Postprint: Extraction Process and Antioxidant Activity of Total Flavonoids from Kadsura coccinea Leaves

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Date: 2020-01-12T00:00:00+00:00

Abstract

To investigate the optimal ultrasonic-assisted extraction process conditions and antioxidant activity of total flavonoids from Kadsura coccinea leaves, this study employed Kadsura coccinea leaves as the experimental material. The ultrasonic extraction method was used to extract total flavonoids, and single-factor experiments were conducted to examine the effects of extraction time, ethanol concentration, extraction temperature, and solid-liquid ratio on the extraction yield. Based on these results, orthogonal experiments were performed to optimize the extraction process conditions. The scavenging capacities of the extracted total flavonoids against DPPH free radicals, •OH radicals, and superoxide anions were evaluated under the optimal conditions. The results demonstrated that the optimal ultrasonic-assisted extraction conditions were: extraction time of 35 min, ethanol concentration of 80%, extraction temperature of 50°C, and solid-liquid ratio of 1:20 $mg \cdot mL^{1}$, with an extraction yield of 4.83%. Antioxidant activity assays revealed that the total flavonoids exhibited favorable scavenging abilities against DPPH free radicals, •OH radicals, and superoxide anions, with the antioxidant capacity following the order: DPPH free radical scavenging > superoxide anion scavenging > •OH radical scavenging. At a concentration of 0.8 $mg \cdot mL^{1}$, the scavenging capacities of the total flavonoids against DPPH free radicals, •OH radicals, and superoxide anions corresponded to 97.6%, 82.1%, and 95.5% of those of vitamin C at the same concentration, respectively, indicating that total flavonoids from Kadsura coccinea leaves represent a promising source of natural antioxidants. This study provides a theoretical foundation for the extraction and utilization of active components from Kadsura coccinea leaves.

Full Text

Preamble

DOI: 10.11931/guihaia.gxzw201908022

Title: Extraction and Antioxidant Activity of Total Flavonoids from *Kadsura coccinea* Leaves

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Abstract

This study investigated the optimal ultrasonic-assisted extraction conditions and antioxidant activity of total flavonoids from *Kadsura coccinea* leaves. Using ultrasonic extraction, we examined the effects of extraction time, ethanol concentration, extraction temperature, and material-to-liquid ratio on the extraction yield through single-factor experiments. Based on these results, orthogonal experiments were conducted to optimize the extraction parameters. The scavenging capacities of the extracted flavonoids against DPPH radicals, •OH radicals, and superoxide anions were evaluated under optimal conditions. The results showed that the optimal extraction conditions were: extraction time 35 min, ethanol concentration 80%, extraction temperature 50°C, and material-to-liquid ratio 1:20 mg·mL ^{1}, achieving a maximum extraction yield of 4.83%. Antioxidant activity tests demonstrated that the total flavonoids exhibited strong scavenging abilities against DPPH radicals, •OH radicals, and superoxide anions, with the following order of potency: DPPH > superoxide anion > •OH. At a concentration of 0.8 mg·mL ^{1}, the flavonoids' scavenging capacities against DPPH, •OH, and superoxide anions reached 97.6%, 82.1%, and 95.5% of those of vitamin C at the same concentration, respectively. These findings indicate that *Kadsura coccinea* leaf flavonoids are a promising source of natural antioxidants and provide a theoretical foundation for the extraction and utilization of active compounds from this plant material.

Keywords: *Kadsura coccinea* leaves; total flavonoids; ultrasonic-assisted extraction; process optimization; antioxidant activity

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Introduction

Kadsura coccinea (Lem.) A.C. Smith, belonging to the family Schisandraceae and genus *Kadsura*, is an evergreen woody liana commonly known as "guoshanlong," "lengfantuan," or "zuandifeng." It is primarily distributed in Jiangxi, Hunan, Fujian, and Guangxi provinces of China. As a medicinal and edible herb, all parts of the plant—including roots, stems, leaves, and fruits—have therapeutic applications, with the roots being the main medicinal component used for promoting blood circulation and relieving pain. Modern pharmacological studies have revealed that various active constituents from *K. coccinea* roots and fruits possess hepatoprotective, anti-inflammatory, antimicrobial, antitumor, anti-HIV, antioxidant, anticoagulant, and lipid-regulating activities. While the leaves remain evergreen throughout the year and have been used traditionally to treat eczema, research on the extraction and pharmacological activities of *K. coccinea* has primarily focused on its roots and fruits, with limited reports on leaf development and utilization.

Antioxidants are classified into two major categories: synthetic and natural. Due to their safety and non-toxic characteristics, natural antioxidants derived from plants have become a research hotspot both domestically and internationally. Flavonoids, widely present in plant leaves and fruits, have been reported to exhibit strong antioxidant properties. Total flavonoids from various plant leaves, such as *Apocynum venetum*, *Agastache rugosa*, and *Physalis alkekengi*, have demonstrated significant antioxidant activity and potential for natural antioxidant development. Current reports on the antioxidant activity of *K. coccinea* have mainly focused on volatile oils from the stems, anthocyanins from the fruits, and phenolic acids from the roots. However, no studies have reported on the flavonoid components from *K. coccinea* leaves as natural antioxidants. Therefore, efficient extraction and antioxidant evaluation of total flavonoids from *K. coccinea* leaves are of great importance.

Various methods exist for flavonoid extraction, including supercritical fluid extraction (SFE), solvent extraction, ultrasonic-assisted extraction (UAE), microwave-assisted extraction (MAE), pulsed electric field (PEF)-assisted extraction, and enzyme-assisted extraction (EAE). Among these, ultrasonic-assisted extraction is widely used due to its high efficiency, short extraction time, low solvent consumption, and operational simplicity. This study employed ultrasonic-assisted extraction to obtain total flavonoids from *K. coccinea* leaves, optimized the process parameters through single-factor and orthogonal experiments, and evaluated the antioxidant activity under optimal conditions to provide technical guidance for extraction and a theoretical basis for developing natural antioxidants.

Materials and Methods

1.1 Materials, Reagents, and Equipment

1.1.1 Materials

Kadsura coccinea leaves were collected in May from the forest cultivation base of Hunan Polytechnic of Environment and Biology and authenticated by Associate Professor YANG Junheng from the College of Medicine & Technology as leaves of *Kadsura coccinea* (Lem.) A.C. Smith (family Schisandraceae, genus *Kadsura*).

1.1.2 Reagents

Rutin standard (purity 98%) was purchased from Beijing Puxi Technology Co., Ltd. 1,1-diphenyl-2-picrylhydrazyl (DPPH), vitamin C, disodium ethylenediaminetetraacetate (EDTA-2Na), hydrochloric acid, hydrogen peroxide, salicylic acid, 95% ethanol, sodium nitrite, aluminum nitrate, pyrogallol, petroleum ether, sodium hydroxide, and other reagents (all analytical grade) were obtained from Sinopharm Chemical Reagent Co., Ltd.

1.1.3 Equipment

UV-2600 UV-Vis spectrophotometer (Shanghai Baihe Instrument Technology Co., Ltd.); KQ-5200E ultrasonic cleaner (Changsha Tianheng Instrument Co., Ltd.); XB-0.3 vacuum rotary concentrator (Shenyang Lehua Biopharmaceutical Equipment Co., Ltd.); FA124 analytical balance (0.1 mg precision, Hunan Xuancai Scientific Instrument Co., Ltd.); DHG-9000-9030A electric thermostatic blast drying oven (Changsha Tianheng Instrument Co., Ltd.).

1.2 Experimental Methods

1.2.1 Extraction of Total Flavonoids from *K. coccinea* Leaves

Fresh leaves were washed, dried at 60°C to constant weight, pulverized, and passed through a 60-mesh sieve to obtain leaf powder. Exactly 2.0 g of powder was weighed, defatted with petroleum ether, and the residue was subjected to ultrasonic-assisted extraction at 250 W under specified conditions of temperature, time, ethanol concentration, and material-to-liquid ratio. The extract was concentrated under reduced pressure and diluted to 100 mL with 70% ethanol to prepare the sample solution.

1.2.2 Preparation of Rutin Standard Curve

The NaNO -Al(NO) -NaOH method was used to prepare the standard curve. Exactly 50 mg of dried rutin was dissolved in 70% ethanol and diluted to 50 mL. Aliquots of 0, 0.2, 0.4, 0.6, 0.8, and 1.0 mL were transferred to 25 mL volumetric flasks, sequentially mixed with 0.8 mL of 5% NaNO and 10% Al(NO) , with 5 min reaction time at room temperature after each addition. Then, 10 mL of 1 mol · L ^{1} NaOH was added, and the volume was adjusted to 25 mL with 70% ethanol. After 15 min at room temperature, absorbance was measured at 510 nm. The standard curve equation was: $y = 4.5411x + 0.0026$, $R^2 = 0.9993$.

1.2.3 Determination and Calculation of Total Flavonoid Content

One milliliter of the sample solution from section 1.2.1 was analyzed according

to the method in 1.2.2. The absorbance value was substituted into the standard curve to calculate the flavonoid concentration C (mg · mL ^{1}). The extraction yield (%) was calculated as: Yield = C × N × V × 10 ^{3} ÷ m × 100%, where V is the extract volume (mL), N is the dilution factor, and m is the leaf powder weight (g).

1.2.4 Single-Factor and Orthogonal Optimization Experiments
Using the method described in 1.2.1, single-factor experiments were conducted by varying one parameter at a time: extraction time (15, 25, 35, 45, 55 min), ethanol concentration (40, 50, 60, 70, 80%), extraction temperature (40, 50, 60, 70, 80°C), and material-to-liquid ratio (1:10, 1:15, 1:20, 1:25, 1:30 g · mL ^{1}).

Based on single-factor results, orthogonal experiments using an L (3) design were performed with extraction time (A), ethanol concentration (B), extraction temperature (C), and material-to-liquid ratio (D) as independent variables, using flavonoid extraction yield as the response. Factor levels are shown in Table 1 .

1.2.5 Antioxidant Activity Assays
Flavonoids extracted under optimal conditions were tested for antioxidant activity using the following methods:

1.2.5.1 DPPH Radical Scavenging Assay
Following the method of Pang et al. (2015), the extracted flavonoids were dissolved in 70% ethanol to prepare solutions of various concentrations (0.025, 0.05, 0.075, 0.1, 0.2, 0.4, 0.6, 0.8 mg · mL ^{1}). One milliliter of each solution was mixed with 2.5 mL of DPPH solution, and absorbance was measured after 30 min. Vitamin C was used as a positive control.

1.2.5.2 • OH Radical Scavenging Assay
Using the salicylic acid method described by Jiang et al. (2018), the scavenging capacity of flavonoids (dissolved in 70% ethanol) at different concentrations (0.025–0.8 mg · mL ^{1}) against • OH radicals was evaluated, with vitamin C as the control.

1.2.5.3 Superoxide Anion Scavenging Assay
Flavonoid solutions (0.025–0.8 mg · mL ^{1}) were prepared in 70% ethanol. The pyrogallol autoxidation method reported by Fan et al. (2017) was used to determine superoxide anion scavenging capacity, with vitamin C as the control.

1.3 Statistical Analysis
All experiments were performed in triplicate, and data are expressed as mean ± standard error. Microsoft Excel 2010, SPSS 20.0, and Design Expert 8.0.6 software were used for data processing, analysis, and graphing.

Results

2.1 Single-Factor Experiments

2.1.1 Effect of Extraction Time

As shown in Figure 1 [Figure 1: see original paper], the extraction yield increased gradually with time from 15 to 35 min, reaching a maximum of 4.31% at 35 min, then decreased with further time extension. This may be because insufficient time results in incomplete extraction, while prolonged heating may degrade flavonoid structures, reducing yield. Therefore, the optimal extraction time range was determined to be 25–45 min.

2.1.2 Effect of Ethanol Concentration

Figure 2 [Figure 2: see original paper] shows that extraction yield increased linearly with ethanol concentration from 40% to 70%, peaking at 4.62% at 70% ethanol, then declined. According to the "like dissolves like" principle, 70% ethanol has similar polarity to the flavonoids. Higher ethanol concentrations increase the extraction of alcohol-soluble impurities that compete with flavonoids, thereby reducing yield. The optimization range was set at 60–80% ethanol.

2.1.3 Effect of Extraction Temperature

As depicted in Figure 3 [Figure 3: see original paper], extraction yield increased with temperature up to 60°C (maximum 4.53%), then decreased. Elevated temperature enhances molecular motion and contact between flavonoids and ethanol, improving extraction efficiency. However, excessive temperature may destroy flavonoid structures. The optimization range was established as 50–70°C.

2.1.4 Effect of Material-to-Liquid Ratio

Figure 4 [Figure 4: see original paper] indicates that extraction yield increased with solvent volume, reaching a maximum of 4.53% at a 1:20 ratio, with no significant improvement beyond this point. While increased solvent volume enhances contact area between material and solvent, beyond a certain ratio the system becomes saturated, and additional solvent only leads to waste without improving yield. The optimization range was set at 1:15–1:25 ($g \cdot mL^{-1}$).

2.2 Orthogonal Optimization

Based on single-factor results, orthogonal experiments were designed according to Table 1. Results and analysis are presented in Table 2 .

Table 2 shows the influence order of factors on extraction yield: ethanol concentration (B) > extraction time (A) > extraction temperature (C) > material-to-liquid ratio (D). The optimal conditions were A2B3C1D2: extraction time 35 min, ethanol concentration 80%, temperature 50°C, and material-to-liquid ratio 1:20 $g \cdot mL^{-1}$, yielding 4.83% total flavonoids.

2.3 Antioxidant Activity

2.3.1 DPPH Radical Scavenging

Figure 5 [Figure 5: see original paper] shows that DPPH scavenging capacity increased with flavonoid concentration from 0 to 0.20 mg·mL ^{1}. At 0.8 mg·mL ^{1}, the scavenging rate reached 94.3%, equivalent to 97.6% of vitamin C activity at the same concentration, demonstrating excellent DPPH radical scavenging ability. The IC value was 0.067 mg·mL ^{1}, which is significantly lower than the 1.14 mg·mL ^{1} reported for *Aquilaria sinensis* leaf flavonoids, indicating superior activity.

2.3.2 •OH Radical Scavenging

As shown in Figure 6 [Figure 6: see original paper], •OH scavenging capacity increased with concentration. At 0.8 mg·mL ^{1}, the scavenging rate was 82.1%, equivalent to 87.2% of vitamin C activity. The IC value was 0.125 mg·mL ^{1}, substantially lower than the 281.89 mg·L ^{1} reported for *Agastache rugosa* leaf flavonoids, confirming strong •OH radical scavenging potential.

2.3.3 Superoxide Anion Scavenging

Figure 7 [Figure 7: see original paper] demonstrates a clear dose-dependent relationship for superoxide anion scavenging. At 0.8 mg·mL ^{1}, the scavenging rate was 93.7%, equivalent to 95.5% of vitamin C activity. The IC value of 0.091 mg·mL ^{1} compares favorably to the 0.425 mg·mL ^{1} reported for maca leaf flavonoids, indicating excellent superoxide anion scavenging capacity.

Discussion and Conclusion

Flavonoids are widely distributed in plant leaves, flowers, and fruits. Extraction methods include supercritical fluid extraction, solvent extraction, ultrasonic-assisted extraction, microwave-assisted extraction, and enzyme-assisted extraction. Ultrasonic-assisted extraction offers high efficiency, short duration, low solvent consumption, and mild conditions by providing high-frequency vibrations that rupture plant cell walls through cavitation and mechanical effects, allowing solvents to diffuse and dissolve intracellular components according to the "like dissolves like" principle. Compared with traditional solvent extraction, the energy from high-frequency ultrasound shortens extraction time, reduces solvent usage, lowers temperature, and improves efficiency. Compared with supercritical fluid extraction, it requires simpler equipment. These advantages have made ultrasonic extraction popular among researchers.

This study focused on *K. coccinea*, a precious resource in Hunan, and optimized the extraction of its leaf flavonoids. The optimal conditions—35 min, 80% ethanol, 50°C, and 1:20 g·mL ^{1} material-to-liquid ratio—yielded 4.83% flavonoids, demonstrating high extraction efficiency.

Scientific research has shown that excessive free radicals produced during metabolism can cause oxidative damage associated with aging, cardiovascular

diseases, and inflammation when not promptly eliminated. Natural antioxidants with low side effects are a current research focus. Flavonoids serve as natural antioxidants through three mechanisms: (1) indirectly scavenging radicals by acting on radical-producing enzymes or transition metal ions; (2) directly scavenging radicals (e.g., DPPH, $\bullet$OH, superoxide anion); and (3) activating endogenous antioxidant systems. This study evaluated *K. coccinea* leaf flavonoids through direct radical scavenging assays. At 0.8 mg $\cdot$ mL ^{1}, the flavonoids achieved 97.6%, 82.1%, and 95.5% of vitamin C' s activity against DPPH, $\bullet$OH, and superoxide anion, respectively, confirming their potential as natural antioxidants. This activity may be attributed to hydroxyl groups on the flavonoid benzene rings that undergo redox reactions with radicals to form stable semiquinone structures, thereby interrupting radical chain reactions.

Kadsura coccinea is a valuable medicinal and edible plant in Guangxi and Hunan. Current research has focused on its roots and fruits, with minimal development of its leaves. This study establishes an efficient extraction process for leaf flavonoids and demonstrates their in vitro antioxidant activity, providing a theoretical basis for applications in food supplements and skincare products. Future research should investigate in vivo antioxidant activity, formulation development for pharmaceutical or nutraceutical applications, and other biological activities such as anti-inflammatory and antitumor effects.

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