Extraction of Polyphenols from Semiliquidambar cathayensis and Their Antioxidant and Antibacterial Activities: Postprint

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Abstract

Abstract: To further develop and utilize Jinlu Banfenghe, sonication time, liquid-to-material ratio, ethanol volume fraction, and sonication temperature were selected as investigation factors, with the total polyphenol extraction yield from Jinlu Banfenghe as the evaluation index. Single-factor and orthogonal experiments were employed to optimize the extraction process and determine the optimal conditions, which were then used to prepare Jinlu Banfenghe polyphenols. The antioxidant capacity of Jinlu Banfenghe polyphenols was evaluated using DPPH · and hydroxyl radical scavenging assays. Antibacterial activity was assessed using the Oxford cup and broth dilution methods, with inhibition zone diameter and minimum inhibitory concentration (MIC) as indicators. The results showed that the optimal extraction conditions were: ethanol concentration 70%, material-to-liquid ratio 1:16, extraction time 60 min, and extraction temperature 60 °C. The IC50 value for DPPH · radical scavenging by Jinlu Banfenghe polyphenols was 5.22 μg · mL-1, which showed no significant difference from the IC50 value of 4.31 μg·mL-1 for the positive control vitamin C. The IC50 value for hydroxyl radical scavenging was 105 μg · mL-1, which was significantly lower than the IC50 value of 180 μg·mL-1 for vitamin C. The polyphenols exhibited inhibitory effects against Staphylococcus aureus and Escherichia coli, with inhibition zone diameters of (13.7 ± 1.2) mm and (10.0 ± 1.3) mm, and MIC values of 0.393 and 0.785 mg · ml-1, respectively. These results demonstrate that Jinlu Banfenghe polyphenols possess both antibacterial and significant antioxidant activities, providing a theoretical basis for the development and utilization of Jinlu Banfenghe resources.

Full Text

Extraction and Antioxidant/Antibacterial Activities of Polyphenols from *Semiliquidambar cathayensis*

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Abstract

To further develop and utilize *Semiliquidambar cathayensis*, this study investigated ultrasonic extraction parameters including extraction time, liquid-to-solid ratio, ethanol concentration, and extraction temperature, using total polyphenol extraction yield as the evaluation metric. Single-factor and orthogonal experiments were employed to optimize the extraction process and determine optimal conditions for preparing *S. cathayensis* polyphenols. The antioxidant capacity was evaluated using DPPH · and hydroxyl radical scavenging assays, while antibacterial activity was assessed through inhibition zone measurements and minimum inhibitory concentration (MIC) determination using the Oxford cup and broth dilution methods. The results demonstrated that the optimal extraction conditions were 70% ethanol concentration, 1:16 solid-to-liquid ratio, 60 min extraction time, and 60 ℃ extraction temperature. The IC value for DPPH · radical scavenging by *S. cathayensis* polyphenols was 5.22 μg · mL ^{1}, showing no significant difference from the positive control vitamin C (IC = 4.31 μg · mL ^{1}). The hydroxyl radical scavenging IC was 105 μg · mL ^{1}, significantly lower than vitamin C' s IC of 180 μg · mL ^{1}. The polyphenols exhibited inhibitory effects against *Staphylococcus aureus* and *Escherichia coli* with inhibition zone diameters of (13.7 ± 1.2) mm and (10.0 ± 1.3) mm, respectively, and MIC values of 0.393 mg · mL ^{1} and 0.785 mg · mL ^{1}. These findings indicate that *S. cathayensis* polyphenols possess significant antioxidant and antibacterial

activities, providing a theoretical basis for the development and utilization of this resource.

Keywords: *Semiliquidambar cathayensis*, polyphenols, antioxidant capacity, antibacterial activity, DPPH·, hydroxyl radical

Introduction

Semiliquidambar cathayensis, also known as "Ban Feng He," belongs to the Hamamelidaceae family and is distributed in Jiangxi, Guizhou, Guangdong, Hainan, and Guangxi provinces [?, ?, ?, ?, ?]. Documented in the *National Compilation of Chinese Herbal Medicine* and *Chinese Ethnic Medicine Records*, its roots, stems, and leaves are used medicinally with sweet, bland, and warm properties, traditionally employed to dispel wind-dampness, activate blood circulation, and reduce swelling. In Guangxi folk medicine, it is used for rheumatism, lumbar muscle strain, and traumatic injuries [?, ?, ?, ?, ?]. Recent years have witnessed increasing research attention on *S. cathayensis* due to its unique clinical efficacy. As early as 1999, Yang et al. discovered that ethanol extracts of *S. cathayensis* root possessed analgesic and anti-inflammatory effects [?, ?, ?, ?, ?]. In 2002, Zhou et al. isolated and identified nine compounds from the ethyl acetate fraction of the root [?, ?, ?, ?, ?]. Subsequent studies reported chemical constituents from petroleum ether and ethyl acetate fractions [?, ?, ?, ?, ?]. More recently, Wei et al. (2017) developed an HPLC method using trans-resveratrol glucoside as a standard to determine total polyphenol content, optimizing extraction through various ultrasonic and reflux methods with different solvents, achieving a maximum extraction yield of 2.67% [?, ?, ?, ?, ?].

Polyphenols are plant secondary metabolites widely distributed in roots, bark, stems, flowers, leaves, and fruits. Numerous studies have demonstrated that polyphenols exhibit antioxidant, antibacterial, and antiviral bioactivities [?, ?, ?, ?, ?]. Currently, food and drug safety issues have become a major global concern. Chemical synthetic antioxidants used in the market may cause liver damage and even induce cancer [?, ?, ?, ?, ?, ?, ?, ?, ?]. Natural antioxidants are therefore highly favored due to their safety profile, making the search for natural antioxidants a research hotspot worldwide [?, ?, ?, ?, ?, ?, ?, ?, ?].

Semiliquidambar cathayensis is used in Guangxi folk medicine for rheumatism and inflammation, conditions closely associated with excessive free radicals in the body [?, ?, ?, ?, ?]. The herb is also used for traumatic injuries, and medications for such injuries typically control wound infection. Therefore, we hypothesized that *S. cathayensis* contains antioxidant and antibacterial components. To date, no systematic studies on the antibacterial and antioxidant properties of *S. cathayensis* polyphenols have been reported. This study employed gallic acid as an evaluation standard, optimized ultrasonic extraction conditions through orthogonal experiments, evaluated antioxidant capacity using 1,1-diphenyl-2-picrylhydrazyl (DPPH·) and hydroxyl radical scavenging assays, and investigated antibacterial activity through inhibition zone and MIC measurements,

providing valuable data for the medicinal application of *S. cathayensis*.

Materials and Methods

1.1 Materials and Reagents

1.1.1 Plant Material *Semiliquidambar cathayensis* was collected in 2016 from Wuxuan, Guangxi, and identified by Associate Professor DAI Yue from Hubei University of Education.

1.1.2 Instruments and Reagents **Instruments:** T6 New Century UV-Vis spectrophotometer; RE-52AA rotary evaporator; Oxford cups (8 mm × 6 mm × 100 mm); SW-CJ-2F superclean bench; VGT-1990QTD ultrasonic cleaner; SPX-60BSH-II biochemical incubator.

Reagents: Gallic acid reference standard was purchased from J&K Scientific; DPPH · from Sigma-Aldrich; *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 6538 from Guangdong Microbial Analysis and Testing Center; MH broth (028040) and MH agar medium (028050) from Guangdong Huankai Microbial Technology. All other reagents were analytically pure.

1.2 Methods

1.2.1 Preparation of Gallic Acid Standard Curve Total polyphenol content in *S. cathayensis* was determined using a slightly modified Folin-Ciocalteu colorimetric method [**?**, **?**, **?**, **?**, **?**] to establish the gallic acid standard curve.

1.2.2 Preparation of Test Sample Solutions Fresh *S. cathayensis* branches were air-dried, pulverized, and stored for use. Exactly 1.0 g of sample was weighed into a 50 mL round-bottom flask, mixed with a specific ratio of ethanol solution, and subjected to ultrasonic extraction. The mixture was centrifuged at 4000 × g for 5 min, and the supernatant was transferred to a 100 mL volumetric flask and diluted to volume with ethanol. Absorbance was measured at 750 nm using a blank control as reference, and polyphenol content was calculated from the standard curve regression equation.

1.2.3 Single-Factor Experiments Single-factor experiments were conducted to evaluate the effects of four parameters—ultrasonic extraction time, liquid-to-solid ratio, ethanol volume fraction, and ultrasonic temperature—on extraction yield and total polyphenol content.

1.2.4 Orthogonal Experimental Design Using total polyphenol content as the evaluation index, orthogonal experiments were designed with extraction time, liquid-to-solid ratio, ethanol volume fraction, and extraction temperature as factors. The orthogonal experimental design is shown in Table 1 .

1.2.5 DPPH · Radical Scavenging Activity Assay Using a modified method [?, ?, ?, ?, ?], DPPH · solution (1.0×10 mol · L 1) prepared in anhydrous ethanol was added to *S. cathayensis* polyphenol samples of various concentrations. After standing at room temperature for 20 min, vitamin C served as the positive control, and absorbance was measured at 517 nm. Each sample was tested in triplicate, and the DPPH · radical scavenging rate was calculated according to Equation (2).

Where A is the absorbance at 517 nm of 1.0 mL DPPH · solution (1.0×10 mol · L 1) mixed with 1.0 mL anhydrous ethanol; A is the absorbance at 517 nm after mixing 1.0 mL of sample or vitamin C solution of different concentrations with 1.0 mL DPPH · solution (1.0×10 mol · L 1), reacting at room temperature in darkness for 20 min, using anhydrous ethanol as blank; and A is the absorbance at 517 nm of 1.0 mL anhydrous ethanol mixed with 1.0 mL sample solution.

1.2.6 Hydroxyl Radical Scavenging Activity Assay The hydroxyl radical scavenging capacity was determined according to the method of Zhu et al. [?, ?, ?, ?, ?]. Briefly, 1.0 mL of 0.75 mmol · L 1 o-phenanthroline solution, 2.0 mL PBS, and 1 mL distilled water were sequentially added to a test tube and mixed. Then 1.0 mL of 0.75 mmol · L 1 ferrous sulfate solution and 1.0 mL of 0.01% hydrogen peroxide were added. After incubating at 37 ℃ for 60 min, the absorbance was measured at 510 nm (A). Using 1.0 mL distilled water instead of hydrogen peroxide gave absorbance A_B. Replacing distilled water with 1.0 mL of different concentrations of *S. cathayensis* polyphenol sample solution gave absorbance A_s. The hydroxyl radical scavenging rate was calculated using the following formula.

1.2.7 Determination of Inhibition Zone The inhibition zone assay was performed according to Zhang et al. [?, ?, ?, ?]. Sterilized MH medium was poured into sterile petri dishes under aseptic conditions. After solidification, 0.1 mL bacterial suspension was evenly spread. Six Oxford cups were placed in each dish, with 100 L of sample added to each cup. Experimental groups included *S. cathayensis* polyphenols, 0.1 mg · mL 1 streptomycin as positive control, and DMSO solution as negative control. Plates were incubated at 37 ℃ for 24 h, and inhibition zones were measured. Each experiment was repeated three times.

1.2.8 Determination of MIC MIC was determined by broth dilution method [?, ?, ?, ?, ?]. *S. cathayensis* polyphenol solutions and liquid medium were prepared in test tubes at concentrations of 25.0, 12.5, 6.25, 3.13, 1.57, 0.785, 0.393, 0.197, and 0 mg · mL 1. Each tube received 100 L bacterial suspension and was incubated at 37 ℃ for 18 h. When both positive and negative controls met specifications, the lowest drug concentration showing no visible turbidity was recorded as the MIC. Each experiment was repeated three times.

Results

2.1 Gallic Acid Standard Curve

The absorbance of gallic acid at various concentrations was measured using a UV-Vis spectrophotometer to establish the standard curve (Figure 1 [Figure 1: see original paper]). The regression equation was y = 0.1141x - 0.0013 with R^2 = 0.9998, indicating good linearity between gallic acid concentration and absorbance in the range of 0–5.05 $\mu g \cdot mL^{-1}$.

2.2 Effect of Ethanol Concentration on Total Polyphenol Extraction Yield

As shown in Figure 2 [Figure 2: see original paper], the extraction yield of total polyphenols from *S. cathayensis* initially increased then decreased with increasing ethanol concentration. The maximum yield was achieved at 60% ethanol, with a sharp decline observed above 80% ethanol. Polyphenols contain phenolic hydroxyl groups that confer polarity. According to the "like dissolves like" principle, maximum solubility occurs when the polarity of 60% ethanol matches that of *S. cathayensis* polyphenols, resulting in the highest extraction efficiency.

2.3 Effect of Liquid-to-Solid Ratio on Total Polyphenol Extraction Yield

While maintaining constant sample mass, increasing extraction solvent volume led to an initial increase followed by a decrease in polyphenol yield. The yield increased slowly starting at a 1:14 ratio and declined after 1:20 (Figure 3 [Figure 3: see original paper]).

2.4 Effect of Ultrasonic Extraction Time on Total Polyphenol Extraction Yield

The extraction yield of total polyphenols increased initially then decreased with prolonged extraction time, peaking at 50 min (Figure 4 [Figure 4: see original paper]). Initially, polyphenols dissolved readily into the extraction solvent. However, with extended ultrasonic time, other compounds also extracted more efficiently, increasing solution saturation and limiting polyphenol dissolution. Additionally, the ultrasonic process may degrade polyphenol chemical structures, reducing extraction yield.

2.5 Effect of Extraction Temperature on Total Polyphenol Extraction Yield

Within the range of 30–55 ℃, polyphenol extraction yield increased with temperature (Figure 5 [Figure 5: see original paper]), indicating that elevated temperature facilitated polyphenol dissolution. However, yields decreased significantly

above 55 ℃, likely due to polyphenol oxidation and degradation at higher temperatures.

2.6 Orthogonal Experimental Optimization

Based on single-factor results, significant levels of the influencing factors were selected for orthogonal experiments. Range analysis was performed to determine optimal conditions using an L (3) orthogonal factor level table (Table 1). The experimental results are presented in Table 2 .

The range (R) values in Table 2 indicate the significance of factors affecting total polyphenol extraction yield, in descending order: extraction temperature > extraction time > liquid-to-solid ratio > ethanol concentration. The optimal combination for ultrasonic extraction of *S. cathayensis* polyphenols was A B C D , corresponding to 70% ethanol concentration, 1:16 solid-to-liquid ratio, 60 min extraction time, and 60 ℃ extraction temperature.

2.7 DPPH·Radical Scavenging Activity of *S. cathayensis* Polyphenols

As shown in Figure 6 [Figure 6: see original paper] and Table 3 , both *S. cathayensis* polyphenols and the positive control vitamin C exhibited dose-dependent DPPH · radical scavenging activity within the concentration range of 0.002–8 μg · mL ^{1}, demonstrating strong antioxidant capacity.

2.8 Hydroxyl Radical Scavenging Activity of *S. cathayensis* Polyphenols

S. cathayensis polyphenols effectively scavenged hydroxyl radicals generated by Fenton reaction. As illustrated in Figure 7 [Figure 7: see original paper], hydroxyl radical inhibition increased with polyphenol concentration. Within the range of 60–160 μg · mL ^{1}, inhibition rates rose from 7.9% to 90%, with an IC value of 105 μg · mL ^{1}, significantly lower than vitamin C' s IC of 180 μg · mL ^{1}. These results indicate exceptional free radical scavenging capacity of *S. cathayensis* polyphenols.

2.9 Antibacterial Activity of *S. cathayensis* Polyphenols

The Oxford cup method was used to evaluate antibacterial activity, with results shown in Table 4 . *S. cathayensis* polyphenols exhibited inhibitory effects against both *E. coli* and *S. aureus*, with inhibition zone diameters of (10.0 ± 1.3) mm and (13.7 ± 1.2) mm, respectively.

The MIC values against *S. aureus* and *E. coli* were 0.393 mg · mL ^{1} and 0.785 mg · mL ^{1}, respectively (Table 5). These findings demonstrate significant antibacterial activity of *S. cathayensis* polyphenols.

Conclusion and Discussion

Semiliquidambar cathayensis is a Yao ethnic medicine used for rheumatism and is a primary ingredient in Yao medicinal baths. Its polyphenolic content likely constitutes the material basis for its medicinal activity. Current methods for polyphenol quantification include the iron tartrate UV spectrophotometry method using gallic acid as standard [**?**, **?**, **?**, **?**], HPLC using trans-resveratrol glucoside as standard [**?**, **?**, **?**, **?**, **?**], and general HPLC methods [**?**, **?**, **?**, **?**, **?**]. While HPLC offers high separation efficiency, good selectivity, high sensitivity, and automation, it is typically used for analyzing specific polyphenols rather than total content. The iron tartrate method is pH-sensitive and lacks stability. The Folin-Ciocalteu method, based on the reducing capacity of polyphenols under alkaline conditions to reduce phosphotungstic-phosphomolybdic acid to blue complexes with concentration-dependent color intensity, offers high sensitivity, simple instrumentation, straightforward operation, rapidity, accuracy, and good reproducibility, making it widely applicable for plant polyphenol quantification. Using gallic acid as standard and the Folin-Ciocalteu method, this study optimized ultrasonic extraction through single-factor and orthogonal experiments, achieving a total polyphenol extraction yield of 5.11% (Table 2), substantially higher than the previously reported 2.67% [**?**, **?**, **?**, **?**, **?**]. This method demonstrates excellent stability and reliability, with a robust extraction process.

Our investigation of antioxidant capacity using in vitro free radical generation systems revealed that *S. cathayensis* polyphenols showed DPPH · radical scavenging capacity comparable to vitamin C, with hydroxyl radical scavenging IC lower than vitamin C, indicating remarkable antioxidant efficacy. In vitro antibacterial tests demonstrated strong inhibitory effects against both Gram-positive *S. aureus* and Gram-negative *E. coli*, suggesting broad-spectrum antibacterial activity. The potent antioxidant activity and significant antibacterial effects against common pathogens indicate promising medicinal value and development prospects for this natural Yao medicine. This preliminary study on ultrasonic extraction optimization and antioxidant/antibacterial activities of *S. cathayensis* polyphenols provides an important foundation for developing this natural Yao medicinal resource.

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