A QUALITATIVE AND QUANTITATIVE INVESTIGATION OF SOME ANTIOXIDANT COMPOUNDS IN PENNYWORT (*Centella asiatica)*

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ABSTRACT

Pennywort (Centella asiatica) is a renowned herb for antioxidant, anti-inflammatory, antibacterial, and wound-healing properties. Therefore, it is necessary to exploit its medicinal potential fully. This study aimed to assess its bioactive compounds (flavonoids, tannins, saponins, terpenoids), quantify total flavonoids and polyphenols, and evaluate antioxidant activity. The extraction parameters, including material-to-solvent ratio (1:1, 1:3 and 1:5), temperature (30, 40 and 50°C), and time (1, 2 and 3 h), were optimized. Results confirmed the presence of target compounds in pennywort extracts. Optimal extraction conditions were determined as a 1:3 leaves-to-solvent ratio at 40°C for 2 hours, yielding 1.73 mgGAE/g total polyphenols and 4.26 mgQE/g total flavonoids. Antioxidant capacity, measured by DPPH radical scavenging activity, exhibited an IC_{50} value of 89.16 mg/mL.

1. INTRODUCTION

Pennywort (*Centella asiatica*), a widely distributed and affordable herb, possesses valuable medicinal properties, including wound healing, anti-inflammatory, and circulatory enhancement. These benefits are attributed to its rich phytochemical profile, encompassing saponins, asiaticoside, madecassoside, flavonoids, polyphenols, and tannins (Shin et al., 2021; Chandrika and Kumara, 2015). Numerous studies have explored the biological activities of pennywort. For instance, supercritical water extraction of asiatic acid and asiaticoside demonstrated increased yields at elevated temperatures and pressures, with optimal conditions at 250°C and 40 MPa (Kim et al., 2009). Furthermore, ultrasound-assisted extraction significantly enhanced the extraction efficiency of bioactive compounds within 10 minutes (Seong et al., 2023). A comparative study by Idris et al. (2021) highlighted the influence of extraction method, solvent, time, and target compounds on the overall extraction efficiency of bioactive compounds from pennywort.

Furthermore, domestic research has made significant strides in characterizing the chemical composition of pennywort. Employing chromatographic techniques alongside recrystallization, Vinh et al. (2020) successfully isolated and identified palmitic acid within pennywort extracts based on physicochemical properties and H-NMR spectral analysis. Moreover, studies investigating essential oil extraction from pennywort through steam distillation, irradiation, and microwave methods, coupled with subsequent chromatographic-mass spectrometry analysis, have yielded valuable insights into the chemical constituents of the herb and paved the way for further investigations into its biological activities (Hanh and Thach, 2021).

The aim of this study was to investigate the presence of bioactive compounds in pennywort. Additionally, the study sought to optimize the extraction conditions including the ratio of pennywort to solvent, extraction time, and temperature, in order to maximize the content of total flavonoids and polyphenols. Additionally, the study evaluated antioxidant activity using DPPH radical scavenging assays. The findings provide a foundation for future research on applying pennywort extract in various forms, such as soluble powders and bottled juices, ultimately contributing to a wider range of products enriched with bioactive compounds.

2. RESEARCH METHODOLOGY

2.1. Materials

Pennywort was purchased at markets in An Hoa ward, Ninh Kieu district, Can Tho city. The chemicals used include sodium hydroxide, aluminium chloride, sodium nitrite, sodium carbonate, iron (III) chloride, sulfuric acid, chloroform, gallic acid (China), 2,2-diphenyl-1-picrylhydrazyl (DPPH, Japan), quercetin, and Folin-Ciocalteu (Germany). The equipment used include UV-VIS spectrophotometer (Libra S60PC, USA), water bath (Memmert WNB 22, Germany), blender (Vietnam), fume hood (Vietnam), analytical balance (Mettler Toledo, USA).

2.2. Determination of the presence of some groups of bioactive compounds in pennywort extract

Fresh pennywort was carefully prepared by washing and discarding wilted or damaged leaves. Subsequently, the qualified leaves were ground with distilled water in a 1:3 (w/v) ratio of leaves to solvent. The resulting mixture was incubated in a water bath at 40°C for 2 hours to facilitate extraction. The extract was then obtained by filtering the mixture through filter paper. The extract's presence of flavonoids, tannins, saponins, and terpenoids was qualitatively assessed using specific reagents. Each experiment was independently replicated three times to ensure reproducibility.

2.3. Investigation of the influence of solvent ratio on total flavonoid and total polyphenol content in pennywort extract

Pennywort extracts were prepared as mentioned above in three different ratios: 1:1, 1:3, and 1:5 (w/v, weight of leaves to volume of water). Each extract's total flavonoid and polyphenol content were then analyzed to determine the optimal leaves-to-solvent ratio for maximizing the yield of these bioactive compounds. Each experiment was independently replicated three times to ensure reproducibility.

2.4. Investigation of the influence of temperature and extraction time on total flavonoid and total polyphenol content in pennywort extract

Pennywort extracts were prepared as mentioned above according to the optimal leaves-to-solvent ratio at three different temperatures (30, 40, and 50°C) for varying durations (1, 2, and 3 hours). Each extract's total flavonoid and polyphenol content was then analyzed to determine the most suitable extraction temperature and time for maximizing the yield of these bioactive compounds. Each experiment was independently replicated three times to ensure reproducibility.

2.5. Determination of antioxidant activity in pennywort extract

A pennywort extract was prepared as mentioned above according to the optimal leaves-to-solvent ratio at the optimal extraction temperature and time. The antioxidant activity of the extract was evaluated by assessing its ability to neutralize DPPH free radicals with an initial concentration of 34.9 µg/mL. Vitamin C (15 µg/mL) served as a positive control. The IC_{50} value which represents the concentration of the extract required to neutralize 50% of the DPPH radicals, was determined. Each experiment was independently replicated three times to ensure reproducibility.

2.6. Analytical methods and data processing

Table 1 presents the criteria and corresponding analytical methods employed to assess the bioactive components in the pennywort extract.

Table 1. Analytical methods

Criteria	Methods
Qualitative analysis	
Flavonoid	Coloration using a 10% solution of sodium hydroxide (Dat et al., 2022)
Tannin	Coloration using a 10% solution of ferric chloride (Yadav and Agarwala, 2011)
Saponin	Foaming effect (Dat et al., 2022)
Terpenoid	Coloration using chloroform and sulfuric acid (Yadav and Agarwala, 2011)
Quantitative analysis	
Total flavonoid content	Coloration using aluminum chloride in an alkaline medium (Marinova et al., 2005)
Total polyphenol content	Folin-Ciocalteu method (Feduraev et al., 2019)
Antioxidant capacity	DPPH radical scavenging activity (Tabart et al., 2007)

The data were compiled using Excel 2016. Statistical analysis was performed using ANOVA in conjunction with the LSD test through Minitab 17 software to determine statistically significant differences.

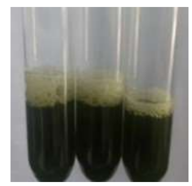
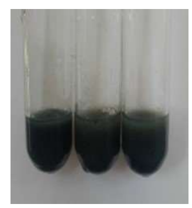
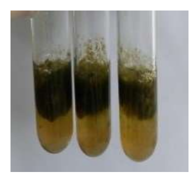
3. RESULTS AND DISCUSSION

3.1. The presence of some groups of bioactive compounds in pennywort extract

Determining the presence of bioactive compounds is a crucial first step in investigating the content and activity of these substances within pennywort. This study surveyed to identify the presence of compounds including flavonoids, saponins, tannins, and terpenoids. The qualitative results of this survey are presented in Table 2.

The study findings confirmed the presence of all four major bioactive compound groups (flavonoids, tannins, saponins, and terpenoids) within the pennywort extract. As reported in previous literature (Huong and Bach, 2011), these compounds are water-soluble. The use of aqueous solvents facilitates the extraction process by enhancing the diffusion of active ingredients into the solvent. Water, a polar solvent, effectively interacts with and dissolves polar compounds through dipole-dipole interactions and hydrogen bond formation (Rahman et al., 2013). These results align with a previous study by Roy and Bharadvaja (2017), which also demonstrated the presence of flavonoids, terpenoids, saponins, and tannins in water extracts of pennywort.

Table 2. Qualitative analysis of bioactive compounds in pennywort extract

Target compounds	Reactions	Results	Images
Flavonoid	Concentrated yellow color	+	
Saponin	A foam layer about 1 cm high	+	
Tannin	Dark green or black color	+	
Terpenoid	Red-brown color	+	

Results from the authors' analysis. +: the presence of the target compound.

3.2. The influence of solvent ratio on total flavonoid and total polyphenol content in pennywort extract

The ratio between plant materials and solvent significantly influences the extraction process. A higher solvent volume enhances the solvent's contact with the plant material, thereby improving the solubility of bioactive compounds. This study investigated the impact of three different leaves-to-solvent ratios (1:1, 1:3, and 1:5) on the total flavonoid and total polyphenol contents in pennywort extracts, while maintaining constant extraction conditions (40°C for 2 hours). The statistical results of this investigation are summarized in Table 3.

Table 3 reveals that the leaves-to-solvent ratio significantly influences the total flavonoid and polyphenol content within the pennywort extract. An increase in the solvent volume from a 1:1 to a 1:3 ratio resulted in a significant increase in both total flavonoid and total polyphenol content. However, a further increase in the solvent volume to a 1:5 ratio did not significantly alter the flavonoid content, while the total polyphenol content exhibited a statistically significant decrease compared to the 1:3 ratio.

The solvent volume significantly influences the extraction efficiency of compounds from plant materials. The driving force behind the extraction process is the concentration gradient between the solutes within the plant cells and the solvent. Solute transport from the plant cells into the solvent primarily occurs through diffusion. Increasing the solvent volume enhances the diffusion rate, facilitating faster and more thorough extraction of target compounds.

Table 3. Total flavonoid and total polyphenol contents at various leaves-to-solvent ratios

Criteria	Leaves-to-solvent ratios (w/v)		
	1:1	1:3	1:5
Total flavonoid content (mgQE/g)	1.79±0.02[b]	3.08±0.11[a]	3.06±0.16[a]
Total polyphenol content (mgGAE/g)	0.96±0.01[c]	1.72±0.05[a]	1.54±0.04[b]

Results from the authors' analysis. The values are the average of 3 replicates ± standard deviation. Distinct letters following values in the same row indicate statistically significant differences ($p < 0.05$).

However, the relationship between solvent volume and extraction yield is not linear. At low solvent volumes (e.g., 1:1 ratio), insufficient solvent may limit the dissolution of compounds like polyphenols and flavonoids, resulting in low extraction yields. Conversely, excessive solvent volumes can also diminish efficiency. While initially promoting diffusion and maximizing extraction, further increases in solvent volume beyond a certain point do not significantly enhance the yield. This phenomenon is attributed to the attainment of equilibrium, where the solute concentration in the solvent remains relatively constant despite further increases in solvent volume. Moreover, the excessive solvent use reduces the economic efficiency of the extraction process (Hoa and Phuong, 2022, Duc et al., 2022).

3.3. The influence of extraction temperature and time on the total flavonoid and polyphenol content in pennywort extract

Temperature and extraction time are critical parameters influencing the efficiency of compound extraction from plant materials (Rahmawati, 2021). Elevated temperatures accelerate molecular motion, enhancing the dissolution and diffusion of target compounds from the plant matrix into the solvent. Higher temperatures reduce solvent viscosity, facilitating deeper penetration into the plant material and increasing the contact surface area between the solvent and the plant matrix (Putra et al., 2023). However, excessive heat can induce unwanted chemical reactions within the extract, potentially leading to compound degradation and loss (de Souza et al., 2019). Prolonged extraction time generally increases the recovery of target compounds. However, beyond a certain point, further increases in extraction time yield diminishing returns, with minimal gains in extraction efficiency (Man et al., 2019).

Pennywort extracts were prepared at three different temperatures (30°C, 40°C, and 50°C) and for varying durations (1, 2, and 3 hours). The resulting extracts were then analyzed for their total flavonoid and polyphenol content. The results of this analysis are presented in Table 4.

The findings in Table 4 reveal statistically significant variations in flavonoid and polyphenol content across different extraction conditions (temperature and time) at a 95% confidence level. Notably, there's an interactive effect between these two factors. The highest flavonoid content (4.26 mgQE/g) was observed at 40°C for 3 hours, while the peak polyphenol content (1.73 mgGAE/g) was achieved at 40°C for 2 hours. This suggests that prolonged extraction times might degrade or denature certain polyphenols not categorized as flavonoids. These results align with previous research by Lien et al. (2014) on soybeans. Their study, which examined extraction times (2, 3, and 4 hours) and temperatures (30, 40, 50, and 60°C), also demonstrated significant effects of these factors on flavonoid and polyphenol content. The optimal conditions in their study were 3 hours and 40°C, yielding 2.97 ± 0.04 mgQE/g of flavonoids and 2.18 ± 0.06 mgGAE/g of polyphenols. This phenomenon can be explained by Fick's second law of diffusion, which posits that an equilibrium in solute concentration between the solid matrix and the solvent is eventually reached. While increased temperature and time generally enhance extraction efficiency, exceeding certain thresholds can lead to a decline in flavonoid and polyphenol content.

Table 4. Total flavonoid and total polyphenol contents at various extraction temperatures and times

Temperature (°C)	Time (hours)	Total flavonoid content (mgQE/g)	Total polyphenol content (mgGAE/g)
30	1	$2.84^{d} \pm 0.07$	$1.38^{c} \pm 0.01$
30	2	$3.18^{c} \pm 0.10$	$1.58^{b} \pm 0.01$
30	3	$3.64^{b} \pm 0.04$	$1.38^{c} \pm 0.08$
40	1	$1.94^{g} \pm 0.02$	$0.92^{e} \pm 0.02$
40	2	$3.31^{c} \pm 0.11$	$1.73^{a} \pm 0.05$
40	3	$4.26^{a} \pm 0.24$	$1.08^{d} \pm 0.04$
50	1	$2.72^{de} \pm 0.00$	$1.35^{c} \pm 0.01$
50	2	$2.41^{f} \pm 0.04$	$1.60^{b} \pm 0.04$
50	3	$2.46^{ef} \pm 0.12$	$1.32^{c} \pm 0.01$

Results from the authors' analysis. The values are the average of 3 replicates ± standard deviation. Distinct letters following values in the same column indicate statistically significant differences ($p < 0.05$).

3.4. Antioxidant activity in pennywort extract

Given that the optimal extraction conditions for total flavonoid and polyphenol content differed, it was crucial to assess the antioxidant activity of the extracts obtained under these specific conditions: 1:3 solvent-to-material ratio, 40°C, and either 2 or 3 hours of extraction. To evaluate this, the DPPH free radical scavenging activity of the extracts was determined. Figure 1 illustrates the DPPH radical scavenging activity of the samples extracted at 1:3, 40°C, and 3 hours, as well as those extracted at 1:3, 40°C, and 2 hours, with vitamin C serving as a control.

The findings in Figure 1 demonstrate a significant difference in free radical scavenging capacity between the two extraction conditions. The extract obtained under the 1:3 leaves-to-solvent ratio, 40°C, and 2-hour extraction exhibited a radical scavenging activity of 30.30%, whereas the extract prepared under the same conditions but with a 3-hour extraction time achieved a radical scavenging activity of 21.17%. This suggests that the extract obtained with the 2-hour extraction time possesses superior antioxidant capacity in this experimental context. Consequently, the pennywort extract prepared under the 1:3 leaves-to-solvent ratio, 40°C, and 2-hour extraction was selected for subsequent IC_{50} value determination.

The IC_{50} value represents the concentration of the extract required to neutralize 50% of the initial DPPH radicals. Figure 2 illustrates the relationship between the concentration of the extract and the corresponding DPPH scavenging level. The R^2 value of the linear regression equation, 0.9859, indicates a strong correlation between concentration and scavenging within the tested range of 50-350 mg/mL. This suggests a direct proportionality between the extract's concentration and its ability to neutralize DPPH radicals within this concentration range.

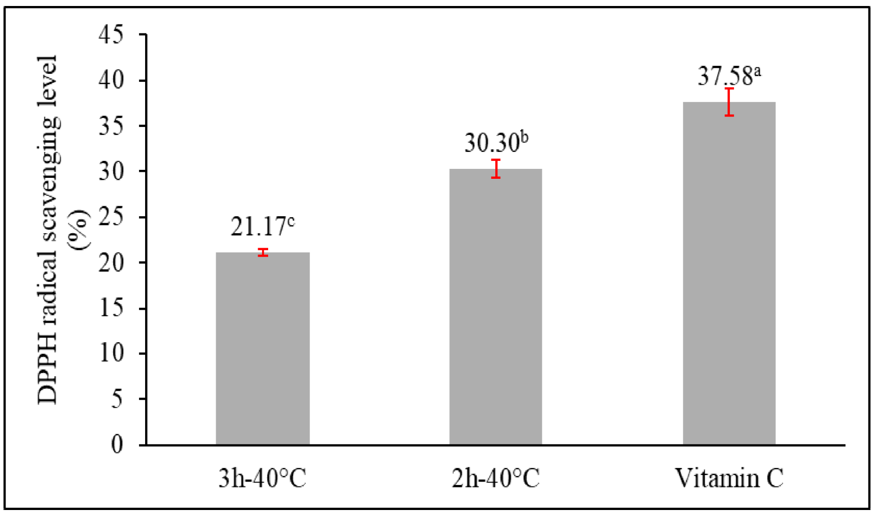


Figure 1. DPPH radical scavenging activity of pennywort extract at 40°C – 3 hours and 40°C – 2 hours (vitamin C as control)

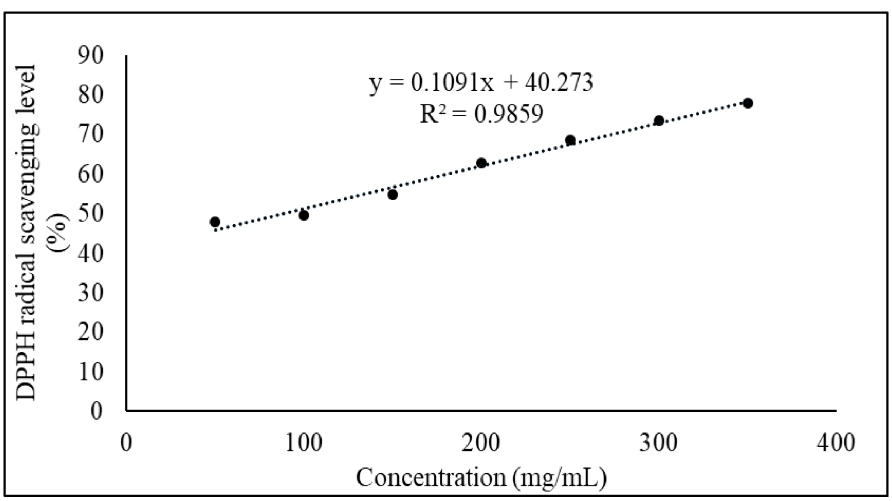


Figure 2. The antioxidant capacity of pennywort extract (1:3 - 40°C - 2 hours)

The IC_{50} value for the pennywort extract was determined to be 89.16 mg/mL, as calculated from the regression equation y = 0.1091x + 40.273. This value represents the concentration of the extract required to neutralize 50% of the initial DPPH radicals. A previous study by Quyen et al. (2020) compared the antioxidant capacity of two types of pennywort extracts obtained from dried powder using water and ethanol as solvents. Their results showed that the ethanol extract had an IC_{50} of 1744.77 µg/mL, while the water extract had an IC_{50} of 2324.26 µg/mL. These values suggest that the antioxidant activity of the pennywort extract in this study, using fresh material and water as the solvent, is lower compared to the ethanol extracts in the previous study. However, the use of water as the solvent offers advantages for certain applications, such as the production of pennywort juice or soluble powders for food processing.

4. CONCLUSION

This study identified four key compound groups within the water-based pennywort extract: flavonoids, saponins, tannins, and terpenoids. Furthermore, the research pinpointed several factors that significantly influence the total flavonoid and polyphenol content of the extract, including the ratio of pennywort leaves to solvent, extraction temperature, and extraction time. Based on the findings, the optimal extraction conditions were determined to be a 1:3 ratio of leaves to solvent, an extraction temperature of 40°C, and an extraction time of 2 hours. Under these conditions, the extract exhibited a total flavonoid content of 4.26 mgQE/g and a total polyphenol content of 1.73 mgGAE/g. The antioxidant activity of the extract under these conditions was demonstrated by an IC_{50} value of 89.16 mg/mL, indicating its ability to neutralize DPPH free radicals. These findings provide a solid foundation for future research aimed at further optimizing the extraction of bioactive compounds from pennywort and exploring their potential applications in food products.

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KHẢO SÁT ĐỊNH TÍNH VÀ ĐỊNH LƯỢNG MỘT SỐ HỢP CHẤT CÓ HOẠT TÍNH KHÁNG OXY HÓA TRONG RAU MÁ (Centella asiatica)

TÓM TẮT

Rau má (Centella asiatica) là một loại thảo dược quen thuộc với nhiều công dụng tốt như kháng oxy hóa, kháng viêm, kháng khuẩn và tác dụng hỗ trợ làm lành vết thương. Vì vậy việc khai thác hết tiềm năng dược lý của rau má là cần thiết. Nghiên cứu này nhằm khảo sát một số hợp chất có hoạt tính sinh học trong rau má (như flavonoid, tannin, saponin, terpenoid), đồng thời xác định hàm lượng flavonoid và polyphenol tổng số và đánh giá hoạt tính kháng oxy hóa. Các thông số trích ly bao gồm tỷ lệ dung môi, nhiệt độ và thời gian được tối ưu hóa thông qua thí nghiệm với các sự kết hợp khác nhau. Kết quả cho thấy trong dịch trích rau má có chứa cả bốn nhóm hợp chất mục tiêu. Điều kiện trích ly tốt nhất là tỷ lệ lá rau má: dung môi 1:3, nhiệt độ 40°C và thời gian 2 giờ, khi đó hàm lượng polyphenol tổng số là 1,73 mgGAE/g và flavonoid tổng số là 4,26 mgQE/g. Hoạt tính kháng oxy hóa thông qua khả năng trung hòa gốc tự do DPPH thể hiện giá trị IC_{50} là 89,16 mg/mL.

Từ khóa: *Centella asiatica, định lượng, định tính, kháng oxy hóa, rau má.*