Research Article

Determination of Heavy Metals in *Alpinia oxyphylla* Miq. Collected from Different Cultivation Regions

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20 batches of *Alpinia oxyphylla* Miq. were collected from Yunnan, Guangdong, Guangxi, and Hainan province in China. The contents of heavy metals of As, Hg, Pb, Cd, and Cu were determined and compared. The results indicated that geographical source might be a major factor to influence the contents of heavy metals of arsenic (As), mercury (Hg), lead (Pb), cadmium (Cd), and copper (Cu) in *Alpinia oxyphylla* Miq. Compared to the criteria of heavy metals, the contents of As, Hg, Pb, and Cd in almost all the samples were in accordance with The Green Trade Standards. The contents of Cu were higher than the criteria for heavy metals except the samples from Changxing town, Qiongzhong county, Maoyang town, Qiongzhong county, Wupo town, Tunchang county, and Nanlv town, Tunchang county, Hainan province. The best cultivation regions of *Alpinia oxyphylla* Miq. were from Changxing town, Qiongzhong county, Maoyang town, Qiongzhong county, Wupo town, Tunchang county, and Nanlv town, Tunchang county, in Hainan province. This research would provide the scientific basis for quality control and standardization of *Alpinia oxyphylla* Miq.

1. Introduction

*Alpinia oxyphylla* Miq., known as Yizhi in Chinese, was a dry ripe fruit of herbaceous perennial belonging to Zingiberaceae, mainly produced in Hainan, Guangdong, Guangxi, and Yunan province [1]. It was one of the four big and sough medicines in China [2].

Modern pharmacological studies had shown that *Alpinia oxyphylla* Miq. had many pharmacological effects such as anti-inflammatory activities, antiallergy, antiulcer, and neuroprotective roles [3, 4], and it was commonly used in traditional East Asian medicine for the treatment of diarrhea, intestinal disorders, diuresis, frequent ruination, and urinary incontinence [5–7].

Chemical analysis revealed that *Alpinia oxyphylla* Miq. contained flavonoids, diarylheptanoids, sesquiterpenes, volatile oil, steroids, and their glycosides, and so forth [8]. Our lab had reported the content levels of pharmacologically active chemicals including flavonoids, diarylheptanoids, and sesquiterpenes, between the seeds and pericarps of *Alpinia oxyphylla* capsular fruit gathered from different production [5]; also, both individual and total contents of 10 nucleobases and nucleosides in the fruit of *Alpinia oxyphylla* Miq. collected from their famous regions in southern China were reported [9]. The results indicated that there was a large variation in the contents of pharmacologically active chemicals among the herbs from different regions.

While many investigations on the quality values of *Alpinia oxyphylla* Miq. were being reported, less emphasis had been made on the heavy metals. Besides, concentrations of heavy metals present in medicinal plants were, aside from other organic plant compounds, of great importance in order to understand their pharmacological actions [10].

The present study was conducted to establish baseline information for heavy metals in *Alpinia oxyphylla* Miq. The results are compared to the limitation of heavy metals in The Green Trade Standards issued by the State Department of Commerce in 2001 [11]. 20 batches of *Alpinia oxyphylla* Miq. gathered from their famous regions in southern China were
selected. The contents of arsenic (As), mercury (Hg), lead (Pb), and cadmium (Cd) were determined by atomic fluorescence spectroscopy (AFS), and the contents of copper (Cu) were determined by atomic absorption spectroscopy (AAS). The information gained may help upcoming research to focus on the site of nutrition, pharmacology, and toxicology.

2. Experimental

2.1. Apparatus. For measurements, 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. A model AFS 830 sequential injection vapor generation double-channel nondispersive 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.

2.2. Regents. Chemicals and reagents used throughout this work were of suprapure grade. Ultrapure water was used throughout the experiment. Certified stock standards (1000 mg L\(^{-1}\)) for As, Hg, Pb, Cd, and Cu were purchased from the National Research Center for Certified Reference Materials (NRCCRM, China). Dilutions of standards and samples were done using deionized water (18.2 MΩ cm). These reagents, such as hydrochloric acid, sulfuric acid, dithizone, carbon tetrachloride, potassium hydroxide, cobalt chloride, thiourea, oxalic acid, potassium ferricyanide, ascorbic acid, and lanthanum nitrate, were purchased from Guangzhou chemical reagent factory. Potassium borohydride was purchased from Sinopharm Chemical Reagent Co., Ltd.

2.3. Sample Collection. Twenty samples of *Alpinia oxyphylla* Miq. were collected from different cultivation regions such as Hainan, Guangdong, Guangxi, and Yunnan province of China, as given in Table 1. After collection, the samples were dried at 45\(^\circ\)C for 6 days. Botanical identification and authentication of the collected species with depositions of herbarium specimens had been done by Jianping Tian, associate professor of Hainan medical university. The voucher specimens were deposited at the Herbarium of Hainan Medical University.

2.4. Sample Preparation. Approximately 1.000 g of the powdered samples was weighed and transferred into 100 mL conical flask and mixed with 10 mL HNO\(_3\) and 2 mL HClO\(_4\). After addition of all digestion reagents, the samples were soaked overnight. 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, ensuring that the digestion solution cannot be heated to dryness. After cooling, the digested solution was transferred to a 25 mL volumetric flask, and 5 mL of 100 g L\(^{-1}\) thiourea solution was mixed and then diluted to 25 mL with 1.5% HCl. This solution was set aside for 30 min at room temperature for the determination of As, Hg, and Cu [12].

1.000 g of the powdered samples was weighed and prepared according to the same method for preparing for the solution of As. Then, the samples were digested until the digestion solution was heated to dryness. After cooling, the rest of the material was dissolved by 1.5% HNO\(_3\). The digesting solution was transferred to a 25 mL volumetric flask, and 5 mL of 100 g L\(^{-1}\) thiourea solution was mixed and then diluted to 25 mL with 1.5% HCl. This solution was set aside for 30 min at room temperature for the determination of As, Hg, and Cu [12].

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Table 1: The samples of *Alpinia oxyphylla* Miq. from different cultivation regions.

| Samples | Cultivation regions                          | Collecting time |
|---------|---------------------------------------------|-----------------|
| S1      | Yangjiang county, Guangdong province        | 13 July         |
| S2      | Daba town, Yangdong county, Guangdong province | 4 July         |
| S3      | Nanning Medicinal Herb Botanical Garden, Guangxi province | 14 June       |
| S4      | Xishuangbanna Tropical Botanical Garden, Yunnan province | 18 May       |
| S5      | Yacha town, Baisha county, Hainan province  | 2 June          |
| S6      | Yacha town, Baisha county, Hainan province  | 23 June         |
| S7      | Yingjeling town, Baisha county, Hainan province | 27 May       |
| S8      | Wushi town, Qiongzhou county, Hainan province | 26 May       |
| S9      | Changxing town, Qiongzhou county, Hainan province | 25 July       |
| S10     | Changxing town, Qiongzhou county, Hainan province | 23 June       |
| S11     | Changxing town, Qiongzhou county, Hainan province | 25 July       |
| S12     | Limushang town, Qiongzhou county, Hainan province | 30 May       |
| S13     | Maoyang town, Qiongzhou county, Hainan province | 11 May       |
| S14     | Xinglong town, Wanning county, Hainan province | 12 May       |
| S15     | Sangengluo town, Wanning county, Hainan province | 29 May       |
| S16     | Changfeng town, Wanning county, Hainan province | 29 May       |
| S17     | Nanlin town, Wanning county, Hainan province | 29 May       |
| S18     | Xingxing town, Tunchang county, Hainan province | 3 June       |
| S19     | Wupotown, Tunchang county, Hainan province  | 21 July         |
| S20     | Nanlv town, Tunchang county, Hainan province | 7 August      |
flask and then diluted to 25 mL with 1.5% HNO₃. This solution was set aside for 30 min at room temperature for the determination of Pb.

1.000 g of the powdered samples were weighed and prepared and digested according to the same method for preparing for the solution of Pb. After cooling, the rest of the material was dissolved by 0.2 mol·L⁻¹ H₂SO₄. The digesting solution was transferred to a 25 mL volumetric flask, and 5 mL of 0.5 g·L⁻¹ dithizone carbon tetrachloride solution was mixed. After shocking fiercely for 2 min, 10 mL of 50 g·L⁻¹ sulfuric acid solution and 0.5 mL of 0.10 μg·L⁻¹ cobalt chloride solution were mixed and then diluted to 25 mL with 0.2 mol·L⁻¹ H₂SO₄. This solution was set aside for 30 min at room temperature for the determination of Cd.

Blanks solutions were prepared in a similar way, achieving the same final concentrations.

1 mL of certified stock standards (1000 mg·L⁻¹, each) of As, Hg, Pb, and Cd was mixed and then diluted to 1.000 mg·L⁻¹ with 3% HNO₃ as calibration solution.

### Table 2: The preparation of calibration solutions of heavy metals.

| Element | 1 | 2  | 3  | 4  | 5  | 6  |
|---------|---|----|----|----|----|----|
| As      | 0 | 2  | 5  | 10 | 20 | 50 |
| Hg      | 0 | 0.1| 0.2| 0.5| 1.0| 2.0|
| Pb      | 0 | 5  | 10 | 20 | 40 | 80 |
| Cd      | 0 | 0.5|    | 2  | 4  | 8  |
| Cu      | 0 | 0.2|    |    | 1.0| 1.5| 2.0|

### Table 3: The calibration curves and correlation coefficients of each element.

| Element | The standard curve | Correlation coefficient | Linearity range   |
|---------|--------------------|------------------------|-------------------|
| As      | 𝐈= 172.9𝐶 + 40.29  | 𝑟= 0.9999              | 0~50 μg·L⁻¹       |
| Hg      | 𝐈= 857.1𝐶 + 20.58  | 𝑟= 0.9997              | 0~2 μg·L⁻¹        |
| Pb      | 𝐈= 1482𝐶 + 213.9   | 𝑟= 0.9985              | 0~8 μg·L⁻¹        |
| Cd      | 𝐈= 1240𝐶 − 162.0   | 𝑟= 0.9991              | 0~8 μg·L⁻¹        |
| Cu      | 𝐴= 0.001438 + 0.136546𝐶 | 𝑟= 0.9998 | 0~2 mg·L⁻¹       |

2.5. Atomic Fluorescence Spectroscopy (AFS) Measurement. 20 g·L⁻¹ potassium borohydride solution was used as reductant and 5% HCl was used as carrier liquid for the determination of As. 20 g·L⁻¹ potassium borohydride solution was used as reductant and 2% HNO₃ was used as carrier liquid for the determination of Hg. 30 g·L⁻¹ potassium borohydride solution and 100 g·L⁻¹ potassium ferricyanide solution were used as reductant and 1.6% HCl was used as carrier liquid for the determination of Pb. 30 g·L⁻¹ potassium borohydride was used as reductant and 0.20 mol·L⁻¹ H₂SO₄ was used as carrier liquid for the determination of Cd.

2.6. Atomic Absorption Spectroscopy (AAS) Measurement. The measured spectral element lines were Cu 324.7 nm. The current of Cu hollow cathode lamps was 2 mA. Slit was 0.2 nm. The ratio of combustion air to acetylene was 8:1.5.

2.7. Sample Analysis. Calibration solutions of each element were prepared according to Table 2. The content of Cu was measured by AAS, and the contents of other four elements, that is, As, Hg, Pb, and Cd, were determined by AFS. The contents of each element of the samples were calculated by the standard curve.

Each sample was analyzed 3 times for the same condition. Relative standard deviation (RSD) of the data was less than 2%.

### 3. Results and Discussion

3.1. Methodological Validation

3.1.1. Calibration Curve. The calibration curves, correlation coefficients, and linearity ranges of As, Hg, Pb, Cd, and Cu were listed in Table 3.

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

3.2. Accuracy and Repeatability Tests. The accuracy of the method was also evaluated by recovery experiments carried out using samples with known concentrations of As, Hg, Pb, Cd, and Cu and analyzed 6 times according to the test method. The RSD of 6 parallel tests was between 0.0% and 2.7% (Table 4).
Table 5: The results of recovery tests ($n = 9$).

| Element | Content of sample ($\mu g \cdot g^{-1}$) | Mass of added certified element ($\mu g \cdot g^{-1}$) | Content by analyzed ($\mu g \cdot g^{-1}$) | Recoveries (%) | Average recoveries (%) | RSD (%) |
|---------|-----------------------------------------|----------------------------------------------------|------------------------------------------|----------------|------------------------|---------|
| As      | 0.3754                                 | 0.4500                                             | 0.7824                                   | 90.5           | 93.4                   | 2.6     |
|         | 0.5500                                 | 0.9265                                             |                                          | 108.8          |                        |         |
|         | 0.6600                                 | 1.0134                                             |                                          | 92.9           |                        |         |
| Hg      | 0.0409                                 | 0.0180                                             | 0.0626                                   | 94.4           | 102.8                  | 1.8     |
|         | 0.0230                                 | 0.0677                                             |                                          | 101.3          |                        |         |
|         | 0.0270                                 | 0.0718                                             |                                          | 112.8          |                        |         |
| Pb      | 0.3791                                 | 0.3000                                             | 0.7086                                   | 109.8          | 101.9                  | 1.3     |
|         | 0.3800                                 | 0.7643                                             |                                          | 101.4          |                        |         |
|         | 0.4600                                 | 0.8132                                             |                                          | 94.4           |                        |         |
| Cd      | 0.0379                                 | 0.0120                                             | 0.0514                                   | 112.8          | 104.2                  | 2.2     |
|         | 0.0150                                 | 0.0519                                             |                                          | 93.5           |                        |         |
|         | 0.0190                                 | 0.0582                                             |                                          | 106.3          |                        |         |
| Cu      | 21.50                                  | 3.000                                              | 24.75                                    | 108.3          | 101.6                  | 2.1     |
|         |                                        | 4.000                                              | 24.13                                    | 93.8           |                        |         |
|         |                                        | 5.000                                              | 26.63                                    | 102.5          |                        |         |

...and the final results highlighted the variations in heavy metal content across different regions, with conclusions drawn based on these findings.
Table 6: The contents of As, Hg, Pb, Cd, and Cu of the investigated samples.

| Sample | As (mg kg⁻¹) | Hg (mg kg⁻¹) | Pb (mg kg⁻¹) | Cd (mg kg⁻¹) | Cu (mg kg⁻¹) |
|--------|--------------|--------------|--------------|--------------|--------------|
| S1     | 0.0067       | 0.0095       | 0.0278       | 0.1405       | 27.50        |
| S2     | 0.0115       | 0.0046       | 0.1214       | 0.0724       | 29.25        |
| S3     | 0.0008       | 0.0230       | 0.0302       | 0.0640       | 155.5        |
| S4     | 0.0650       | 0.0001       | 0.0092       | 0.0076       | 51.85        |
| S5     | 0.0404       | 0.0344       | 0.0181       | 0.0423       | 67.50        |
| S6     | 0.1025       | 0.0702       | 0.1244       |              |              |
| S7     | 0.0000       | 0.0358       |              |              |              |
| S8     | 0.0000       | 0.0117       |              |              |              |
| S9     | 0.0034       |              |              |              |              |
| S10    | 0.0678       | 0.0801       | 0.0095       | 6.080        |
| S11    | 0.0634       | 0.0347       | 6.080        |
| S12    | 0.0190       |              |              |              |              |
| S13    | 0.0910       | 0.0437       |              |              |              |
| S14    | 0.0495       | 0.0991       | 0.0282       | 37.53        |
| S15    | 0.0284       | 0.0200       | 0.0165       | 99.75        |
| S16    | 0.0053       | 0.0253       | 99.75        |
| S17    | 0.1888       | 0.0554       | 69.25        |
| S18    | 0.0076       | 0.0564       | 24.75        |
| S19    | 0.0652       | 0.1662       | 7.830        |
| S20    | 0.1055       | 0.0599       | 7.530        |

The results indicated that geographical source might be a major factor to influence the contents of heavy metals of As, Hg, Pb, Cd, and Cu in *Alpinia oxyphylla* Miq. The best cultivation regions of *Alpinia oxyphylla* Miq. were from Changxing town, Qiongzhong county, Maoyang town, Qiongzhong county, Wupo town, Tunchang county, and Nanlv town, Tunchang county, in Hainan province.

This research had established the test method of heavy metals in *Alpinia oxyphylla* Miq, which would be helpful for monitoring heavy metals in the growth stage of *Alpinia oxyphylla* Miq. Besides, it would have some reference for the analysis of heavy metals in other herbs.

This research would provide the scientific basis for quality control and standardization of *Alpinia oxyphylla* Miq.

Competing Interests

The authors declare that they have no competing interests.

Authors’ Contributions

Dan Zhou and Yurong Fu are equal contributors.

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