Postprint: Analysis of Content Differences of Gallic Acid and Catechin in Rosa laevigata Root and Its Processed Products from Different Producing Areas in Guangxi

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Abstract

To establish a method for determining the contents of gallic acid and catechin in Rosa laevigata root and to analyze the variations in these compounds in samples from different producing areas of Guangxi and their processed products, this study employed gallic acid and catechin contents as indicator components. HPLC was used to determine the contents in raw, stir-fried, wine-fried, salt-fried, and vinegar-fried products of Guangxi-produced Rosa laevigata root, and SPSS 23.0 software was utilized for variance analysis and cluster analysis. The results demonstrated that the contents of gallic acid and catechin varied among samples from different producing areas and processed products. The catechin content was higher than that of gallic acid in all samples. The contents of both compounds in the southern region (except Guigang Guiping) were generally higher than those in the northern region. Among processed products, the vinegar-fried product exhibited the highest contents of gallic acid and catechin. This study indicates that the HPLC method is simple and feasible, and that variations in gallic acid and catechin contents in Rosa laevigata root are primarily associated with geographical origin and processing method, providing a scientific basis for the rational utilization of Rosa laevigata root resources, formulation of quality standards, and research on clinical applications.

Full Text

Difference Analysis on Contents of Gallic Acid and Catechin in Roots of Rosa laevigata and Its Processed Products from Different Habitats of Guangxi

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Abstract

This study aimed to establish a method for determining gallic acid and catechin contents in the roots of *Rosa laevigata* and analyze variations in these compounds across different habitats and processing methods in Guangxi. Using gallic acid and catechin as marker components, we employed HPLC to analyze raw, fried, wine-processed, salt-processed, and vinegar-processed samples from Guangxi. SPSS 23.0 software was used for analysis of variance (ANOVA) and hierarchical clustering analysis (HCA). The results revealed significant differences in gallic acid and catechin contents among samples from different habitats and processing methods. Catechin content exceeded gallic acid in all samples. Overall, southern regions (except Guiping, Guigang) showed higher contents than northern regions. Among processed products, vinegar-processed samples exhibited the highest levels of both compounds. This study demonstrates that the HPLC method is simple and feasible, and that variations in gallic acid and catechin contents in *R. laevigata* roots primarily reflect differences in geographical origin and processing methods. These findings provide a scientific basis for rational resource utilization, quality standard formulation, and clinical application research of *R. laevigata* roots.

Keywords: high performance liquid chromatography (HPLC); *Rosa laevigata* roots; processed products; gallic acid; catechin; analysis of variance (ANOVA); hierarchical clustering analysis (HCA)

Introduction

Rosa laevigata root (Zhuang name: Makgoij), derived from the root of *Rosa laevigata* Michx. (Rosaceae), is widely distributed across central, southern, eastern, and southwestern China. As a genuine medicinal material in Guangxi, it has been extensively used as both food and medicine among local populations and serves as a major specialty product supporting the development of traditional Chinese medicine in the region.

Modern pharmacological studies have demonstrated that *R. laevigata* root exhibits significant potential for development and utilization in anti-inflammatory, antibacterial, antitumor, immune-enhancing, antioxidant, and anti-arrhythmic

applications. Gallic acid, one of its chemical constituents, possesses multiple activities including antioxidant, antimicrobial, antitumor, and cardiovascular protective effects, while catechin exhibits antitumor, antioxidant, antimicrobial, and cardioprotective properties. These two compounds represent key pharmacodynamic components that play dominant roles in the clinical pharmacology of *R. laevigata* root.

Despite its importance as the principal drug in Guangxi-characteristic proprietary Chinese medicines such as Jinji Tablets and Sanjin Tablets, few studies have investigated the material basis of *R. laevigata* root, and the effects of different habitats and processing methods on its active constituent contents remain unclear. Although some researchers have examined total tannins and flavonoids in processed *R. laevigata* root using UV spectrophotometry, these studies involved limited indicators and small sample sizes. Other studies have compared total flavonoid contents in roots and stems or analyzed triterpenoid acids from 22 habitats nationwide, revealing significant variations. However, a comprehensive quality control standard for *R. laevigata* root is still lacking, as is systematic research on process quality control and pharmacodynamic component relationships. This study addresses these gaps by employing HPLC to analyze gallic acid and catechin—key pharmacodynamic components—in *R. laevigata* roots from nine Guangxi habitats and their processed products. Through ANOVA and cluster analysis, we compared and analyzed variations in these marker compounds to provide references for further research, development, and quality standard formulation.

Materials and Methods

1.1 Materials and Instruments

Instruments: Ultimate 3000 HPLC system (Thermo Fisher Scientific, USA), KQ-500DB ultrasonic cleaner (Kunshan Ultrasonic Instrument Co., Ltd.), HH-4 electric thermostatic water bath (Beijing Keweiyongguang Instrument Co., Ltd.), METTLER TOLEDO MS204TS electronic balance (Mettler-Toledo), electric blast drying oven (Shanghai Precision Instrument Co.), and induction cooker (Midea).

Reagents: Methanol (analytical grade, Tianjin Fuyu Fine Chemical Co., Ltd.), phosphoric acid (analytical grade, Tianjin Fuyu Fine Chemical Co., Ltd.), hydrochloric acid (guaranteed reagent, Kunshan Jincheng Reagent Co., Ltd.), purified water (UPW-50N ultrapure water system), ultrapure water (UPW-50N system), methanol (HPLC grade, Tedia Company, USA), gallic acid reference substance (National Institutes for Food and Drug Control, Batch No. 110831-201605), and catechin reference substance (National Institutes for Food and Drug Control, Batch No. 110877-201203).

Samples: *R. laevigata* roots were collected from nine habitats in Guangxi and authenticated by Associate Chief Pharmacist Shu Ke at Guizhou Institute for Food and Drug Control as the roots of *Rosa laevigata* Michx. (Rosaceae).

A total of 45 samples of raw and processed products were prepared in our laboratory (Table 1).

1.2 Methods

1.2.1 Preparation of Standard Solutions Gallic acid standard stock solution (0.024 $mg \cdot mL^{-1}$) was prepared by accurately weighing 2.40 mg of gallic acid reference standard, dissolving in methanol, and diluting to 100 mL. Catechin standard stock solution (0.290 $mg \cdot mL^{-1}$) was prepared by accurately weighing 5.81 mg of catechin reference standard, dissolving in 50% ethanol, and diluting to 20 mL.

1.2.2 Preparation of Different Processed Products Following the processing guidelines in the 2020 edition of Chinese Pharmacopoeia (General Rules), nine batches of *R. laevigata* roots (1,000 g each) from different habitats were divided into five portions (200 g each) for preparation as raw, fried, wine-processed, salt-processed, and vinegar-processed products.

Raw product: Nine batches of crude drug were cleaned, cut, and dried at 40°C.
Fried product: Appropriate amounts (200 g each) were placed in a heated pan and stir-fried with gentle heat until the surface color deepened, then cooled.
Wine-processed product: Crude drug (200 g each) was mixed with rice wine (drug:wine = 100:20), moistened until thoroughly permeated, stir-fried with gentle heat until the surface color deepened, then cooled.
Salt-processed product: Crude drug (200 g each) was mixed with salt water (drug:salt = 100:20), moistened until thoroughly permeated, stir-fried with gentle heat until the surface color deepened, then cooled.
Vinegar-processed product: Crude drug (200 g each) was mixed with rice vinegar (drug:vinegar = 100:20), moistened until thoroughly permeated, stir-fried with gentle heat until the surface color deepened, then cooled.

1.2.3 Preparation of Sample Solutions **For gallic acid determination:** Samples from Section 1.2.2 were dried at low temperature, pulverized, and passed through a No. 3 sieve. Approximately 0.1 g of powder was accurately weighed into a stoppered conical flask, mixed with 50 mL of 4 $mol \cdot L^{-1}$ HCl, weighed, heated under reflux for 4 h, cooled, and replenished with 4 $mol \cdot L^{-1}$ HCl to the original weight. After filtration, 10 mL of the filtrate was evaporated to dryness, and the residue was dissolved in methanol and transferred to a 10 mL volumetric flask for determination.

For catechin determination: Samples were prepared similarly but using 0.5 g of powder mixed with 50 mL of methanol-HCl (50:1), ultrasonicated (250 W, 40 kHz) for 45 min, cooled, replenished with methanol to the original weight, filtered, and the filtrate used for determination.

1.2.4 HPLC Chromatographic Conditions **Gallic acid:** Thermo Scientific C18 column (250 mm × 4.6 mm, 5 m); mobile phase: methanol (A) - 0.1% phosphoric acid (B) (5:95); detection wavelength: 271 nm; column temperature: 30°C; flow rate: 0.6 mL · min^{-1}; injection volume: 5 L.

Catechin: Waters C18 column (250 mm × 4.6 mm, 5 m); mobile phase: methanol (A) - 0.2% phosphoric acid (B) (10:90); detection wavelength: 279 nm; column temperature: 30°C; flow rate: 1 mL · min^{-1}; injection volume: 10 L.

Results

2.1 Method Validation

2.1.1 Linearity, Detection Limit, and Quantitation Limit Aliquots of 1, 3, 5, 10, and 15 L of the standard stock solutions were injected to record peak areas. Standard curves were plotted with injection amount (X) versus peak area (Y). Regression equations, correlation coefficients, linear ranges, detection limits, and quantitation limits are shown in Table 2 .

2.1.2 Precision Test Standard solutions were injected six times consecutively under the chromatographic conditions described in Section 1.2.4. The relative standard deviations (RSD) for gallic acid and catechin were 0.91% and 0.40%, respectively, indicating good instrument precision.

2.1.3 Repeatability Test Six samples (S1) were prepared according to Section 1.2.3.1 (0.1 g) and Section 1.2.3.2 (0.5 g), respectively, and analyzed. The average contents of gallic acid and catechin were 4.902 mg · mL^{-1} and 19.318 mg · mL^{-1}, with RSD values of 0.80% and 1.50%, demonstrating good repeatability for both methods.

2.1.4 Stability Test Sample solutions (S1) were stored at room temperature and analyzed at 0, 2, 4, 8, 12, and 24 h. The RSD values for gallic acid and catechin peak areas were 1.95% and 0.96% (n=6), respectively, indicating good stability within 24 h.

2.1.5 Spike Recovery Test Known amounts of gallic acid standard solution (0.2266 mg · mL^{-1}, 1 mL) and catechin standard solution (1.9462 mg · mL^{-1}, 1 mL) were added to accurately weighed S1 samples, evaporated to dryness, and prepared according to Section 1.2.3. Analysis under the chromatographic conditions showed good recovery rates for both compounds (Table 3).

2.2 Content Determination Results

Samples prepared according to Section 1.2.3 were analyzed under the chromatographic conditions in Section 1.2.4. Chromatograms are shown in Figure 1 [Figure 1: see original paper]. The content determination results (Table 4) revealed

that catechin content was significantly higher than gallic acid content across all samples, with catechin levels being 2–9 times greater. This characteristic may serve as an important feature for identifying *R. laevigata* roots from Guangxi.

2.3 Cluster Analysis of Different Producing Areas

Using gallic acid and catechin contents in raw products from nine habitats as variables, cluster analysis was performed with SPSS 23.0. The dendrogram (Figure 2 [Figure 2: see original paper]) showed that at $5 < \lambda \leq 10$, samples S1, S6, S11, S16, S21, and S26 clustered as Group I; S36 and S41 as Group II; and S31 as Group III. At $10 < \lambda < 25$, all samples divided into two groups: S1, S6, S11, S16, S21, S26, S36, and S41 as one group, and S31 alone as another. The results indicate distinct geographical distribution patterns, with northern habitats (Yizhou, Liuzhou, Guilin, and Hezhou) clustering together, southern habitats (Pingnan, Guigang and Pingji, Qinzhou) forming another group, and Guiping, Guigang showing unique characteristics, possibly due to environmental factors or genetic variations.

The average contents of gallic acid and catechin in the three clustered groups are compared in Table 5 . Significant differences ($P < 0.05$) were observed among groups. Southern regions (except Guiping) showed higher contents than northern regions, while Guiping exhibited the lowest levels for both compounds.

2.4 Variance Analysis of Gallic Acid and Catechin Contents

Multivariate ANOVA was performed with gallic acid and catechin contents as dependent variables. The results (Table 6) showed highly significant differences ($P < 0.01$) among habitats for both compounds, confirming that geographical environment substantially influences their accumulation. Processing methods also significantly affected contents: gallic acid showed highly significant differences ($P < 0.01$), while catechin showed significant differences ($P < 0.05$).

The distribution of gallic acid and catechin contents across different habitats and processed products is illustrated in Figures 3 [Figure 3: see original paper] and 4 [Figure 4: see original paper]. While some processing methods in certain habitats caused slight decreases, vinegar processing consistently increased both compounds across all habitats, reaching (0.66 ± 0.16) mg$\cdot$g^{-1} for gallic acid and (2.83 ± 0.87) mg$\cdot$g^{-1} for catechin. Further ANOVA (Table 7) confirmed that vinegar-processed products differed significantly from other processing methods ($P < 0.05$ or $P < 0.01$), indicating that vinegar processing is optimal for enhancing gallic acid and catechin contents.

Discussion

3.1 Investigation of Chromatographic Conditions and Extraction Methods

During method development, various mobile phase systems (methanol-phosphoric acid, acetonitrile-phosphoric acid, methanol-formic acid, acetonitrile-formic acid) and wavelengths (200–400 nm) were evaluated. Different C18 columns (Waters, Dikma, Elite, Thermo Scientific) were tested at column temperatures of 20, 30, and 40°C and flow rates of 0.8, 1.0, and 1.2 $mL \cdot min^{-1}$. Given that gallic acid and catechin are acidic phenolic compounds with high polarity, and that *R. laevigata* root contains predominantly polar components, acidic modifiers were added to the mobile phase to optimize separation and peak shape. The final conditions selected were: for gallic acid, methanol-0.1% phosphoric acid (5:95) on a Thermo Scientific C18 column at 271 nm, 0.6 $mL \cdot min^{-1}$, and 30°C; for catechin, methanol-0.2% phosphoric acid (10:90) on a Waters C18 column at 279 nm, 1 $mL \cdot min^{-1}$, and 30°C. These conditions provided good resolution, stable baselines, and minimal interference.

Extraction methods were optimized by evaluating solvents (various concentrations of methanol, ethanol, HCl, and methanol-HCl mixtures), extraction methods (reflux, ultrasonication), time (30 min to 4 h), and sample amounts (0.1–0.5 g). The optimal conditions selected were: 0.1 g sample with 4 $mol \cdot L^{-1}$ HCl refluxed for 4 h for gallic acid; and 0.5 g sample with methanol-HCl (50:1) ultrasonicated for 45 min for catechin.

While most previous studies on *R. laevigata* root used UV methods, this study established an HPLC method that is simple, sensitive, reliable, and reproducible, enabling accurate qualitative and quantitative analysis with baseline separation from interfering peaks.

3.2 Quality Evaluation and Processing Method Optimization Based on Gallic Acid and Catechin Contents

Previous studies on *R. laevigata* root content determination have focused on single chemical classes, such as total tannins or flavonoids, often with limited sample sizes and single indicators. Gallic acid and catechin are fundamental pharmacodynamic substances in *R. laevigata* root, both containing phenolic hydroxyl groups that confer acidity. Catechin also contributes bitterness and astringency, suggesting that the sour and astringent properties of *R. laevigata* root derive from these compounds. This study selected nine habitats across Guangxi and used gallic acid and catechin as indicators, providing a more comprehensive evaluation of quality and optimal processing methods.

As plant secondary metabolites, gallic acid and catechin synthesis is influenced by climate and geography. Southern Guangxi (subtropical climate with abundant rainfall and sunlight) showed higher contents than northern regions, consistent with reports that increased light intensity enhances polyphenol accumula-

tion. However, Guiping exhibited unique characteristics, possibly due to specific environmental or genetic factors. Further research with expanded sample sizes and additional parameters (soil composition, altitude) is needed.

Variance analysis revealed that processing methods significantly affected contents, likely due to heating and excipient effects. Vinegar processing consistently increased both compounds, aligning with our previous findings on metal element contents. The increase may result from decomposition of tannin or glycoside components under acidic, heated conditions, releasing gallic acid, while catechin stability is enhanced under acidic pH conditions, preventing degradation that occurs in neutral or alkaline environments.

3.3 Conclusion

This study established an HPLC method for determining gallic acid and catechin contents in *R. laevigata* root, providing a scientific basis for rational resource utilization and quality standard development in Guangxi. By elucidating the effects of processing on these key components, this work lays a foundation for further investigation into the relationship between chemical changes during processing and pharmacological activity.

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