ICP-MS Determination of 22 Metal Elements in Guangxi Rosa laevigata Root and Processed Products: Postprint

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Abstract

An inductively coupled plasma mass spectrometry (ICP-MS) analytical method was established for 22 metal elements in Guangxi Jin Ying Gen (Rosa laevigata root) medicinal material, and comparative analysis of metal element contents in medicinal materials from different producing areas and their processed products was performed to evaluate medicinal material quality and optimize appropriate processing methods. The ICP-MS method was used to determine the contents of 22 metal elements and generate characteristic metal element bar charts, while systematic cluster analysis and principal component analysis were employed for discriminant studies on 45 samples of Jin Ying Gen medicinal materials from different producing areas and processed products. The results indicated that metal element contents varied among the 45 samples of Guangxi Jin Ying Gen from different producing areas and their processed products, with Al element content being notably elevated. Cluster analysis and principal component analysis classified the producing areas into three categories, with Al, Pb, Ba, Zn, As, and Sr identified as characteristic elements by principal component analysis. This study, through investigating variation patterns of metal elements in Jin Ying Gen from different producing areas and its processed products, demonstrated that the vinegar-roasting processing method is optimal for Jin Ying Gen, and can provide theoretical basis and methodological reference for clinical safe application and quality control of Jin Ying Gen.

Full Text

Determination of 22 Metal Elements in the Roots of *Rosa laevigata* and Its Processed Products from Guangxi by ICP-MS

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Abstract: This study established an inductively coupled plasma mass spectrometry (ICP-MS) method for the simultaneous analysis of 22 metal elements in the roots of *Rosa laevigata* from Guangxi, and compared metal element contents across different habitats and processed products to evaluate 药材 quality and optimize processing methods. ICP-MS was used to determine the contents of 22 metal elements, and characteristic metal element histograms were constructed. Hierarchical clustering analysis and principal component analysis were employed to discriminate among 45 samples from different habitats and processed products. The results demonstrated significant variations in metal element contents among the 45 samples from different habitats and processed products of Guangxi *R. laevigata*, with notably high aluminum (Al) content. Clustering and principal component analyses classified the samples into three groups, with Al, Pb, Ba, Zn, As, and Sr identified as characteristic elements. This investigation reveals the variation patterns of metal elements across different habitats and processed products, indicating that vinegar stir-frying represents the optimal processing method for *R. laevigata*. The findings provide theoretical and methodological references for the clinical safety application and quality control of this medicinal material.

Keywords: *Rosa laevigata* roots, processed products, metal elements, ICP-MS, hierarchical clustering analysis, principal component analysis

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Introduction

Rosa laevigata (Zhuang name: Makgoij), the root of *Rosa laevigata* Michx., is a traditional Chinese medicinal material with astringent, sour, and neutral properties. It is used to treat spermatorrhea, enuresis, dysentery, diarrhea, uterine bleeding, and uterine prolapse (Zhong et al., 2013). Currently, this 药材 serves as a raw material for Guangxi-characteristic Chinese patent medicines such as Jinji Tablets and Sanjin Tablets, which are widely used in clinical practice. Consequently, in-depth research on the scientific development, fundamental exploration, and clinical application of *R. laevigata* roots is particularly important.

At present, *R. laevigata* roots are primarily wild-harvested, and quality evaluation and control studies remain limited. Existing research has mainly focused on organic chemical constituents such as tannins, phenols, alkaloids, flavonoids, polysaccharides, and triterpenoids (Shan et al., 2011; Liu et al., 2018). However, systematic investigations into metal element composition are scarce. Metal elements constitute an important component of traditional Chinese medicines, and their types and concentrations directly influence therapeutic efficacy and toxic side effects (Wu, 2017; Zhang et al., 2018). Therefore, controlling metal element content is directly related to 中药 quality. Modern research has demonstrated that processing can alter trace element composition in 中药, thereby modifying medicinal properties (Wu et al., 2003) and affecting therapeutic outcomes (Chen and Li, 2002). Furthermore, excessive heavy metal content in Chinese medicinal materials has become a bottleneck for international trade (Han et al., 2015), making 中药 safety a hot-button issue in the global community.

This study employed ICP-MS to determine metal element contents in *R. laevigata* roots and their processed products from different habitats in Guangxi. Hierarchical clustering analysis and principal component analysis were used to construct dendrograms based on quantitative results, visually demonstrating differences in metal elements across habitats and processing methods. The optimal processing method was selected to provide scientific evidence for the safety and rational clinical application of *R. laevigata* roots and their processed products, while also offering reference value for quality control standards.

1.1.1 Instruments

The instrumentation included an iCAP Q inductively coupled plasma mass spectrometer (Thermo Fisher Scientific), a Milli-Q ultrapure water system (Millipore, USA), a microwave digestion system (CEM, USA), an electronic analytical balance (Mettler Toledo, Switzerland), an electric thermostatic drying oven (Shanghai Precision Instrument Company), and an induction cooker (Midea).

1.1.2 Reagents and Materials

Standard stock solutions of 22 elements (Al, Fe, Cu, Pb, As, Cd, Cr, Ni, V, Sb, Sn, Tl, Ag, B, Ba, Co, Mn, Mo, Se, Sr, Ti, and Zn) at 1,000 $\mathrm{ng \cdot mL^{-1}}$ were obtained from the National Research Center for Certified Reference Materials. Nitric acid (analytical grade) and ultrapure water (laboratory-prepared) were used. *R. laevigata* roots were collected from nine habitats in Guangxi and identified by Associate Chief Pharmacist SHU Ke from the Guizhou Institute for Food and Drug Control as the roots of *Rosa laevigata* Michx. (Rosaceae).

A total of 45 samples of raw and processed *R. laevigata* roots were prepared in the laboratory, as detailed in Table 1 .

1.2.1 Preparation of Standard Solutions

Standard solutions with an original concentration of 1,000 $\mathrm{ng \cdot mL^{-1}}$ were diluted to the required concentrations before use to ensure that the concentrations of analytes fell within the standard series range, as specified in Table 2 .

1.2.2 Preparation of Processed Products

Following the preparation methods for various processed products specified in the 2015 edition of the Chinese Pharmacopoeia (General Rules), nine batches of *R. laevigata* roots from different habitats (1,000 g each) were divided into five portions of 200 g each for preparation as raw, stir-baked to yellow, wine-processed, salt-processed, and vinegar-processed products.

Raw product: Nine batches of *R. laevigata* roots were cleaned, cut, and dried at 40°C for later use. **Stir-baked to yellow:** Appropriate amounts (200 g each) of the cleaned materials were placed in a heated wok and stir-baked with gentle heat until the surface color deepened, then removed and cooled. **Wine-processed product:** Appropriate amounts (200 g each) were mixed with a specified quantity of rice wine (medicinal material:wine = 100:20), stirred evenly, and left until the wine was completely absorbed. The materials were then placed in a heated wok, stir-baked with gentle heat until the surface color deepened, removed, and cooled. **Salt-processed product:** Appropriate amounts (200 g each) were mixed with a specified quantity of salt water (medicinal material:salt = 100:20), stirred evenly, and left until the salt water was completely absorbed. The materials were then placed in a heated wok, stir-baked with gentle heat until the surface color deepened, removed, and cooled. **Vinegar-processed product:** Appropriate amounts (200 g each) were mixed with a specified quantity of rice vinegar (medicinal material:vinegar = 100:20), stirred evenly, and left until the vinegar was completely absorbed. The materials were then placed in a heated wok, stir-baked with gentle heat until the surface color deepened, removed, and cooled.

1.2.3 Preparation of Sample Solutions

The processed products from nine habitats were dried at low temperature, pulverized, and passed through a 65-mesh sieve. Accurately weighed powder (0.5 g) was placed in a polytetrafluoroethylene digestion vessel, and 8 mL of concentrated nitric acid was precisely added. After standing in a fume hood for 25 minutes, the vessel was placed in a microwave digestion system and digested according to the following program: heated to 150°C over 10 minutes and maintained for 2 minutes, then heated to 200°C over 3 minutes and maintained for 8 minutes. After cooling to room temperature, the vessel was removed, and the acid was evaporated to dryness in a fume hood. The residue was diluted to 100 mL with ultrapure water. Sample blank solutions were prepared simultaneously using the same method.

1.2.4 ICP-MS Operating Conditions

The operating parameters were as follows: RF power 1,555 W; cooling gas flow rate 14 $L \cdot min^{-1}$; carrier gas flow rate 0.8 $L \cdot min^{-1}$; auxiliary gas flow rate 0.8 $L \cdot min^{-1}$; nebulizer gas flow rate 1 $L \cdot min^{-1}$; sample uptake rate 1.5 $L \cdot min^{-1}$; scan mode: peak hopping; number of scans: 10; replicates: 3; number of sampling channels: 3; dwell time: 0.01 s.

1.2.5 Method Validation

1.2.5.1 Linearity, Detection Limits, and Quantitation Limits

According to the procedure described in section 2.1, standard solutions of the 22 metal elements were sequentially analyzed. Calibration curves were constructed with standard concentration as the abscissa (X) and peak intensity as the ordinate (Y). The regression equations, correlation coefficients, linear ranges, and detection limits for each element are presented in Table 2.

1.2.5.2 Precision

Each of the 22 metal element standard solutions was injected six times consecutively. The relative standard deviations (RSDs) ranged from 0.36% to 2.86%, as shown in Table 2, indicating good instrument precision.

1.2.5.3 Repeatability

Six samples of S1 *R. laevigata* root were prepared according to the method described in section 1.2.3, and the contents of 22 metal elements were determined. The RSDs ranged from 0.46% to 2.96%, as shown in Table 2, demonstrating good method repeatability.

1.2.5.4 Stability

One sample of S1 *R. laevigata* root was prepared according to section 1.2.3 and analyzed at 0, 2, 4, 8, 16, and 24 hours. The RSDs ranged from 0.52% to 2.71%, as shown in Table 2, indicating that the elements remained stable within 24 hours.

1.2.5.5 Recovery

Accurately weighed samples of S1 (0.5 g, n=5) with known element contents were spiked with known amounts of standard solutions, prepared according to section 1.2.3, and analyzed under the conditions described in section 1.2.4. The recoveries ranged from 87.34% to 108.33%, with RSDs less than 3%.

2.1 Analysis of Heavy Metal Element Contents

This study determined the contents of 22 metal elements in 45 batches of *R. laevigata* root samples and processed products from different habitats in Guangxi using ICP-MS. The analytical results are presented in Table 3 . According to the 限量 indicators specified in the current *Green Trade Standards for Importing and Exporting Medicinal Plants and Preparations (WM2-2001)* (Ministry of Foreign Trade and Economic Cooperation of the People’ s Republic of China, 2005): Pb $\leq$ 5.0 mg $\cdot$ kg^{-1}, Cd $\leq$ 0.3 mg $\cdot$ kg^{-1}, Hg $\leq$ 0.2 mg $\cdot$ kg^{-1}, Cu $\leq$ 20.0 mg $\cdot$ kg^{-1}, and As $\leq$ 2.0 mg $\cdot$ kg^{-1}, the contents of Pb, Cd, Hg, Cu, and As in all 45 samples of processed *R. laevigata* roots were within the standard limits. The heavy metal contents in both raw and processed *R. laevigata* roots complied with relevant Chinese safety regulations for Chinese medicinal materials. These results may be closely related to the absorption and enrichment capacity of *R. laevigata* for heavy metal elements during growth and the heavy metal content in the soil, suggesting that Guangxi’ s soil and climatic conditions are favorable for *R. laevigata* cultivation.

2.2 Cluster Analysis of Metal Elements in Processed *R. laevigata* Roots from Different Habitats

Hierarchical cluster analysis was performed using the between-groups linkage method as the clustering approach and squared Euclidean distance as the distance measurement method. A data matrix of 22 × 45 dimensions was constructed from the metal element contents of 45 batches of samples from nine habitats and different processed products. SPSS 23.0 software (Windows 23.0, SPSS Inc., USA) was used to construct a dendrogram based on the metal element analysis results, as shown in Figure 1 [Figure 1: see original paper].

The dendrogram in Figure 1 clearly shows that when $\lambda < 5.0$, the 45 samples can be distinctly divided into three groups. The first group includes processed products from Rongshui (Liuzhou), Huaiyuan (Yizhou), Luodong (Yizhou), and Quanzhou (Guilin). The second group comprises processed products from Guanyang (Guilin), Xindou (Hezhou), Guiping (Guigang), and Pingji (Qinzhou). The third group consists of processed products from Pingnan (Guigang). Analysis of Table 3 reveals that Al content was high in all three groups, with the highest range observed in the third group. For Fe, Ba, B, and Zn, the second group showed the highest average content, followed by the first group, while the third group fell within the ranges of the first and second groups. When $\lambda > 10.0$, all samples aggregated into two major clusters: the first and second groups merged into one cluster, primarily collected from northwestern, northeastern, and southeastern Guangxi, while samples from Pingnan County (Guigang) formed a separate cluster. The second cluster exhibited higher Al content than the first cluster, while other element contents fell within the range of the first cluster. These findings indicate that metal element contents in *R. laevigata* roots from northeastern and southeastern Guangxi are higher than those from northwestern regions, with Guigang area forming a distinct cluster. This may be attributed to the non-selective absorption of enriched elements from soil by plants while meeting their own elemental requirements, a process dependent on soil element content, physicochemical properties, and environmental factors such as soil conditions, temperature, and humidity, leading to regional variations in metal element composition in Chinese medicinal materials. The analysis results demonstrate that regional differences, environmental conditions, and climate factors in Guangxi significantly influence the enrichment of metal elements in processed *R. laevigata* roots.

2.3 Principal Component Analysis of Metal Elements

To determine the principal component eigenvalues and contribution rates of the 22 metal elements in Guangxi *R. laevigata*, the content data of 22 metal elements from 45 batches of samples collected from nine habitats and different processed products were analyzed. Five metal elements had contents close to or equal to zero; therefore, 17 metal elements were included in the statistical analysis using SPSS 23.0 software. Eigenvalues and contribution rates are the primary criteria for principal component selection. Six principal components were obtained, with a cumulative contribution rate of 85.432%. The first principal component accounted for 32.44% of the variance, primarily reflecting information from Al (0.726), Pb (0.797), Ba (0.845), and Zn (0.797). The second principal component contributed 15.43%, mainly from As (0.797). The third principal component contributed 14.17%, primarily from Sr (0.829). According to principal component analysis principles and the eigenvector table, Al, Pb, Ba, Zn, As, and Sr can be considered characteristic metal elements of *R. laevigata* and its processed products, essentially representing all information from the indicators.

Three principal components were extracted and used to generate a 3D scatter plot using SPSS 23.0, as shown in Figure 2 [Figure 2: see original paper]. The plot shows that the 45 batches of samples can be divided into three categories: processed products from Rongshui (Liuzhou), Huaiyuan (Yizhou), Luodong (Yizhou), and Quanzhou (Guilin) as the first category; those from Guanyang (Guilin), Xindou (Hezhou), Guiping (Guigang), and Pingji (Qinzhou) as the second category; and those from Pingnan (Guigang) as the third category. The principal component analysis results are completely consistent with the cluster analysis results.

2.4 Histogram of Characteristic Metal Elements

Principal component analysis identified Al, Pb, Ba, Zn, As, and Sr as characteristic metal elements of processed *R. laevigata* roots, capable of representing the information from all 22 metal elements in these samples. The results showed high Al content. To facilitate analysis of the distribution of characteristic metal elements across the 45 batches, the high-concentration Al values were reduced by a factor of 10 to match the magnitude of other characteristic elements before constructing the histogram, as shown in Figure 3 [Figure 3: see original paper].

Figure 3 shows that Pb, As, and Sr contents did not change significantly after different processing methods in *R. laevigata* roots from the nine habitats. However, Al content decreased significantly after vinegar processing, while Zn content increased markedly. These two elements showed no significant changes with other processing methods. Ba content varied significantly after processing only in samples from Rongshui (Liuzhou), Guiping (Guigang), and Pingji (Qinzhou), with no significant changes observed in other habitats. The reduction in Al during vinegar processing may be due to the formation of complexes between certain components in *R. laevigata* and Al under acidic conditions, leading to decreased Al content. The increase in Zn may result from substances in *R. laevigata* that promote the dissociation of Zn salts in acidic environments. The irregular changes observed in other elements across different habitats and processing methods may be related to the influence of regional component composition or physicochemical changes occurring during specific processing procedures.

3 Discussion and Conclusion

Among the processed products of *R. laevigata* roots, the major elements Al and Fe were most abundant. Al content in various processed products ranged from 33.9 to 270 $\mathrm{mg \cdot kg^{-1}}$, which may serve as an important marker for Guangxi *R. laevigata* 药材. The generally high Al content may be related to the genetic characteristics of the 药材 and the presence of certain components capable of active Al absorption. Fe content exceeded 1 $\mathrm{mg \cdot kg^{-1}}$ in raw products, though irregular decreases or increases were observed in some habitats after processing, likely due to combined effects of regional soil, climate, and processing methods. Trace elements including B, Ba, Mn, Zn, and Sr were relatively abundant, all of which are essential for human health.

Modern medical research indicates that normal human Al content ranges from 45 to 150 mg, with relatively low toxicity and antagonistic effects against certain lead toxicities. However, Al levels must be controlled within necessary limits, as excessive Al can hinder phosphorus absorption and inhibit digestive enzymes (Ye et al., 1999). Zinc possesses heat-clearing, blood-cooling, anti-inflammatory, and astringent properties, and can antagonize Cd, Hg, and Al while enhancing immunity (Zhang et al., 2002; Guan, 2008). These effects align with the anti-inflammatory, heat-clearing, and astringent functions of *R. laevigata* roots (Tan et al., 2012). The therapeutic efficacy of *R. laevigata* is correlated with these trace elements; therefore, appropriately increasing Zn content while reducing Al content may create synergistic effects. The experimental results show that vinegar processing produces a decreasing trend in Al and an increasing trend in Zn. Vinegar processing not only effectively controls and reduces Al content but also appropriately increases Zn content. Furthermore, vinegar’ s sour taste and astringent properties in traditional Chinese medicine create synergistic effects with the therapeutic functions of *R. laevigata*.

This study established an ICP-MS method for determining 22 metal elements in *R. laevigata* roots and their processed products from Guangxi. The method is rapid and accurate, providing necessary reference value for establishing quality standards for *R. laevigata* 药材. Statistical analysis of metal element data from 45 batches revealed that cluster analysis enabled preliminary grouping of samples, demonstrating chemical differences among *R. laevigata* roots from different habitats and processing methods. Principal component analysis further validated the accuracy of cluster analysis results while screening characteristic metal elements to provide methodological references for quality control research. Additionally, this study offers scientific evidence for selecting artificial cultivation bases, producing decoction pieces, and ensuring clinical medication safety.

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Note: Figure translations are in progress. See original paper for figures.