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Study on Antibacterial and Antioxidant Activity of Extracts from Hydroponic Pennywort Tubers (*Centella Asiatica* (L.) Urban)

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ABSTRACT: Several plant species have been found to contain bioactive compounds that contribute positively to health and are utilized across food, pharmaceutical, and cosmetic industries. The purpose of this research is to investigate the antibacterial and antioxidant activities of extracts derived from hydroponically grown pennywort tubers (*Entella asiatica* (L.) Urban). The results show that the antioxidant activity of the extract (IC50) was 6.85 mg/mL. The extract demonstrates antibacterial efficacy against foodborne pathogen *Escherichiacoli* and the pathogenic bacterium*Staphylococcus aureus* at the extract concentration of 250 mg/mL. These results underscore the potential of hydroponically cultivated pennywort tubers as a source of bioactive compounds with beneficial applications in the food, pharmaceutical, and cosmetic industries. Further research is warranted to fully explore the hydroponically grown pennywort tubers, aiming to harness their medicinal attributes for disease prevention and human health enhancement in the future.

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1. INTRODUCTION

The bioactive compounds found in plants offer many important benefits for current and future medical applications (Wawrosch and Zotchev, 2021). These compounds, presentin foods such as vegetables, fruits, and whole grains,are essential for human health (Pai *et al.,* 2022). Because of their nutritional, health and therapeutic benefits, extensive research has been conducted on bioactive compoundsfor their potential applications across variousfields (Huang and Chen, 2022).

Pennywort, scientifically known as *Centella asiatica* (L.) Urban, is a traditional herbal remedy used in Southeast Asia (Barge *et al*., 2023). *Centella asiatica* is characterized by its rich content of active substances from various chemical groups, including triterpenoids, carotenoids, glycosides, flavonoids, alkaloids, essential oils, and fatty oils. These compounds exhibit antibacterial, antiviral, antioxidant, wound healing, and antidepressant effects. They aid in improving cognitive function and support the treatment of cardiovascular, gastrointestinal, diabetic, cancerous, and gynecological diseases (Wiciński *et al.,* 2024).

Recent studies consider pennywort as an antioxidant, reducing the effects of stress both in vitro and in vivo. The high antioxidant contents present in this plant suggest that it could serve as a potential alternative source of natural antioxidants (Abdul Rahim *et al.,* 2021). Moreover, pennywort has demonstrated antibacterial properties, effectivelyresist bacteria that cause disease and food poisoning (Norzaharaini *et al.*, 2011; Pitinidhipat and Yasurin 2012).

Studies conducted in the world and in Vietnam have focused on chemical composition and biological activity of pennywort (Seevaratnam et al., 2012) as well as exploring the pharmacological application of pennywort leaves in the production of commercial products (Ploenkutham et al., 2018; Chuthaputti, 2011). However, research on potential bioactivecompounds of pennywort tubers is still limited. Hence, this study aims to determine the antibacterial and antioxidant activities of extracts derived from hydroponic pennywort tubers (*Centella asiatica* (L.) Urban). This investigation serves as a basis for in-depth research on pennywort tubers for potential applications in food, pharmaceuticals, and cosmetics production in the future.

2. MATERIAL AND METHODS

2.1 Plant material

Hydroponically grown pennywort tubers were collected from Hoang Long Farm - BC Vu Long Company Limited in Village 4, MyLong commune, CaoLanh district, DongThap province. These tubers were typically harvested 6 months after planting. The tubers were transported to the processing area where they underwent sorting to remove damaged and rotten tubers. The substrate was cleaned and fluffed before the tubers were further processed. A thorough rinse within water lasting 3-5 minutes was conducted, followed blydraining for approximately30 minutes.

2.2 Methods

2.2.1 Sample preparation

Centella asiatica tubers were extracted according to the research of Kumar and Kavita (2020) with modifications. 50.0 g of hydroponic pennywort root were used to remove the roots, wash, and cut into pieces of 0.5-1.0 cm, and mince with extraction solvent (ratio 1:1, w/ v) to obtain a mixture. Remove solvent from the mixture using a rotary vacuum evaporator. The collected mixture was filtered through Whatman filter paper No. 4 (hole size 20-25 µm) and then centrifuged at 6000 rpm to collect the extract. The used solvent was Ethanol 60%. The control solvent was u distilled water.

2.2.2 Measurement of antioxidant activity

Antioxidant activity of hydroponic pennywort tuber extract was performed by the DPPH (2,2-diphenyl-1-picryl hydrazyl) radical scavenging activity measurement (Ye *et al.,* 2013) with modifications. 200 µL of the extract (with different concentrations) was dissolved in 1 mL of 0.1 mM DPPH and then shaken well to obtain a reaction mixture. The reaction mixture was incubated in the dark at 30 ºC for 30 minutes, and then was measured absorbance at 517 nm using UV/Vis Spectrophotometer. Vitamin C (ascorbic acid) was used as positive control. The experiment was repeated 3 times.

The DPPH inhibition ability was calculated according to the following formula:

$$\%I = \left(1 - \frac{A_{sample}}{A_0}\right) \times 100$$

Note: *%I: % of antioxidant activity.*
A_0: Control reaction absorbance
A_{sample}: Testing specimen absorbance

The control solution (vitamin C) was prepared stock at a concentration of 1 mg/mL (1000 ppm) in ethanol, and then was diluted 100 times to obtain a concentration of 10 µg/mL (10 ppm). Dilution of vitamin C solutions from initial concentration of 10 ppm to obtain a vitamin C solution concentration of 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10 ppm.

Add 1 mL of diluted vitamin C to 1 mL of 0.1 mM (DPPH) in ethanol 95%. The mixture was mixed and left for 30 min at room temperature in the dark. The absorbance of the reaction mixture was measured at 517 nm by using a UV-VIS spectrophotometer. A standard curve is established y = ax + b with the percentage of DPPH inhibition at different concentrations. And then, the IC_{50} value of vitamin C and hydroponic pennywort extract were calculated.

2.2.3 Antibacterial activity testing

Evaluation of antibacterial activity of hydroponic pennywort tuber extract was determined according to the study of Mythili *et al* (2012) with modifications. *Escherichia coli* and *Staphylococcus aureus* were cultured on LB media (Luria Bertani) at 37°C for 24 hours. Transfer bacterial colonies to 4 mL of LB media and then shake overnight at 37°C. The extract was made different concentrations (250, 500, and 1000 mg/mL). The positive control was Ampicillin (100 µg/mL). Dimethyl sulfoxide (DMSO) 30% was as the negative control.

The activity test' plate was prepared by spreading 100 µL of bacterial solution (10^6 CFU/mL) onto the surface of LB agar, let it dry for 15 minutes, and made holes in the agar surface with a diameter of 6 mm (agar wells). Add 50 µL of the diluted extract at different concentrations (or control) into the prepared agar wells, and then the plate were incubated for 24 hours at 37°C. Measure the diameter of the antibacterial ring after 24 hours, each experiment repeated 3 times. The diameter of the antibacterial rings were determined by the formula: H = D-d (mm), in which: D was the antibacterial ring's diameter calculated from the central's hole (mm); d was the agar hole's diameter (mm). Antibacterial ability was measured as following: no antibacterial activity (<1 mm), weak antibacterial activity (1 – 5 mm), moderate antibacterial activity (1 - 20mm), strong antibacterial activity (>20 mm).

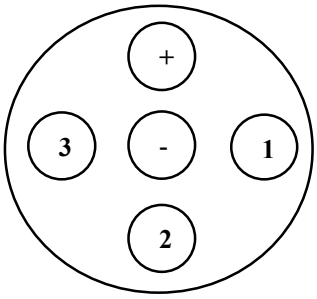

Figure 1: Diagram of injecting extract, positive control, and negative control into agar wells at different treatment concentrations

Note: 1, 2, 3: concentrations of diluted extract; (+): Positive control – Ampicillin 100 µg/mL ; (-): Negative control – DMSO 30 % .

2.2.4 Statistical analysis

Excel 2016 software and Statgraphics XIX software were used to analyze variance (ANOVA), standard deviation (SD), and LSD test.

3. RESULTS

3.1 Antioxidant activity of hydroponic pennywort tuber extract

Table 1 presents the DPPH (2,2-diphenyl-1-picryl hydrazyl) radical scavenging activity of hydroponic pennywort tuber extract. The results show a linear increase inDPPH free radical removal efficiency of the extractconcentration. In particular, the concentration of the extract rosefrom 1mg/mL to 10mg/mL, the free radical removal efficiency increased from 24% to 65%. Notably, the DPPH free radical removal efficiency at concentration of 10 mg/mL was the highest and significantly different from other concentrations ($p<0.05$).

The IC_{50} value of hydroponic pennywort tuber extract was analyzed and presented in **Table 1**. The lower IC 50 value indicates higher antioxidant activity. The IC_{50} value of hydroponic pennywort extract was 6.85 mg/ mL and the IC_{50} of vitamin C (6.12 µg/ mL) displayed the antioxidant activity of hydroponic pennywort extract was lower than the antioxidant activity of vitamin C.

Table 1: DPPH free radical scavenging activity of the extract and vitamin C

Hydroponic pennywort extract		**Vitamin C**	
Concentration (mg /mL)	**DPPH activity * (%)**	**Concentration (µg/ml)**	**DPPH activity * (%)**
1	24.96^{i} ±1.53	1	8.87^{j} ±1.10
2	28.59^{h} ±0.90	2	16.47^{i} ±1.16
3	31.80^{g} ±1.66	3	24.94^{h} ±0.84
4	35.95^{f} ±1.05	4	33.63^{g} ±0.71
5	39.29^{e} ±1.16	5	41.01^{f} ±0.94
6	46.86^{d} ±2.49	6	49.38^{e} ±0.88
7	49.02^{d} ±1.29	7	57.07^{d} ±1.00
8	54.89^{c} ±2.16	8	68.44^{c} ±0.86
9	61.69^{b} ±0.98	9	73.14^{b} ±1.11
10	65.71^{a} ±1.6 0	10	78.14^{a} ±0.88
Regression equation: y = 5.45x + 12.63 (R^2 = 0.93)		**Regression equation: y = 8.03x + 0.84 (R^2 = 0.99)**	
IC_{50} = 6.85 mg/mL		**IC_{50} = 6.12 (µg/mL)**	

(*) The values were the average values of three repetitions. In the same column, values followed by a different letter represent statistically significant differences ($p<0.05$).

Polyphenol and flavonoid compounds in pennywort were the main ingredients with antioxidant properties (Jhansi and Kola (2019). According to the study of Pittella (2009), the extract from pennywort leaves had the ability to remove DPPH free radicals and the IC_{50} value was 31.25 µg/mL, while the IC_{50} of vitamin C) was 2.50 µg/mL.

3.2 Antibacterial activities of hydroponic pennywort tuber extract for Escherichia coli and Staphylococcus aureus

Antibacterial activities *of Escherichia coli* and *Staphylococcus aureus* of hdroponic pennywort tuber were investigated and evaluated by the diameter of the antibacterial rings. The appearance of antibacterial rings around the wells in the agar plate is due to the antibacterial active substances of the extract diffused from the center of the wells to the surrounding wells and inhibited the growth of bacteria.

The results depicted in Figure 2 show that the extract derived from the hydroponic pennywort tuber extracts (samples extracted with ethanol: A and C) exhibited antibacterial activity against*Escherichia coli* and *Staphylococcus aureus.*Meanwhile, samples extracted with water (B and D) did not demonstratecorresponding antibacterial properties. The size of thesterile zone diameter correlates with the antimicrobial activity resulting fromthe extraction process, while smaller diameters suggest weaker antimicrobial effects.

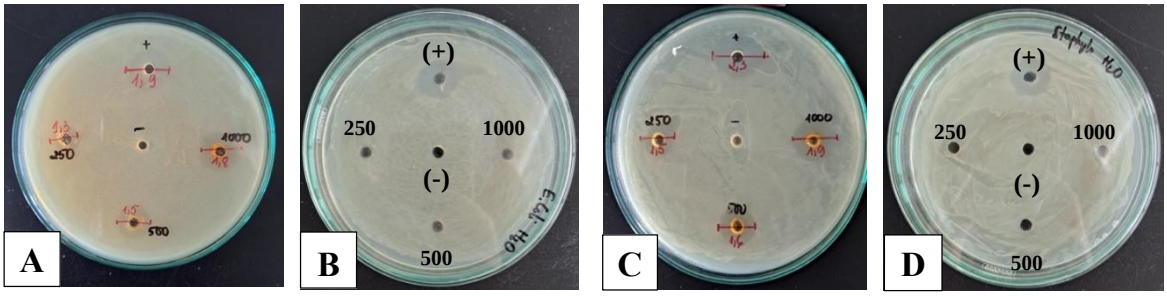

Figure 2: Antibacterial rings of the hydroponic pennywort tuber extract for Escherichia coli and Staphylococcus aureus

A, B – Antibacterial ability for Escherichia coli of samples extracted with ethanol and water at different concentrations (250 mg/mL, 500 mg/mL, and 1000 mg/mL).

C, D – Antibacterial ability for Staphylococcus aureus of samples extracted with ethanol and water at different concentrations 250 mg/mL, 500 mg/mL, and 1000 mg/mL).

(+) the positive control, Ampicillin 100 µg/mL.

(-) the negative control, DMSO 30%.

Table 2: Antibacterial ring's diameters of Escherichia coli and Staphylococcus aureus of the hydroponic pennywort tuber extracts

Extract concentration	**Antibacterial ring's diameter for *E. coli** (mm)**	**Antibacterial ring's diameter for *S. aureus** (mm)**
250 mg/mL	$7.33^{b} \pm 1.53$	$8.00^{c} \pm 1.00$
500 mg/mL	$9.67^{b} \pm 2.08$	$10.67^{b} \pm 0.58$
1000 mg/mL	$12.67^{a} \pm 0.58$	$14.33^{a} \pm 1.15$
Ampicillin 100 µg/mL	$14.33^{a} \pm 1.15$	$15.67^{a} \pm 1.53$
CV%	27.95	27.11

(*): the average value of three repetitions.

Negative control: DMSO 30%. Positive control: Ampicillin 100 µg/mL.

Table 2 showsthat the extract concentrations at/or more than 250 mg/mL had the ability to fight against *Escherichia coli*. The extract' antibacterial ability at a concentration of 1000 mg/mL (12.67 ± 0,58 mm) was outstanding compared to other concentrations (250 mg/mL – 7.33 ±1,53 mm and 500 mg/mL - 9,67 ± 2,08 mm) and lower than the positive control Ampicillin 100 µg/mL ($14.33^{a} \pm 1.15$ mm), no statistically significant difference ($p>0.05$).

For *Staphylococcus aureus* (Table 2), the extract displayed the antibacterial activities for all the extract concentrations and the antibacterial ring's diameter values were statistically significant difference ($p>0.05$). The extract at a concentration of 1000 m g/mL showed the highest antibacterial ability, the antibacterial ring's diameter of 14.33 ± 1,15 mm. The antibacterial ability of the extract for *Staphylococcus aureus* was lower than the positive control (Ampicillin 100 µg/mL, the ring's diameter of 15,67 ± 1,53 mm) and no statistically significant difference ($p>0.05$).

According to the study of Mamtha *et al* (2004), the antibacterial properties of Indian pennywort leaf extract ccould fight bacteria that cause intestinal diseases. The minimum concentration to inhibit *Escherichia coli* (with an antibacterial ring's diameter of 15 – 20 mm) was 100mg/mL; and for *taphylococcus aureus* at the minimum concentration (with an antibacterial ring's diameter of less than 15 mm) was 100mg/mL.

Extracts from the leaves and roots of pennywort had the antibacterial effects for pathogenic bacteria. Using ethanol solvent to extract compounds from the pennywort had higher efficiency than chloroform and water. The minimum inhibitory concentration for *Escherichia coli* was 2.5 mg/mL and for *Staphylococcus aureus* was 5.0 mg/mL (Nasution *et al*., 2018).

The antibacterial activity of pennywort extract was attributed to the compounds such as alkaloids, cardiac glycosides, saponins, tannins, flavonoids, terpenoids, and phenols, that inhibit Gram-negative bacteria such as *Escherichia coli, Vibrio cholera, Pseudomonas aeruginosa, Samolnella senftenberg, Shigella dysenteriae* and Gram-positive bacteria such as *Staphylococcus aureus, Streptococcus mutans, Streptococcus pneumoniae, Bacillus anthracis,* and *Bacillus subtilis*. Therefore, the pennywort extract could be used as a substitute for tetracycline (Sieberi *et al*,. 2020).

4. CONCLUSION

The study has identified the antibacterial and antioxidant activities of extracts derived from hydroponic pennywort tuber (*Centella asiatica* (L.) Urban). The the extract (IC_{50}) demonstrated an antioxidant activity with an IC value of 6.85 mg/mL. The extract also exhibited efficacy againstfoodbornebacteria such as *Escherichiacoli* and pathogenicbacteria - *Staphylococcus aureus* at a concentration of 250 mg/mL. The results show that the hydroponic pennywort tuber extract may contain bioactive compounds. Given these potential results, it is necessary to conduct furtherresearch on hydroponically grown pennywort tubers. This research could lead to development of new applications in food, pharmaceutical, and cosmetic fields, makingthe most of its valuable medicinal properties of pennywort to create high-value products.

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