 DOI: 10.19600/j.cnki.issn2152 - 3924.2026.01.001

Content Determination of Zhuang Medicine *Sauropus spatulifolius* Beille from Guangxi

Lixin SUN[1△], Yongshan JIN[2△], Wenjing YANG[1△], Aipeng LI[1], Yarong WEI[2], Liangna SU[3], Chengxu CHEN[2], Fangyuan SHEN[2], Yanmin LU[2], Suoyi HUANG[2*], Lixiang LU[3*]

1. School of Basic Medical Sciences, Youjiang Medical University for Nationalities, Baise 533000, China; 2. School of Pharmacy, Youjiang Medical University for Nationalities, Baise 533000, China; 3. School of Clinical Medicine, Youjiang Medical University for Nationalities, Baise 533000, China

Abstract [**Objectives**] To determine the content of Zhuang medicine *Sauropus spatulifolius* Beille from Guangxi. [**Methods**] The amino acid content of *S. spatulifolius* Beille was determined by ultraviolet spectrophotometry (UVs). The content of kaempferol-3-O-gentiobioside in *S. spatulifolius* Beille was determined by liquid chromatography-mass spectrometry (LC-MS). Pesticide residues in *S. spatulifolius* Beille were detected by gas chromatography-mass spectrometry (GC-MS). Heavy metal elements arsenic (As), cadmium (Cd), and lead (Pb) in *S. spatulifolius* Beille were detected by inductively coupled plasma mass spectrometry (ICP-MS). [**Results**] The amino acid content in *S. spatulifolius* Beille was 3.233 mg/g, with a relative standard deviation (*RSD*) of 0.36%. The content of kaempferol-3-O-gentiobioside was 1.15 μg/mL. No pesticide residues or heavy metals were detected in the *S. spatulifolius* Beille medicinal material. [**Conclusions**] This study improves the quality control system for *S. spatulifolius* Beille and provides a reference basis for the quality standard control of Zhuang medicine *S. spatulifolius* Beille from Guangxi.

Key words Zhuang medicine, *Sauropus spatulifolius* Beille, Liquid chromatography-mass spectrometry (LC-MS), Gas chromatography-mass spectrometry (GC-MS), Inductively coupled plasma mass spectrometry (ICP-MS), Content determination

1 Introduction

Sauropus spatulifolius Beille, also known as Longliye, Longganye, Longsheye, Longweiye, and Niuerye, belongs to the genus *Sauropus* in the Euphorbiaceae family. It is an important ethnic medicinal material and traditional dietary therapy material in the Lingnan region[1]. Native to Sumatra Island, Indonesia, it is cultivated in Guangdong, Guangxi, Yunnan, Fujian, Hainan, and other regions, with the most cultivation in Guangdong and Guangxi. *S. spatulifolius* Beille contains chemical components including sugars, polysaccharides, glycosides, saponins, tannins, organic acids, alkaloids, flavonoids, anthraquinones, lactones, oils, volatile oils, *etc.*[2]. In traditional Chinese medicine (TCM), its leaves are used medicinally for clearing heat and moistening the lungs, resolving phlegm and relieving cough. It is mainly used to treat lung heat with excessive phlegm and cough, constipation, dry mouth syndrome, ear, nose, and throat diseases, *etc.*[3].

S. spatulifolius Beille is also a cultivated medicinal herb, often planted around houses and in rural vegetable plots. Farmers in the Lingnan region commonly use it to make soup with pork. This dietary therapy has the effects of clearing heat, moistening the lungs, relieving cough, and resolving phlegm[4]. Currently, domestic and foreign scholars have conducted some research on the chemical constituents[5-6], microscopic identification[7-8], pharmacological effects[9-10], and fingerprinting[11-12] of *S. spatulifolius* Beille, and certain progress has been made. The results indicate that *S. spatulifolius* Beille possess anti-inflammatory[13], antitussive (cough-relieving)[4, 14], antibacterial[15], antitumor[16], and anti-allergic[17] effects.

This paper determined the amino acid content and kaempferol-3-O-gentiobioside content in *S. spatulifolius* Beille, as well as pesticide residues in *S. spatulifolius* Beille; it also detected the heavy metal elements arsenic (As), cadmium (Cd), and lead (Pb) in *S. spatulifolius* Beille. The quality of *S. spatulifolius* Beille medicinal material was further investigated, hoping to provide a reference for its quality standards.

2 Materials

2.1 Medicinal materials *S. spatulifolius* Beille was purchased from the Chinese Medicinal Materials Market in Yulin City, Guangxi. The medicinal materials were identified by Chief TCM Pharmacist Zhou Shiyou from the Department of Pharmacy, School of Clinical Medicine, Youjiang Medical University for Nationalities, as the leaves of *S. spatulifolius* Beille (Euphorbiaceae).

2.2 Reagents Arsenic standard (Batch No.: 20180821), Lead standard (Batch No.: 20180420), Chromium standard (Batch No.: 20181105), Quintozene (PCNB) standard (Batch No.: 18001), alpha-BHC standard (Batch No.: 18002), beta-BHC standard (Batch No.: 18002), gamma-BHC (Lindane) standard (Batch No.: 18002), delta-BHC standard (Batch No.: 19001), p, p'-DDE standard (Batch No.: 18001), p, p'-DDD standard (Batch No.: 18001), o, p'-DDT standard (Batch No.: 18001), p, p'-DDT standard (Batch No.: 18001).

Received: July 13, 2025 Accepted: October 30, 2025

Supported by Guangxi Key Research and Development Program Project (GuiKe AB18221095); Open Fund Project of Guangxi Key Laboratory of Research on Ethnic Medicinal Plants in the Youjiang River Basin (yykf2024-01); High-level Talent Research Project of Youjiang Medical University for Nationalities (1002018079); 2023 National-level College Student Innovation and Entrepreneurship Training Program Project (202310599008).

△These authors contributed equally to this study.

*Corresponding authors. Suoyi HUANG, bachelor's degree, Professor, Level 2; Lixiang LU, bachelor's degree, associate chief pharmacist.

All other reagents used in this experiment were of chromatographic grade.

2.3 Instruments FA1204B Electronic Balance (Shanghai Tianmei Tianping Instrument Co., Ltd.), HC-5002S CNC Benchtop Ultrasonic Cleaner, DL-1 Universal Electric Furnace (Beijing Yongguangming Medical Instrument Co., Ltd.), DHG-9070A Electric Thermostatic Blast Drying Oven (Shanghai Jinghong Laboratory Equipment Co., Ltd.), Shimadzu GCMS-TQ8040 (Triple Quadrupole Gas Chromatograph-Mass Spectrometer, Shimadzu Corporation, Japan), Xiangyi H1650 Benchtop High-Speed Centrifuge, Agilent 1290 Infinity II UPLC-QTOF 6550 (Quadrupole Time-of-Flight Liquid Chromatograph-Mass Spectrometer, Agilent Technologies, USA), PerkinElmer NexION 300X[Inductively Coupled Plasma Mass Spectrometer (ICP-MS), PerkinElmer Inc., USA], CEM DISCOVER SP-D Single-Mode Microwave Digester (CEM Corporation, USA).

3 Experimental methods and results

3.1 Determination of amino acid content

3.1.1 Preparation of test and reference solutions. *S. spatulifolius* Beille powder (4.051 g) was accurately weighed and placed in a 250 mL stoppered conical flask. Purified water (100.0 mL) was added, and the mixture was soaked for 1 h. After weighing, reflux extraction was performed for 2 h. The mixture was cooled to room temperature, weighed again, and the lost weight was replenished with purified water. The mixture was shaken well, allowed to stand, and filtered through a 0.45 μm microporous membrane to obtain the test stock solution. The test stock solution (5.0 mL) was precisely measured, placed in a 50 mL volumetric flask, and diluted to volume with purified water to obtain the test solution. L-Glutamic acid (0.025 g) was accurately weighed, placed in a 25 mL volumetric flask, dissolved, and diluted to volume with purified water to obtain the reference stock solution. The reference stock solution (5.0 mL) was precisely measured, placed in a 50 mL volumetric flask, and diluted to volume with purified water to obtain the reference solution.

3.1.2 Selection of wavelength. The *S. spatulifolius* Beille test solution and the glutamic acid reference solution (1.0 mL each) were precisely pipetted into separate 10 mL test tubes. pH 6.0 buffer solution (1 mL) and 2% ninhydrin solution (5.0 mL) were added sequentially. The mixtures were shaken well, incubated in a water bath until color development occurred, and then cooled to room temperature. Using purified water as the blank reference, the absorption curves were scanned in the range of 400 – 800 nm by ultraviolet spectrophotometry. The maximum absorption wavelength for both the *S. spatulifolius* Beille test solution and the glutamic acid reference solution was found to be 566.5 nm. Therefore, 566.5 nm was determined as the detection wavelength.

3.1.3 Linearity test. Glutamic acid reference solution (2.0, 3.0, 4.0, 5.0, 6.0, and 7.0 mL) was precisely pipetted into separate 10 mL volumetric flasks and diluted to the mark with purified water. From each flask, 1.0 mL of solution was taken and placed in separate 10 mL test tubes. pH 6.0 buffer solution (1.0 mL) and 2% ninhydrin solution (5.0 mL) were added sequentially. The mixtures were shaken well, incubated in a water bath until color development occurred, and then cooled to room temperature. The absorbance was measured at 566.5 nm using purified water as the blank reference. A standard working curve was plotted with glutamic acid mass concentration as the abscissa (x-axis) and absorbance as the ordinate (y-axis). The regression equation was: $y = 0.010\ 3x + 0.260\ 1$ ($R^2 = 0.998\ 9$). The results indicated a good linear relationship between glutamic acid concentration and absorbance within the range of 20.0 – 70.0 μg/mL.

3.1.4 Determination of total amino acid content. *S. spatulifolius* Beille powder (4.081 g) was accurately weighed, and the test was prepared according to the method described in Section **3.1.1**. The test solution (1.0 mL) was precisely pipetted into a 10 mL test tube and shaken well. After color development according to the method described in Section **3.1.3**, the absorbance was measured at 566.5 nm using the reagent blank as reference. The measurement was performed in triplicate. The amino acid content in *S. spatulifolius* Beille (calculated as glutamic acid) was calculated. The average amino acid content in *S. spatulifolius* Beille was 3.233 mg/g, with an *RSD* of 0.36%.

3.1.5 Test grouping and result analysis. (i) Repeatability Test: Six portions of *S. spatulifolius* Beille powder (approximately 4.00 g each) were taken. The test solution was prepared for each portion according to the method described in Section **3.1.1**. From each test solution, 1.0 mL was precisely pipetted into separate 10 mL test tubes, shaken well, and subjected to color development according to the method in Section **3.1.3**. The absorbance was measured at 566.5 nm using the reagent blank as reference. The *RSD* was 0.64% ($n = 6$), indicating good repeatability.

(ii) Stability Test: *S. spatulifolius* Beille powder (4.021 g) was accurately weighed, and the test solution was prepared according to the method described in Section **3.1.1**. The test solution (1.0 mL) was precisely pipetted into a 10 mL test tube, shaken well, and subjected to color development according to the method in Section **3.1.3**. Using the reagent blank as reference, the absorbance was measured at 566.5 nm at 0, 0.5, 1.0, 1.5, and 2.0 h. The *RSD* was 0.40% ($n = 5$), indicating good stability within 2 h.

(iii) Precision Test: *S. spatulifolius* Beille powder (4.003 g) was accurately weighed, and the test solution was prepared according to the method described in Section **3.1.1**. The test solution (1.0 mL) was precisely pipetted into a 10 mL test tube, shaken well, and subjected to color development according to the method in Section **3.1.3**. Using the reagent blank as reference, the absorbance was measured at 566.5 nm six times consecutively. The *RSD* was 0.94% ($n = 6$), indicating good instrument precision.

3.2 Determination of kaempferol-3-O-gentiobioside content

3.2.1 Chromatographic conditions. Mobile phase A: 0.1% formic acid aqueous solution; Mobile phase B: Acetonitrile solution; Column: Waters BEH C_{18} 2.1 mm × 100 mm 1.7 μm; Flow rate:

0.5 mL/min; Injection volume: 20 μL. The mobile phase gradient program is shown in Table 1.

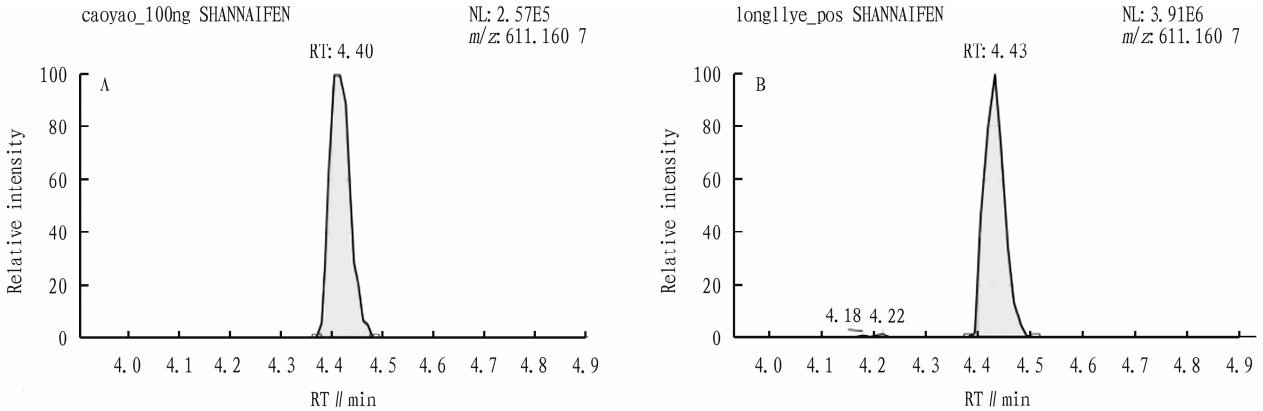
Table 1 LC-MS mobile phase gradient program

Time//min	Phase A proportion//%	Phase B proportion//%
0	90	10
2	90	10
8	60	40
18	2	98
20	2	98
20.1	90	10

3.2.2 Sample pretreatment. *S. spatulifolius* Beille powder (0.5 g) was placed in a 10 mL test tube. Methanol (10 mL) was added for dissolution. The mixture was ultrasonicated at 25 ℃ for 60 min, then taken out and allowed to stand for 5 min. The supernatant (1 mL) was taken and placed in a 2 mL centrifuge tube, centrifuged at 13 000 r/min for 10 min, passed through a 0.22 μm filter membrane, and transferred to a 1.5 mL autosampler vial to obtain the test extract. It was stored at 4 ℃, taken out before analysis, and the storage time did not exceed 24 h. An appropriate amount of kaempferol-3-O-gentiobioside was taken, and the reference solution was prepared according to the above conditions.

3.2.3 Sample content determination. *S. spatulifolius* Beille powder was taken, and the test solution was prepared according to the method described in Section **3.2.2**. The content was determined by injecting 100 μL using a microsyringe. The results showed that the content of kaempferol-3-O-gentiobioside was 1.15 μg/mL.

3.2.4 Chromatograms. The liquid chromatography-mass spectrometry (LC-MS) chromatograms are shown in Fig. 1.



NOTE A. Kaempferol-3-O-gentiobioside reference; B. *Sauropus spatulifolius* Beille sample.

Fig. 1 Sample and reference chromatograms

3.3 Pesticide residue detection

3.3.1 Chromatographic conditions. Injector temperature: 250 ℃; Ion source temperature: 200 ℃; Interface temperature: 200 ℃; Flow rate: 1 mL/min; Column model: SH-Rxi-5Sil MS, 30 m × 0.25 mm × 0.25 μm.

3.3.2 Sample pretreatment. *S. spatulifolius* Beille powder (0.5 g) was placed in a 10 mL test tube. Methanol (10 mL) was added for dissolution. The mixture was ultrasonicated at 25 ℃ for 60 min, then taken out and allowed to stand for 5 min. The supernatant (1 mL) was taken and placed in a 2 mL centrifuge tube, centrifuged at 13 000 r/min for 10 min, passed through a 0.22 μm filter membrane, and transferred to a 1.5 mL autosampler vial to obtain the sample extract. A blank control sample was prepared under the same conditions. Samples were stored at 4 ℃, taken out before analysis, and the storage time did not exceed 24 h. Standard curves were constructed by plotting the peak area of the reference standard (y-axis) against the reference standard concentration (ppm, x-axis), as shown in Table 2.

Table 2 Pesticide residue detection standard curves

Reference standard	Standard curve	Correlation coefficient (r)	LOD//mg/kg
alpha-BHC	$y = 1\,039.749\,6 + 734.723\,98x$	0.998 97	0.1
beta-BHC	$y = 1\,218.971\,93 + 5\,200.015\,51x$	0.998 57	0.1
gamma-BHC(Lindane)	$y = 1\,333.785\,48 + 4\,429.289\,79x$	0.997 82	0.1
delta-BHC	$y = 1\,357.352\,13 + 4\,770.320\,269x$	0.998 39	0.1
p, p'-DDE	$y = 4\,837.509\,34 + 15\,354.083\,24x$	0.998 50	0.1
p, p'-DDD	$y = 1\,613.825\,95 + 24\,676.228\,76x$	0.999 94	0.1
o, p'-DDT	$y = 3\,594.227\,76 + 31\,042.084\,11x$	0.998 89	0.1
p, p'-DDT	$y = 2\,384.895\,61 + 24\,582.378\,12x$	0.999 08	0.1

3.3.3 Pesticide residue detection results. As shown in Table 3, no pesticide residues were detected in any of the samples.

Table 3 Pesticide residue detection results in *Sauropus spatulifolius* Beille medicinal material

Pesticide residue name	Content	LOD	Result
alpha-BHC	mg/kg	0.1	Not detected
beta-BHC	mg/kg	0.1	Not detected
gamma-BHC (Lindane)	mg/kg	0.1	Not detected
delta-BHC	mg/kg	0.1	Not detected
p, p'-DDE	mg/kg	0.1	Not detected
p, p'-DDD	mg/kg	0.1	Not detected
o, p'-DDT	mg/kg	0.1	Not detected
p, p'-DDT	mg/kg	0.1	Not detected
Qμintozene	mg/kg	0.1	Not detected

3.4 Detection of heavy metals (As, Cd, Pb) *S. spatulifolius* Beille powder (0.10 g) was accurately weighed and placed in a quartz digestion tube. Nitric acid (20.0 mL) and hydrogen peroxide (8.0 mL) were added. The mixture was digested in a digester until the solution became clear and transparent, free of any insoluble matter. After filtration, the solution was transferred to a 50.0 mL volumetric flask and diluted to volume. It was then tested by inductively coupled plasma mass spectrometry (ICP-MS). During ICP-MS testing, the standard curve was tested first, followed by the samples. The arsenic content results in the samples could be directly generated by the instrument without substituting into the standard curve for calculation.

The test results are shown in Table 4. No heavy metals were detected in any of the samples.

Table 4 Heavy metal determination results in *Sauropus spatulifolius* Beille medicinal material

Tested component	Content	LOD	Result
As	mg/kg	1	Not detected
Cd	mg/kg	1	Not detected
Pb	mg/kg	1	Not detected

4 Discussion

This experiment evaluated the pharmaceutical properties and quality of *S. spatulifolius* Beille medicinal material by detecting its active components. Using L-glutamic acid as the reference standard, the total amino acid content in *S. spatulifolius* Beille medicinal material was determined. Using kaempferol-3-O-gentiobioside as the reference standard, the fingerprint chromatogram of *S. spatulifolius* Beille was studied. Additionally, pesticide residues in *S. spatulifolius* Beille were detected by gas chromatography in this experiment, and heavy metal elements such as As, Cd, and Pb were detected by inductively coupled plasma mass spectrometry (ICP-MS). This method has advantages such as low detection limits, high accuracy, and simple operation. According to relevant international and domestic standards, this batch of *S. spatulifolius* Beille medicinal material is of excellent quality and safe for use; the experimental results of this study provide a reference for improving the quality standards of Zhuang medicine *S. spatulifolius* Beille medicinal material from Guangxi, and provide a theoretical basis for the quality control of *S. spatulifolius* Beille medicinal material.

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