# Evaluation of *in vitro* Antibacterial, Antioxidant, and α-Glucosidase Inhibitory Effects of *Pilea peltata* Hance

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**Abstract** [**Objectives**] To evaluate the *in vitro* antibacterial, antioxidant, and α-glucosidase inhibitory activities of the ethanol total extract and four different polarity fractions (n-butanol, ethyl acetate, petroleum ether, and water) of *Pilea peltata* Hance, so as to provide a reference for its further development and research. [**Methods**] The antibacterial activity of *P. peltata* was evaluated *in vitro* by determining the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of its ethanol total extract and four different polarity fractions against seven test bacterial strains using the broth microdilution method. The *in vitro* antioxidant activity was investigated through DPPH radical, hydroxyl radical, and superoxide anion radical scavenging assays, with vitamin C (Vit C) as the positive control and the half maximal scavenging concentration ($IC_{50}$) as the evaluation indicator. The *in vitro* α-glucosidase inhibitory activity was assessed by measuring the peak area of p-nitrophenol (PNP), the hydrolysis product of 4-nitrophenyl α-D-glucopyranoside (PNPG), via high-performance liquid chromatography (HPLC), using the half maximal inhibitory concentration ($IC_{50}$) as the evaluation indicator. [**Results**] Both the ethanol total extract and the four different polarity fractions of *P. peltata* exhibited significant *in vitro* anti-Streptococcus pneumoniae activity. The DPPH radical scavenging capacities of the ethanol total extract and the various fractions were all weaker than that of VitC, with the order of efficacy being: n-butanol fraction > ethanol total extract > ethyl acetate fraction > petroleum ether fraction > aqueous fraction. For hydroxyl radical scavenging activity, the efficacy order of *P. peltata* fractions was: n-butanol extract > ethyl acetate extract > ethanol total extract > petroleum ether extract > aqueous extract. Notably, the n-butanol fraction ($IC_{50} = 0.068 \pm 0.001$) demonstrated stronger activity than VitC ($IC_{50} = 0.097 \pm 0.001$). The activity of the ethyl acetate fraction ($IC_{50} = 0.096 \pm 0.004$) was comparable to that of VitC ($IC_{50} = 0.097 \pm 0.001$). The superoxide anion scavenging capacities of the ethanol total extract and different polarity fractions from *P. peltata* were all weaker than that of VitC, with the order of efficacy being: n-butanol fraction > ethyl acetate fraction > ethanol total extract > petroleum ether fraction > aqueous fraction. The ethanol total extract and aqueous fraction of *Pilea peltata* showed no significant *in vitro* α-glucosidase inhibitory activity. Compared with the acarbose group, the $IC_{50}$ values of the ethyl acetate fraction and the n-butanol fraction both showed highly significant differences ($P < 0.01$). [**Conclusions**] This study provides an experimental basis for the pharmacodynamic study and active component study of *P. peltata*.

**Key words** *Pilea peltata* Hance, Antibacterial, Antioxidation, α-glucosidase inhibition

## 1 Introduction

*Pilea peltata* Hance is a plant species belonging to the genus *Pilea* in Urticaceae family. It is also known as Beihuachuang, Afeimang and Dashijie. It is distributed in regions of China including Guangdong, Guangxi, and Guizhou. It possesses therapeutic effects such as clearing heat and detoxifying, eliminating phlegm and resolving stasis. Traditionally, it has been used to treat diseases like cough and asthma due to lung heat, chronic cough in pulmonary tuberculosis, hemoptysis, and sores and ulcerations[1-4]. In this study, we analyzed and evaluated the *in vitro* antibacterial, antioxidant, and α-glucosidase inhibitory activities of the ethanol total extract of *P. peltata* and its four different polarity fractions (petroleum ether, ethyl acetate, n-butanol, and water). The aim was to screen out the fraction with the strongest activity, thereby providing a reference for the pharmacodynamic research and the study of effective active constituents of *P. peltata*.

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## 2 Materials and methods

**2.1 Materials and reagents** *P. peltata* Hance was purchased from Chenghuang Town, Yulin City of Guangxi.

All chemical reagents, bacterial strains, and instruments used in the experiments were provided by the Teaching Experiment and Training Center of Guangxi University of Chinese Medicine. The main instruments and their manufacturer models are as follows: ultrasonic cleaner (Baoshan Ultrasonic Instrument Co., Ltd.), constant temperature incubator (Shaoguan Taihong Medical Equipment Co., Ltd.), rotary evaporator (EYELA SB-1300), UV-Vis spectrophotometer (Shimadzu, Japan; UV1900), electronic balance (Sartorius Scientific Instruments Co., Ltd., SOP), centrifuge (Hunan Xiangyi Laboratory Instrument Development Co., Ltd., H1650), and high-performance liquid chromatograph (Agilent, USA; 1260).

**2.2 Preparation of polarity-based extract fractions** Dried coarse powder of *P. peltata* (1 500 g) was soaked overnight in 80% ethanol at a solid-to-liquid ratio of 1∶10, followed by ultrasonic extraction for 45 min and filtration. This extraction procedure was repeated three times. The combined filtrates were concentrated and dried under reduced pressure to obtain the total ethanolic extract. A portion of this total ethanolic extract was then successively partitioned three times each with petroleum ether, ethyl acetate, and n-butanol. The remaining aqueous phase was

also collected. Each solvent fraction (petroleum ether, ethyl acetate, n-butanol, and aqueous) was concentrated and dried under reduced pressure separately, yielding four distinct polarity-based extract fractions. The extraction yields for these fractions were 1.61%, 3.15%, 2.09%, and 2.74%, respectively. (Note: The total ethanolic extract yield was 4.97%).

**2.3 Preparation of antibacterial test solution**

**2.3.1** Sample solution. An appropriate amount of each polarity-based extract was accurately weighed and dissolved in 0.5‰ DMSO to prepare sample solutions for antibacterial testing, each with an extract concentration of 516 mg/mL. The prepared solutions were stored for subsequent use.

**2.3.2** Strain solution. *Staphylococcus aureus*, *Salmonella* Paratyphi B, *Staphylococcus epidermidis*, *Pseudomonas aeruginosa*, *Candida albicans*, and *Escherichia coli* were suspended separately in sterile physiological saline and adjusted to a 0.5 McFarland standard. These suspensions were then diluted 1∶100 with sterile Mueller-Hinton broth to obtain bacterial solutions with a concentration of $10^6$ CFU/mL. For Streptococcus pneumoniae, a bacterial solution with a concentration of $10^6$ CFU/mL was prepared using fetal bovine serum.

**2.4 Preparation of *in vitro* antioxidant capacity test solution**

**2.4.1** Sample solution. An appropriate amount of the extract from each polar fraction was precisely weighed and dissolved in 80% ethanol to prepare antioxidant test sample solutions of different polar fractions with concentration gradients of 0.2, 0.4, 0.6, 0.8, and 1.0 mg/mL.

**2.4.2** VitC solution. An appropriate amount of VitC was precisely weighed and dissolved in 80% ethanol to prepare VitC solutions with concentrations of 0.2, 0.4, 0.6, 0.8, and 1.0 mg/mL, which were then stored under refrigeration and protected from light.

**2.5 Preparation of *in vitro* α-glucosidase inhibition test solution**

**2.5.1** PNPG solution. An appropriate amount of PNPG was precisely weighed and dissolved in 0.1 mol/L PBS buffer to prepare a PNPG solution with a concentration of 5.0 mmol/L, which was then stored under refrigeration for subsequent use.

**2.5.2** α-glucosidase solution. An appropriate amount of α-glucosidase lyophilized powder was precisely weighed and dissolved in 0.1 mol/L PBS buffer to prepare an α-glucosidase solution with a concentration of 1 U/mL, which was then stored frozen at −20 ℃ for subsequent use.

**2.5.3** Acarbose solution. An appropriate amount of 20 mg of acarbose reference standard was precisely weighed and dissolved in distilled water to prepare acarbose sample solutions at concentrations of 10, 5, 2.5, 1.25, 0.625, and 0.312 5 mg/mL for subsequent use.

**2.5.4** Test solution. Appropriate amounts of extracts from each polar fraction were precisely weighed and dissolved in distilled water to prepare sample solutions for the *in vitro* α-glucosidase inhibitory assay at concentrations of 4, 2, 1, 0.5, 0.25, and 0.125 mg/mL, which were then stored under refrigeration for subsequent use.

**2.6 Antibacterial test method** Each sample solution prepared in Section **2.3.1** was added to sterile MH broth and subjected to two-fold serial dilution to prepare test sample solutions with extract concentrations of 256, 128, 64, 32, 16, 8, 4, 2, 1, and 0.5 mg/mL. Then, 1.8 mL of each diluted sample solution was mixed with 0.2 mL of a bacterial inoculum at a concentration of $10^6$ CFU/mL. The resulting mixtures served as the antibacterial test groups, which were incubated at 37 ℃ for 24 h. A sterile MH broth was used as the blank control group. The antibacterial test groups were compared with the blank control group. If the antibacterial test solution containing bacteria remained clear, indicating no bacterial growth, it demonstrated that the drug possessed antibacterial activity. The lowest drug concentration at which no bacterial growth was observed entirely was defined as the sensitivity of the bacterial strain to the drug, *i. e.*, the Minimum Inhibitory Concentration (MIC) of the drug.

A 0.1 mL aliquot from the *S. pneumoniae* test groups showing no bacterial growth at and above the observed MIC was transferred onto drug-free nutrient agar plates containing 5% sheep blood. The samples from the test groups of other bacterial species were transferred onto drug-free nutrient agar plates. All plates were then incubated at a constant temperature of 37 ℃ for 24 h. The presence or absence of bacterial growth was observed. The absence of colony growth was defined as the Minimum Bactericidal Concentration (MBC) of the drug[6-9].

**2.7 Method of *in vitro* antioxidant test[10-13]**

**2.7.1** DPPH free radical scavenging test. Separately, 2.0 mL each of the sample solutions from Section **2.4.1** and the VitC solution from Section **2.4.2** were taken. To each, 2.0 mL of a 0.1 mmol/L DPPH solution was added. The mixtures were allowed to react in the dark for 15 min, after which their absorbance values were measured at 520 nm (recorded as $A_{sample}$). For control measurements, the DPPH solution was replaced with 80% ethanol following the same procedure, and the absorbance was measured (recorded as $A_{control}$). For the blank group measurement, the sample solution was replaced with 80% ethanol following the same procedure, and the absorbance was measured (recorded as Ablank). The scavenging rate of DPPH radicals was calculated using the Formula (1):

$$\text{DPPH free radical scavenging rate (\%)} = [1 - (A_{sample} - A_{control})/A_{blank}] \times 100\% \quad (1)$$

**2.7.2** Determination of hydroxyl radical scavenging capacity. A 2.0 mL aliquot was taken from each sample solution in Section **2.4.1** and from the VitC solution in Section **2.4.2**. To each aliquot, 1.0 mL of a 7.5 mmol/L 1,10-phenanthroline solution and 4.0 mL of a phosphate buffer solution (pH 7.4) were added sequentially. After mixing, 2.0 mL of a 7.5 mmol/L ferrous sulfate solution and 0.2 mL of a 0.1% hydrogen peroxide solution were added, and the mixture was vortexed. It was then incubated in a 37 ℃ water bath for 60 min, and the absorbance was measured at

a wavelength of 510 nm (recorded as $A_s$). For measurement $A_b$, the hydrogen peroxide solution was replaced with distilled water following the same procedure. For measurement $A_p$, the sample solution was replaced with 80% ethanol following the same procedure. The hydroxyl radical scavenging rate was calculated using the Formula (2):

Hydroxyl radical scavenging (%) = $(A_s - A_p)/(A_b - A_p) \times 100\%$ (2)

**2.7.3** Determination of superoxide anion radical scavenging activity. A 1.0 mL aliquot was taken from each sample solution in Section **2.4.1** and from the VitC solution, respectively. To each aliquot, 4.5 mL of Tris-HCl buffer solution and 0.5 mL of a pyrogallol solution (with a concentration of 25 mmol/L) were added. The mixture was vortexed, allowed to stand for 5 min, and then 1.0 mL of an 8 mmol/L hydrochloric acid solution was added. The absorbance was measured at a wavelength of 320 nm following the same procedure (recorded as $A$). For the blank test, the sample was replaced with 80% ethanol, and the absorbance was measured in the same manner (recorded as $A_0$). The calculation for the superoxide anion radical scavenging rate uses the Formula (3):

Superoxide anion radical scavenging rate (%) = $(A_0 - A)/A_0 \times 100\%$ (3)

### 2.8 *In vitro* α-glucosidase activity inhibition test[13-15]

**2.8.1** Chromatographic conditions. Chromatographic column: Dalian Elite $C_{18}$ (250 mm × 4.6 mm, 5 μm); mobile phase: 0.1% formic acid solution (A)-acetonitrile (B), with gradient elution (0-8 min, 20%-30% B; 8-13 min, 30%-80% B; 13-18 min, 80%-20% B; 18-28 min, 20% B); flow rate: 1.0 mL/min; detection wavelength: 315 nm; column temperature: 30 ℃; injection volume: 10 μL.

**2.8.2** Linear relationship test. An appropriate amount of PNP was precisely weighed and dissolved in 0.1 mol/L PBS buffer to prepare solutions with concentrations of 0.005, 0.01, 0.05, 0.1, 0.2, 0.4, and 0.8 mmol/L. According to the chromatographic conditions specified in Section **2.8.1**, the peak areas were measured using a high-performance liquid chromatograph. A linear regression was performed with concentration as the $X$-axis and peak area as the $Y$-axis. The resulting linear equation for PNP was $y = 505.93x - 527.47$ ($r^2 = 0.9995$). This linear equation indicates that when the concentration of PNP is within the range of 0.005-0.8 mmol/L, there is a good linear relationship between concentration and peak area.

**2.8.3** Method and calculation of *in vitro* α-glucosidase activity inhibition test. The test sample solutions prepared in Section **2.5.4** were mixed with the respective reaction reagents according to Table 1. After the reaction was completed, the peak area of PNP in each test sample was measured using high-performance liquid chromatography (HPLC). The inhibition rate was then calculated using the Formula (4):

Inhibition rate (%) = $[1 - (B - b)/(A - a)] \times 100\%$ (4)

where $A$ denotes the reactant amount in the negative control group, $a$ represents the reactant amount in the negative blank group, $B$ represents the reactant amount in the sample group, and $b$ represents the reactant amount in the sample blank group.

**Table 1 Addition sequence and amount of reagents for different extract fractions** μL

| Reagent | a | A | b | B | Acarbose blank group (PBS dissolved) | Acarbose group (PBS dissolved) |
|---|---|---|---|---|---|---|
| PBS solution | 270 | 220 | 240 | 190 | 210 | 160 |
| Acarbose solution | 0 | 0 | 0 | 0 | 30 | 30 |
| Inhibitor | 0 | 0 | 30 | 30 | 0 | 0 |
| α-glucosidase | 0 | 50 | 0 | 50 | 0 | 50 |
| DMSO | 0 | 0 | 0 | 0 | 30 | 30 |
| After mixing thoroughly, incubated at 37 ℃ for 20 min | | | | | | |
| PNPG substrate | 50 | 50 | 50 | 50 | 50 | 50 |
| After mixing thoroughly, incubated at 37 ℃ for 30 min | | | | | | |
| SDS | 400 | 400 | 400 | 400 | 400 | 400 |

**NOTE** A is the negative control group, a is the negative blank group, b. is the sample group, and B is the sample blank.

## 3 Results and analysis

**3.1 Results of *in vitro* antibacterial test** The experimental results indicated that the total ethanol extract of *P. peltata* and its four different polar fraction extracts exhibited the most significant antibacterial activity against *S. pneumoniae* among the seven tested bacterial strains. The MIC and MBC values are listed in Table 2. Blank control tests confirmed normal growth of all bacterial strains, demonstrating that the experimental conditions did not interfere with the results.

**3.2 Results of *in vitro* antioxidant test**

**3.2.1** Scavenging ability to DPPH radical. The total ethanol extract of *P. peltata* and its different polar fractions all exhibited weaker DPPH radical scavenging capacity compared to VitC. The order of scavenging activity was as follows: n-butanol fraction > total ethanol extract > ethyl acetate fraction > petroleum ether fraction > aqueous fraction (Table 3).

**3.2.2** Scavenging ability to hydroxyl radical (·OH). The ·OH radical scavenging activity of the total ethanol extract and different polar fractions of *P. peltata* was ranked in the following order: n-butanol fraction > ethyl acetate fraction > total ethanol extract > petroleum ether fraction > aqueous fraction. Among them, the n-butanol fraction exhibited stronger hydroxyl radical scavenging

capacity ($IC_{50}$ = 0.068 ± 0.001) than VitC ($IC_{50}$ = 0.097 ± 0.001), while the ethyl acetate fraction showed comparable scavenging capacity ($IC_{50}$ = 0.096 ± 0.004) to VitC ($IC_{50}$ = 0.097 ± 0.001) (Table 3).

**3.2.3** Scavenging ability to superoxide anion radical ($O_2 \cdot^-$). The total ethanol extract of *P. peltata* and its different polar fractions all exhibited weaker superoxide anion scavenging capacity compared to VitC. The order of scavenging activity was as follows: n-butanol fraction > ethyl acetate fraction > total ethanol extract > petroleum ether fraction > aqueous fraction (Table 3).

**Table 2 MIC and MBC of *Pilea peltata* to 7 strains** mg/mL

| Strain | MIC | | | | | MBC | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | I | II | III | IV | V | I | II | III | IV | V |
| *Staphylococcus aureus* | 8 | 2 | 2 | 4 | 4 | 16 | 8 | 4 | 32 | 128 |
| *Escherichia coli* | 8 | 4 | 8 | 8 | 4 | 32 | 32 | 32 | 16 | 32 |
| *Salmonella* Paratyphi B | 8 | 8 | 8 | 8 | 32 | 32 | 32 | 64 | 8 | 64 |
| *Candida albicans* | 8 | 4 | 4 | 8 | 8 | 16 | 8 | 8 | 32 | 8 |
| *Pseudomonas aeruginosa* | 4 | 4 | 4 | 4 | 8 | 16 | 8 | 8 | 32 | 8 |
| *Staphylococcus epidermidis* | 8 | 2 | 4 | 8 | 128 | 8 | 2 | 4 | 32 | 128 |
| *Streptococcus pneumoniae* | 1 | 1 | 0.5 | 1 | 2 | 4 | 2 | 1 | 4 | 8 |

**NOTE** I. Total ethanol extract, II. Petroleum ether fraction, III. Ethyl acetate fraction, IV. Butanol fraction, V. Water fraction.

**Table 3 Comparison of DPPH, hydroxyl radical and superoxide anion radical scavenging activities of *Pilea peltata*** mg/mL

| Polar fraction | DPPH $IC_{50}$ | · OH $IC_{50}$ | $O_2 \cdot^-$ $IC_{50}$ |
|---|---|---|---|
| Total ethanol | 0.276 ± 0.004# | 0.216 ± 0.009# | 0.164 ± 0.006# |
| Petroleum ether | 0.667 ± 0.032# | 0.260 ± 0.002# | 0.251 ± 0.006# |
| Ethyl acetate | 0.384 ± 0.008# | 0.096 ± 0.004 | 0.166 ± 0.038 |
| N-butanol | 0.209 ± 0.001# | 0.068 ± 0.001# | 0.112 ± 0.006 |
| Water | 0.940 ± 0.032# | 0.442 ± 0.052# | 0.244 ± 0.009# |
| VitC | 0.111 ± 0.001 | 0.097 ± 0.001 | 0.086 ± 0.003 |
| *F* | 831.184 | 128.513 | 49.644 |
| *P* | <0.01 | <0.01 | <0.01 |

**NOTE** # denotes compared with VitC, $P < 0.05$.

### 3.3 Results of *in vitro* α-glucosidase inhibitory activity test

The experimental results revealed that neither the total ethanol extract nor the aqueous fraction of *P. peltata* exhibited *in vitro* α-glucosidase inhibitory activity. In contrast, the petroleum ether fraction, ethyl acetate fraction, and n-butanol fraction all demonstrated inhibitory activity. The order of inhibitory potency was as follows: ethyl acetate fraction > n-butanol fraction > petroleum ether fraction.

The half-maximal inhibitory concentration ($IC_{50}$) of each test sample against α-glucosidase was calculated using SPSS 23.0 software. All data are presented as mean ± SD ($\bar{x} \pm s$). Variance analysis was performed on the results, and a $P < 0.05$ was considered statistically significant. The results showed that the $IC_{50}$ values of both the ethyl acetate fraction and the n-butanol fraction showed a significant difference ($P < 0.01$) compared to the acarbose group. Their *in vitro* α-glucosidase inhibitory activities were significantly stronger than that of the positive control drug, acarbose (Table 4).

**Table 4 The $IC_{50}$ and variance analysis of *in vitro* tests** ($n = 3$)

| Name | $IC_{50}$ // mg/mL | *F* |
|---|---|---|
| Petroleum ether fraction | 0.329 ± 0.107 | 466.794 |
| Ethyl acetate fraction | 0.023 ± 0.004c | |
| N-butanol fraction | 0.075 ± 0.004c | |
| Acarbose | 0.405 ± 0.028 | |

**NOTE** Compared with acarbose group, c denotes $P < 0.01$.

## 4 Conclusions and discussion

In this study, we investigated the *in vitro* antibacterial and antioxidant activities, as well as the α-glucosidase inhibitory activity, of the total ethanol extract of *P. peltata* and its four different polar fractions (n-butanol, ethyl acetate, petroleum ether, and aqueous fractions). The active fractions were thereby identified. The results revealed that the n-butanol and ethyl acetate fractions exhibited significant *in vitro* antibacterial and antioxidant capacities, along with notable α-glucosidase inhibitory activity. This may be attributed to a higher concentration of effective active constituents in these fractions, though the specific details require further investigation. We found that the total ethanol extract of *P. peltata* and its four different polar fractions exhibited the most significant antibacterial effect against *S. pneumoniae* among the seven tested bacterial strains. Among these, the ethyl acetate fraction showed the strongest activity, with MIC and MBC values as low as 0.5 and 1 mg/mL, respectively. These results are consistent with the traditional therapeutic effects of *P. peltata* and may provide more options for the prevention and treatment of *S. pneumoniae* infections. It was also observed that the n-butanol fraction demonstrated notable scavenging activity against DPPH radicals, hydroxyl radicals, and superoxide anions, laying a foundation for further research into the antioxidant properties of *P. peltata*. Additionally, the ethyl acetate and n-butanol fractions exhibited stronger *in vitro* α-glucosidase inhibitory activity than acarbose, suggesting potential alternatives for research and applications targeting α-glucosidase inhibition.

The genus *Pilea* (Urticaceae) has evolved into a large genus comprising over 700 species due to its complex and diverse evolutionary pathways and morphological expressions. Currently, there are relatively few research reports on plants within this genus. *P. peltata* is a variety of the *Pilea* genus, widely distributed in Guangxi Zhuang Autonomous Region with a long history of use and well-documented efficacy. Research, development, and application of this plant hold significant potential. The findings of this

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derlie the traditional heat-clearing and analgesic properties attributed to *L. foenum-graecum*. Furthermore, these findings provide a foundational basis for subsequent investigations into the plant's bioactivity.

Principal component analysis demonstrated that the first two principal components, accounting for a cumulative contribution rate of 89.45%, effectively differentiated subtle variations in the chemical characteristics of samples from diverse locations. Furthermore, in conjunction with load matrix analysis, future studies should prioritize chromatographic peaks that contribute significantly to the principal components. These peaks may serve as key markers for characterizing quality differences in *L. foenum-graecum*.

The primary limitation of this study is the relatively small sample size, which is confined to the Guangxi production area. Future research should consider expanding the sample collection scope and integrating content determination, chemometric analysis, and pharmacodynamic correlation analysis. Such an approach would facilitate a more comprehensive exploration of the intrinsic relationship between fingerprint profiles and the quality of medicinal materials, thereby contributing to the enhancement of quality standards for *L. foenum-graecum*.

This study successfully developed a stable, reliable, and specific HPLC fingerprint analysis method for Zhuang medicine *L. foenum-graecum*. Similarity evaluation confirmed that the overall chemical composition of *L. foenum-graecum* from different locations was consistent. Principal component analysis further elucidated the chemical variations present within the samples. The integration of fingerprinting with multimodal chemical analysis offers a more comprehensive and scientific approach to evaluating the quality of *L. foenum-graecum* medicinal materials, thereby providing essential technical support and data references for the future standardization and industrial development of this distinctive Zhuang medicinal resource.

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study are expected to provide an experimental basis for further investigation into the active components and pharmacological effects of *P. peltata*, and may offer valuable insights for the research and development of ethnomedicines.

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