia oxyphylla Miq., and the results were inadequate for the whole quality assurance purposes of Alpinia oxyphylla Miq. Therefore, a powerful analysis approach for identification and simultaneous determination of heavy metals in Alpinia oxyphylla Miq. was urgently needed to ensure the quality control, efficacy and safety of Chinese patent drug.

In the study, the contents of heavy metals in Alpinia oxyphylla Miq. at different growth time were determined. The research would provide the scientific basis for the quality control and safe medication of Alpinia oxyphylla Miq. Meanwhile, it could provide data reference for the acquisition time and GAP (Good Agricultural Practice of Medicinal Plants and Animals) specification planting of Alpinia oxyphylla Miq.

Experimental

Apparatus

3510 atomic absorption spectrophotometry(Agilent Technologies Instrument Co., Ltd, Shanghai, China) equipped with Cu hollow cathode lamps(Beijing Titan Instruments Co., Ltd, Beijing, China) was used in measurement. A model AFS 830 sequential injection vapor generation double-channel non-dispersive atomic fluorescence spectrometry (Beijing Titan Instruments Co., Ltd, Beijing, China) equipped with As, Hg, Pb and Cd hollow cathode lamps (Beijing Titan Instruments Co., Ltd, Beijing, China) was used. A LabTech EG20A electric hot plate (Beijing Titan Instruments Co., Ltd, Beijing, China) was employed for sample preparation.

Reagents

All reagents were of highest available purity and were at least analytical grade. Ultrapure water was used throughout the experiment.

Chemicals and reagents used throughout the work were of hyper-pure grade. Certified stock standards (1000mg·L^{-1}, each) for As, Hg, Pb and Cd were purchased from National Research Center for Certified Reference Materials (NRCCRM, China). Dilutions of standards and samples were done by using deionized water(18.2MΩ cm). These reagents, such as hydrochloride, nitric acid, sulfuric acid, dithizone, carbon tetrachloride, potassium hydroxide, cobalt chloride, thiourea, oxalic acid, potassium ferricyanide, ascorbic acid, lanthanum nitrate, were purchased from Guangzhou Chemical Reagent Factory. Potassium borohydride was purchased from Sinopharm Chemical Reagent Co., Ltd.

Sample collection

14 samples of different parts of Alpinia oxyphylla Miq. (fruit, rhizome, stem and leaf) at different growth stages were collected, respectively including 5^{th}, 10^{th}, 15^{th}, 20^{th}, 25^{th}, 27^{th}, 30^{th}, 35^{th}, 40^{th}, 45^{th}, 50^{th}, 55^{th}, 60^{th} and 65^{th} day. Each sample was 100g.

Botanical identification and authentication of the collected species with depositions of herbarium specimens had been done by Jian-ping Tian, associate professor of Hainan Medical University.

Sample preparation

Powdered samples with the weight of 1000g were weighed and transferred into 100-mL conical flask and mixed with HNO_3 10mL and $HClO_4$ 2mL. The samples were soaked overnight after all digestion reagents were added. Then, the flask was placed on the electric hot plate for digesting until the white smoke appeared. However, this operation had to be handled gently, thereby ensuring that the digestion solution cannot be heated to dryness. The digested solution was transferred to a 25-mL volumetric flask after being cooled, 100g·L^{-1} thiourea solution 5 mL was mixed, which was then diluted to 25 mL with 1.5% HCl. The solution was set aside for 30min at room temperature for the determining As, Hg and Cu[13].

Powdered samples 1000g were weighed and prepared according to the same method for preparing solution of As. Then, the samples were digested until the digestion solution was heated to dryness. The remaining material was dissolved by 1.5% HNO_3 after being cooled. The digesting solution was transferred to a 25-mL volumetric flask, which was then diluted to 25 mL with 1.5% HNO_3 . This solution was set aside for 30min at room temperature for determining Pb.

Powdered samples 1000g were weighed, prepared and digested according to the same method for preparing for the solution of Pb. The remaining material was dissolved by 0.2mol·L^{-1} H_2SO_4 after being cooled. The digesting solution was transferred to a 25-mL volumetric flask, and 0.5g·L^{-1} dithizone carbon tetrachloride solution5 mL was mixed. 50g·L^{-1} sulfuric acid solution 10mL and 0.10μg·L^{-1} cobalt chloride solution 0.5mL were mixed after being shocked fiercely for 2min, which was then diluted to 25 mL with 0.2mol·L^{-1} H_2SO_4. This solution was set aside for 30min at room temperature for determining Cd.

Blank solutions were prepared in a similar way, and the same final concentrations were achieved. Certified stock standards (1000mg·L^{-1}, each) of As,Hg,Pb, Cd and Cu 1mL were mixed, which was then diluted to 1.000mg·L^{-1} with 3% HNO_3 as calibration solution.

Instrument parameters

The content of Cu was determined by atomic absorption spectroscopy(AAS), and the contents of other four elements, i.e. As, Hg, Pb and Cd, were determined by atomic fluorescence spectroscopy(AFS). The instrument parameters of AAS and AFS were respectively shown in Table 1 and Table 2.

Table 1 the parameters for Atomic fluorescence spectroscopy(AFS) measurement

Element	Lamp current	Atomizer temperature	Atomizer height	Reductant	Carrier liquid
As	60mA	200℃	8.0mm	20g·L^{-1} KBH_4	5% HCl
Hg	25mA	200℃	8.0mm	20g·L^{-1} KBH_4	2% HNO_3
Pb	80mA	200℃	8.0mm	30g·L^{-1}KBH_4,100g·L^{-1}$K_3Fe(CN)_6$	1.6% HCl
Cd	80mA	200℃	8.0mm	30g·L^{-1} KBH_4	0.20mol·L^{-1} H_2SO_4

Table 2 the parameters for Atomic absorption spectroscopy(AAS) measurement

Element	Wavelength	Lamp current	Slit	The ratio of combustion air to acetylene
Cu	324.7nm	2mA	0.2nm	8:1.5

The standard curve

Standard solution (1.00mg·L^{-1}) for As with volume of 0.00, 0.20, 0.50, 1.00, 2.00 and 5.00mL was respectively transferred into 100-mL volumetric flask, and standard solution (0.10mg·L^{-1}) for Hg 0.00, 0.10, 0.20, 0.50, 1.00 and 2.00mL was then added into each volumetric flask, which was mixed with hydrochlorie 5.0mL and 100g·L^{-1} ascorbic acid thiourea solution20mL, which was then diluted to 100 mL. The solutions were set aside for 30min at room temperature for determining As and Hg.

Standard solution (1.00mg·L^{-1}) for Pb with volume of 0.00, 0.50, 1.00, 2.00, 4.00 and 8.00mL was respectively transferred into 100-mL volumetric flask, which was then diluted to 100 mL with 1.5% HNO_3. The solutions were set aside for 30min at room temperature for determining Pb.

Standard solution (0.1mg·L^{-1}) for Cd with volume of 0.00, 0.50, 1.00, 2.00, 4.00 and 8.00mL was respectively transferred into 100-mL volumetric flask, which was mixed with dithizone carbon tetrachloride solution. 50g·L^{-1} sulfuric acid solution 20mL and 0.10μg·L^{-1} cobalt chloride solution 2.0mL were mixed after being shocked fiercely for 2min, which wasthen diluted to 100 mL with 0.20mol·L^{-1} H_2SO_4. The solutions were set aside for 30min at room temperature for determining Cd.

Standard solution 100mg·L^{-1} for Cu with volume of 0.00, 0.20, 0.50, 1.00, 1.50 and 2.00mL was respectively transferred into 100-mL volumetric flask, which was mixed with 5.00mL HNO_3 and then diluted to 100 mL. The solutions were set aside for 30min at room temperature for determining Cu by AAS.

These solutions were determined by AAS or AFS. Then the calibration curves for As,Hg,Pb,Cd and Cu were drawn.

Results and Discussion

Method validation

Standard curve

The calibration curves were linear up to 50μg·L^{-1} for As, 2μg·L^{-1} for Hg, 8μg·L^{-1} for Pb and Cd, and 2mg·L^{-1} for Cu. The linear correlations were all between 0.9985 and 0.9999. The favorable correlation showed evidence for the reliability of the proposed method.

Accuracy tests

The accuracy of the method was also evaluated by recovery experiments, which was carried out by using samples with known concentrations of As, Hg, Pb, Cd and Cu, and analyzed for 6 times according to the test method. The RSD of 6 parallel tests was 1.7 % for As, 2.7% for Hg, 1.4% for Pb, 2.1%for Cd and 0.0% for Cu.

Repeatability tests

Powdered samples with the weight of 1000g were weighed, prepared and analyzed for 3 times according to the test method. The RSD of repeatability tests was 3.9% for As, 1.7% for Hg, 0.72% for Pb, 3.9 % for Cd and 2.2% for Cu.

Recovery tests

Powdered samples 0.5000g were weighed, and mixed with different calibration solutions (at higher, middle, and lower levels) of each element, which were then prepared and analyzed according to the test method. Each sample with the same calibration solution was prepared for 3 parallel tests. The results of recovery test were shown in table 3. The recoveries of each element ranged from 93.4% to 104.2%.

Table 3 The result of recovery test （n=9）

Element	Content of sample (μg/g)	Mass of added certified element(μg/g)	Content by analyzed (μg/g)	Recoveries (%)	Average recoveries(%)	RSD (%)
As	0.3754	0.4500	0.7824	90.5		
		0.5500	0.9265	108.8	93.4	2.6
		0.6600	1.0134	92.9		
Hg	0.0409	0.0180	0.0626	94.4		
		0.0230	0.0677	101.3	102.8	1.8
		0.0270	0.0718	112.8		
Pb	0.3791	0.3000	0.7086	109.8		
		0.3800	0.7643	101.4	101.9	1.3
		0.4600	0.8132	94.4		
Cd	0.0379	0.0120	0.0514	112.8		
		0.0150	0.0519	93.5	104.2	2.2
		0.0190	0.0582	106.3		
Cu	21.50	3.000	24.75	108.3		
		4.000	24.13	93.8	101.6	2.1
		5.000	26.63	102.5		

Contents of heavy elements of samples at different growth stages

Contents of heavy elements of different parts of Alpinia oxyphylla Miq.

The accumulation of heavy metals such as As, Hg, Pb, Cd and Cu was determined in fruit, rhizome, stem and leaf of Alpinia oxyphylla Miq. The results were shown in table 4.

Table 4 Contents of heavy metals in different parts of *Alpinia oxyphylla* Miq. ($\mu g \cdot g^{-1}$)

	As	Hg	Pb	Cd	Cu
Fruit	0.2604	0.0220	0.1000	0.0360	7.300
Rhizome	0.7266	0.1450	0.1832	0.0210	8.350
Stem and leaf	0.4413	0.1480	0.0782	0.0080	9.025

The accumulation of heavy metals such as As, Hg, Pb and Cu in Alpinia oxyphylla Miq. was lower in fruit than that in rhizome, stem and leaf. While the content was higher in fruit than that in rhizome, stem and leaf aiming at Cd. In a whole, the contents of heavy metals were lower than that in other parts of Alpinia oxyphylla Miq.

Contents of heavy elements of Alpinia oxyphylla Miq. at different growth stages

Contents of heavy metals of samples at different stages were analyzed. The results were shown in Fig 1.

The changes of contents of As in fruit, rhizome, stem and leaf were listed in Fig.1(a).The contents of As in fruit had changed in the whole growth stages, which reached four peak times. However, the lowest content was discovered in the 40^{th} day. The contents of As in stem and leaf had kept stable.

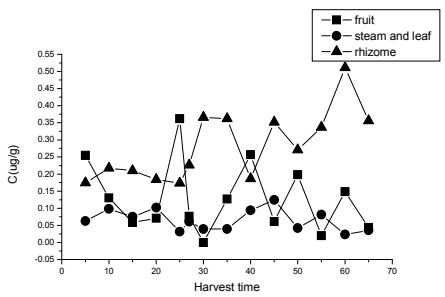
When the fruit was matured, the content of As in fruit seemed higher, but it was much lower than that in rhizome. The result showed that As had migrated from fruit into rhizome, stem and leaf, especially in rhizome.

The results could be drawn from Fig.1(b). In the whole stage, the content of Hg was extremely lower in fruit, steam and leaf, but it had a volatility in rhizome, which was lower between 5th day and 30th day. The content was gradually rising from the 30th to the 40th day, and reached peak in the 40th day, which was then gradually reduced after the 45th day.

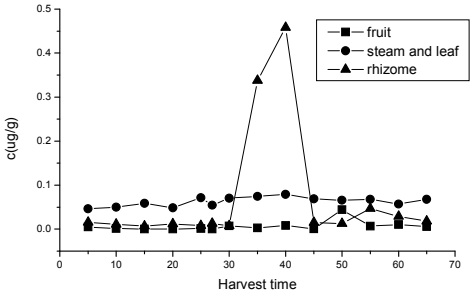
Fig.1(c) shows that the content of Pb was higher in fruit than that in rhizome, steam and leaf in the whole grown stage, and it had four peak times. The content was lower in the 10th, 25th, 45th and 60th day, and it reached the lowest level in the 60th day.

From Fig.1(d) shows that the content of Cd was higher in fruit, steam and leaf than that in rhizome in the whole stage, and it had four peak times. The content was lower in fruit in the 10th, 25th, 30th, 40th, 55th and 60th day. It reached the lowest level in the 30th day in fruit, while the content of Cd was lower in the 15th, 30th, 50th and 60th day in steam and leaf.

Fig.1(e) shows that the content of Cu in fruit was much higher than that in rhizome, steam and leaf. It reached the lowest level in the 40th day.



(a)As



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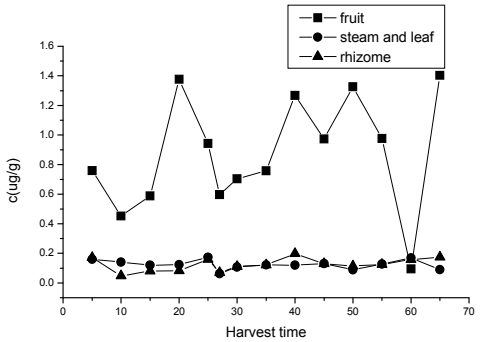
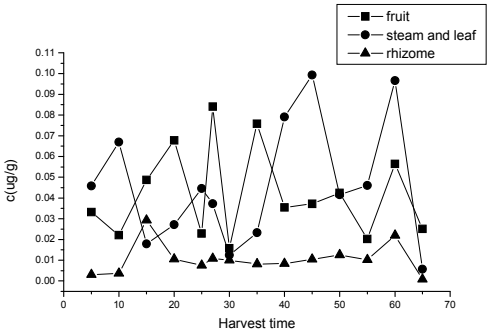
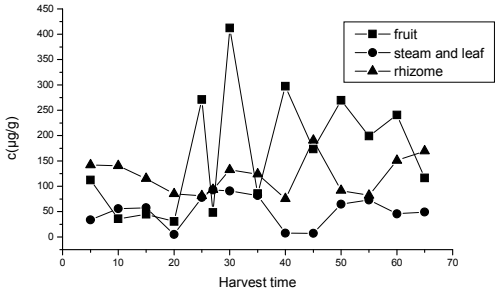


(c) Pb



(d)Cd



(e)Cu

Fig.1 Change of content of As,Hg,Pb,Cd,Cu in different parts of Alpinia oxyphylla Miq. at different grown stages

It is discovered according to changes of contents of heavy metals in fruit, rhizome, steam and leaf in the whole grown stage that the contents of heavy metals were lower in fruit and rhizome. The best harvest time is the 40^{th} day.

Conclusion

The contents of heavy metals such as As, Hg, Pb and Cd were determined by AFS. The content of Cu was determined by AAS. The methodology validation showed that the precision and accuracy of the method were in line with requirements.

The contents of heavy metals in different parts of Alpinia oxyphylla Miq. were determined, and the results showed that the contents of heavy metals were lower in fruit and rhizome. It was safe to use fruit and rhizome of Alpinia oxyphylla Miq. as a medicine.

The best harvest time of Alpinia oxyphylla Miq. was the 40^{th} day and the contents of heavy metals were $0.609 \mu g \cdot g^{-1}$ for As, $0.0007 \mu g \cdot g^{-1}$ for Hg, $0.9729 \mu g \cdot g^{-1}$ for Pb, $0.0372 \mu g \cdot g^{-1}$ for Cd and $173.5 \mu g \cdot g^{-1}$ for Cu.

Green Trade Standards of Importing & Exporting Medicinal Plants & Preparations, which was awarded by the State Department of Commerce of China in 2001, had set bounds to the contents of heavy elements in medicinal plants. As to the phenomenon that the contents of the harmful heavy metals in some traditional Chinese medicines (TCM) were excessive, it was suggested to do from the source, and large cultivation bases of Chinese medicinal materials should be established, which were consistent with GAP. Heavy metals pollution should be strictly controlled from the place, environment control, seed selection, planting, cultivation and acquisition process. At the same time, GMP (good manufacturing practice) should be strictly carried out through the whole process of pharmaceutical production.

The research would provide the scientific basis for the resource utilization of the rhizome, stem and leaf of Alpinia oxyphylla Miq.

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