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INVESTIGATION ON PHYTOCHEMICAL COMPOSITION, ANTIOXIDANT ACTIVITY, TOTAL PHENOLIC AND FLAVONOID CONTENTS OF *LUDWIGIA PERUVIANA*

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Abstract

Ludwigia peruviana as a member of the family Onagraceae has been reported to possess various biological activities; however, information on its antioxidant properties and phytochemical composition remains limited. This study aimed to investigate the phytochemical constituents and evaluate the antioxidant activity as well as the total phenolic and flavonoid contents of the ethanolic extract of L. peruviana. Aerial parts of L. peruviana were collected in Can Tho City, Viet Nam, between October and November 2024 and extracted by ultrasonic-assisted extraction using 99% ethanol. The phytochemical constituents were determined by employing standard qualitative chemical tests. Antioxidant activity was evaluated using the DPPH radical scavenging assay, while total phenolic content (TPC) and total flavonoid content (TFC) were determined by the Folin–Ciocalteu method and the aluminum chloride colorimetric assay, respectively. This study demonstrated the occurrence of various secondary metabolites, including phenolics, flavonoids, alkaloids, tannins, fixed oil, carotenoids, saponins and triterpenoids. The ethanolic extract exhibited weak DPPH radical-scavenging activity, with an IC_{50} value of 229.96 µg/mL. The TPC and TFC were 125.81 ± 7.99 mg GAE/g extract and 39.20 ± 0.49 mg QE/g extract, respectively. This is the first report on phytochemical composition, DPPH radical scavenging activity and phenolic–flavonoid contents of L. peruviana. These findings provide a scientific basis for further phytochemical investigations and evaluation of other biological activities of this species.

Keywords: *antioxidant activity, Ludwigia peruviana, phytochemical screening, total flavonoid content, total phenolic content.*

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KHẢO SÁT THÀNH PHẦN HÓA HỌC, HOẠT TÍNH KHÁNG OXY HÓA, HÀM LƯỢNG PHENOLIC TỔNG VÀ FLAVONOID TỔNG CỦA LOÀI RAU MƯƠNG *LUDWIGIA PERUVIANA*

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Tóm tắt

Ludwigia peruviana, thuộc họ Onagraceae, đã được chứng minh có nhiều hoạt tính sinh học; tuy nhiên, các thông tin về khả năng kháng oxy hóa và thành phần hóa học của loài này vẫn còn hạn chế. Nghiên cứu này nhằm khảo sát sơ bộ thành phần hóa học, đánh giá hoạt tính kháng oxy hóa, xác định hàm lượng phenolic tổng và flavonoid tổng của cao chiết ethanol của loài L. peruviana. Phần trên mặt đất của L. peruviana được thu hái tại thành phố Cần Thơ, Việt Nam, trong khoảng thời gian từ tháng 10 đến tháng 11 năm 2024 và được chiết xuất bằng phương pháp siêu âm với ethanol 99%. Khảo sát sơ bộ thành phần hóa học được thực hiện thông qua các phản ứng định tính đặc trưng. Hoạt tính kháng oxy hóa được đánh giá bằng phương pháp bắt gốc tự do DPPH, trong khi hàm lượng phenolic tổng (TPC) và flavonoid tổng (TFC) được xác định lần lượt bằng phương pháp Folin–Ciocalteu và phương pháp tạo phức với nhôm clorid. Kết quả khảo sát sơ bộ thành phần hóa học cho thấy L. peruviana có chứa nhóm phenolic, flavonoid, alkaloid, tannin, dầu béo, carotenoid, saponin và triterpenoid. Cao chiết ethanol thể hiện hoạt tính bắt gốc DPPH yếu, với giá trị IC_{50} là 229,96 µg/mL. Hàm lượng TPC và TFC lần lượt đạt 125,81 ± 7,99 mg GAE/g cao chiết và 39,20 ± 0,49 mg QE/g cao chiết. Đây là công bố đầu tiên về thành phần hóa học, hoạt tính kháng oxy hóa và hàm lượng phenolic–flavonoid của L. peruviana. Những kết quả này góp phần cung cấp cơ sở khoa học cho các nghiên cứu tiếp theo về thành phần hóa học cũng như đánh giá các hoạt tính sinh học khác của loài cây này.

Từ khóa: *hoạt tính kháng oxy hóa, Ludwigia peruviana, khảo sát thành phần hóa học hàm lượng flavonoid tổng, hàm lượng phenolic tổng.*

1. Introduction

Natural sources with antioxidant potential have attracted considerable interest due to their roles in preventing oxidative stress-related diseases. The genus *Ludwigia* (Onagraceae) comprises numerous species that have been reported to exhibit antioxidant, antibacterial, anti-inflammatory, and cytoprotective activities. Phytochemical investigations of several *Ludwigia* species have revealed the presence of flavonoids, phenolic acids, triterpenoids, coumarins, and related secondary metabolites, which are considered responsible for these biological effects (Averett et al., 1990; Ayinampudi et al., 2013). In particular, compounds such as quercetin, luteolin, and gallic acid have been identified in *L. hyssopifolia* and *L. octovalvis*, supporting their reported antioxidant potential (Averett et al., 1990; Ayinampudi et al., 2013).

Furthermore, recent studies on *Ludwigia peruviana* have mainly focused on phytochemical profiling and *in silico* pharmacological evaluation. Midhuna et al. (2024) identified several bioactive constituents from the methanolic extract of *L. peruviana* using GC–MS analysis, while a subsequent study (Midhuna et al., 2025) further characterized compounds present in the ethyl acetate fraction. These studies suggested that *L. peruviana* may possess diverse biological activities, including antioxidant, antibacterial, and anti-inflammatory potentials. However, experimental evidence regarding the antioxidant activity and polyphenolic composition of this species remains limited, particularly in Vietnam. Therefore, further investigation of antioxidant-related phytochemicals in *L. peruviana* is still necessary. In Vietnam, current research is mostly restricted to ecological distribution and morphological descriptions. This indicates a clear research gap in the comprehensive investigation of the phytochemical composition and biological activities of *L. peruviana*, emphasising the necessity for further scientific exploration. Therefore, this study investigates the preliminary phytochemical constituents, evaluates the antioxidant activity, and quantifies the total phenolic (TPC) and flavonoid (TFC) contents of *L. peruviana*, providing baseline data for further biological research.

2. Materials and methods

2.1. Research subjects

Ludwigia peruviana was collected from October to November 2024 in Can Tho City, Vietnam. The identification was conducted by Dr. Dang Minh Quan, School of Education, Can Tho University, Viet Nam. A voucher specimen with the code No LP-24 was deposited in the herbarium of the Faculty of Basic Sciences, Can Tho University of Medicine and Pharmacy, Can Tho, Viet Nam.

2.2. Research methods

2.2.1. Phytochemical constituents screening

The ethanol extract of *L. peruviana* was prepared using an ultrasonic-assisted extraction (UAE) procedure. Briefly, 20 g of ground, air-dried material was exhaustively extracted with 500 mL of ethanol. The mixture was filtered through Whatman® qualitative paper and concentrated using a rotary evaporator (Heidolph™ Hei-VAP, Germany) under reduced pressure (175 mbar, 40°C) at a speed of 50 rpm. The extraction yield was calculated based on the dry extract weight relative to the dried plant material weight.

The phytochemical constituents of the *L. peruviana* ethanol extract were screened using standard qualitative methods. The presence of alkaloids was confirmed by Dragendorff's and Mayer's tests, while phenolics and flavonoids were identified using ferric chloride and lead acetate tests, respectively. Saponins were detected via the foam test, and

triterpenoids were evidenced by Salkowski's test. The extract was also screened for tannins (gelatin test), carbohydrates (Fehling's test), and glycosides (Liebermann's & Salkowski's tests). Additionally, the presence of carotenoids, fixed oils, and amino acids/proteins was determined using antimony trichloride, the filter paper absorption test, and the concentrated nitric acid test, respectively. All procedures were conducted following established protocols (Audu et al., 2007; Tiwari et al., 2011; Silva et al., 2017).

The ethanol extract was evaluated for its antioxidant activity and quantified for total phenolic content (TPC) and total flavonoid content (TFC) using standard spectrophotometric methods. The total phenolic content was determined using the Folin–Ciocalteu reagent according to the method described by Singleton et al. (1999). The total flavonoid content was measured by the aluminum chloride colorimetric method following Bhaigyabati et al. (2015). The antioxidant activity was evaluated using the DPPH radical scavenging assay as described by Brand-Williams et al. (1995) with slight modifications. Relevant methodological information and antioxidant data reported for *Ludwigia* species were also referred to in Shawky et al. (2023) to ensure methodological consistency and comparability with previous studies.

2.2.2. Determination of antioxidant activity

The antioxidant activity was determined based on the ability of the extract to scavenge the stable free radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) in methanol, following the method described by Brand-Williams et al. (1995) with slight modifications. A 100 µL aliquot of the extract solution at different concentrations was added to 100 µL of 0.1 mM DPPH solution in each well of a 96-well plate. The mixture was incubated in the dark at room temperature for 30 minutes, and the absorbance was measured at 517 nm. Each measurement was carried out in triplicate, and the mean value was recorded. Vitamin C (ascorbic acid) was used as a positive control under the same conditions.

The percentage of DPPH radical inhibition was calculated using the formula:

$$\text{Inhibition (\%)} = \frac{AbS_c - AbS_s}{AbS_c} \; x \; 100$$

Where:

AbS_c is the absorbance value of the negative control sample (containing only the solvent).

AbS_s is the absorbance value of the test sample.

The IC_{50} value (the concentration required to inhibit 50% of the radicals) was determined from the regression curve plotted between the percentage inhibition and extract concentration.

2.2.3. Determination of total phenolic content (TPC)

The total phenolic content was determined using the Folin–Ciocalteu colorimetric method described by Singleton et al. (1999) with slight modifications. Gallic acid standard solutions were prepared at concentrations ranging from 2 to 20 µg/mL to establish the calibration curve. A reaction mixture consisting of 250 µL of the extract (or gallic acid standard solution), 250 µL of distilled water, and 250 µL of Folin–Ciocalteu reagent was prepared. After 5 minutes, 250 µL of 10% Na_2CO_3 was added, and the mixture was incubated at 40°C for 30 minutes. The absorbance was measured at 765 nm. Each measurement was performed in triplicate, and the average value was used for calculation.

The total phenolic content was expressed as milligrams of gallic acid equivalent per gram of extract (mg GAE/g extract) and calculated using the equation:

$$Total\ Phenolic\ content = \frac{AbS_S - b}{a\ x\ C}\ x\ 1000$$

Where:
AbS_s is the absorbance value of the sample;
a and b are the constants obtained from the calibration curve of gallic acid;
C is the concentration of the sample (50 µg/mL);
1000 is the conversion factor to express the results as mg GAE/g extract.

2.2.4. Determination of total flavonoid content (TFC)

The total flavonoid content was determined using the aluminum chloride colorimetric method described by Bag et al. (2015) with slight modifications. Quercetin standard solutions were prepared at concentrations ranging from 5 to 100 µg/mL for calibration curve construction. A reaction mixture containing 200 µL of the extract (or quercetin standard solution), 200 µL of distilled water, and 40 µL of 5% $NaNO_2$ solution was prepared. After 5 minutes, 40 µL of 10% $AlCl_3$ was added and allowed to react for 6 minutes, followed by the addition of 400 µL of 1 M NaOH and distilled water to bring the total volume to 1 mL. The absorbance was measured at 510 nm. Each test was carried out in triplicate. The total flavonoid content was expressed as milligrams of quercetin equivalent per gram of extract (mg QE/g extract) and calculated as follows:

$$Total\ Flavonoid\ content = \frac{AbS_S - b}{a\ x\ C}\ x\ 1000$$

Where:
AbS_s is the absorbance value of the sample;
a and b are the constants obtained from the calibration curve of quercetin;
C is the concentration of the sample (50 µg/mL);
1000 is the conversion factor to express the results as mg GAE/g extract.

2.2.5. Statistical analysis

All experiments were carried out in triplicate and the results were expressed as mean ± standard deviation (SD). Linear regression analysis was performed using Microsoft Excel 2021 to construct calibration curves and determine IC_{50} values. The coefficient of determination (R^2) was used to evaluate the linearity of the calibration models.

3. Results

3.1. Phytochemical constituents screening

Preliminary phytochemical screening of the *L. peruviana* extract indicated the presence of diverse secondary metabolites, including phenolics, flavonoids, alkaloids, tannins, fixed oils, carotenoids, saponins, and triterpenoids (Table 1). Among these, phenolics and flavonoids were identified as the predominant classes of compounds.

Table 1. Phytochemical screening of *L. peruviana*

Phytochemical group	Ludwigia peruviana
Alkaloids	+

Phenolics	+
Flavonoids	+
Saponins	+
Triterpenoids	+
Tannins	+
Carbohydrates	-
Glycosides	-
Carotenoids	+
Fixed oil	+
Amino acids and proteins	-

(-): negative; (+): positive

3.2. Antioxidant activity

The results of the DPPH free radical scavenging activity of the ethanolic extract from the aerial parts of *L. peruviana* are presented in Table 2.

Table 2. DPPH radical scavenging activity of *L. peruviana* extract compared with ascorbic acid

Ludwigia peruviana		**Ascorbic acid**	
Concentration (µg/mL)	**Percentage (%)**	**Concentration (µg/mL)**	**Percentage (%)**
50	8.89 ± 0.58	1	11.10 ± 1.23
100	23.13 ± 0.53	2	19.99 ± 0.67
250	56.62 ± 1.12	3	29.78 ± 0.24
350	77.79 ± 0.56	5	47.53 ± 0.37
400	83.74 ± 0.04	7	64.80 ±0.65
IC_{50}	229.96 ± 0.09	IC_{50}	5.34 ± 0.06

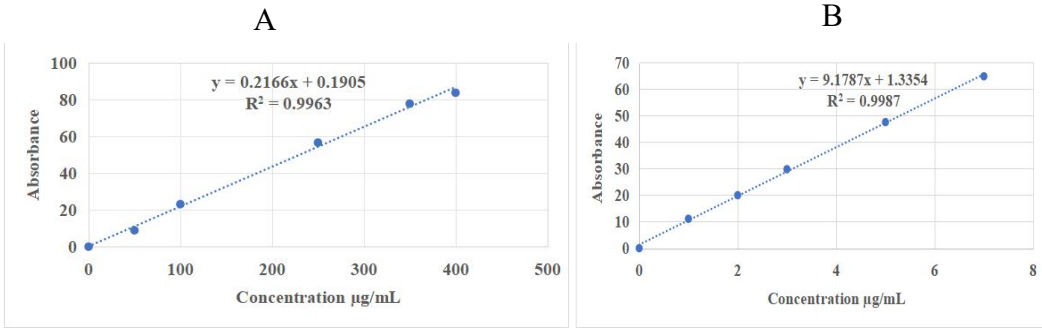

Figure 1. DPPH calibration curves: (A) *L. peruviana* extract and (B) ascorbic acid

The DPPH radical scavenging activity of the ethanolic extract from the aerial parts of *L. peruviana* was evaluated, and the regression equation obtained was y = 0.2166x + 0.1905 with a determination coefficient (R^2 = 0.9963). The extract exhibited a weak antioxidant activity with an IC_{50} value of 229.96 ± 0.09 µg/mL (Figure 1A).

In comparison, ascorbic acid (vitamin C), used as the positive control, showed a markedly stronger antioxidant capacity with an IC_{50} value of 5.34 ± 0.06 µg/mL. Thus, the IC_{50} of the *L. peruviana* extract was approximately 43 times higher than that of vitamin C, indicating substantially lower free-radical scavenging efficiency (Figure 1B). According to Nurmazela et al. (2022), antioxidant strength is classified as very strong (<50 µg/mL), strong (50–100 µg/mL), moderate (101–150 µg/mL), weak (151–200 µg/mL), and very weak (>200 µg/mL).

3.3. Determination of total phenolic content (TPC) and total flavonoid content (TFC)

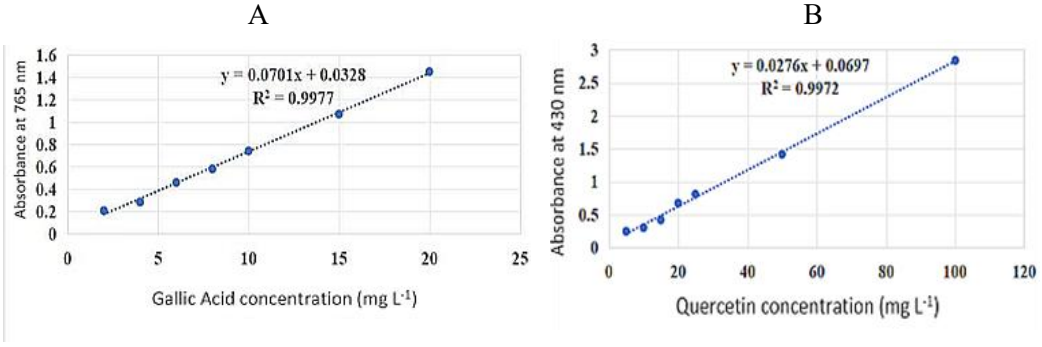

Figure 2. Representative standard curve for 96-well assay: (A) total phenolic content, and (B) total flavonoid content

Table 3. Total phenolic content in the ethanolic extract of the aerial parts of *L. peruviana*

Extract	Extract concentration (ppm)	Absorbance (765 nm)	Total phenolic equivalent concentration (µg GAE/mL)	Mean phenolic content (mg GAE/g extract)	Total phenolic content
L. peruviana	200	1.92	134.60	125.81	125.81 ± 7.99
	300	2.54	118.98		
	400	3.50	123.83		

The total phenolic content (TPC) of the *L. peruviana* ethanolic extract was quantified using the Folin–Ciocalteu method, with results calculated from the gallic acid calibration curve ($y = 0.0701x + 0.0328$, $R^2 = 0.9977$) (Figure 2A). The extract exhibited a TPC value of 125.81 ± 7.99 mg GAE/g extract, indicating a considerable presence of phenolic compounds (Table 3).

Table 4. Total flavonoid content in the ethanolic extract of the aerial parts of *L. peruviana*

Extract	Extract concentration (ppm)	Absorbance (510 nm)	Total flavonoid equivalent concentration (µg QE/mL)	Mean flavonoid content (mg QE/g extract)	Total flavonoid content
L. peruviana	120	0.19	38.99	39.20	39.20 ± 0.49
	140	0.22	38.89		
	160	0.24	39.77		

Similarly, the total flavonoid content (TFC) was determined using the aluminum chloride ($AlCl_3$) colorimetric assay. The quercetin calibration curve ($y = 0.0276x + 0.0697$, $R^2 = 0.9972$) (Figure 2B) was used for calculation, yielding a TFC value of 39.20 ± 0.49 mg QE/g extract (Table 4).

4. Discussion

4.1. Phytochemical constituents screening

The detection of diverse classes of secondary metabolites in the *L. peruviana* extract indicates a wide range of potential pharmacological properties. Notably, synergistic interactions among phenolics, flavonoids, and alkaloids are considered key contributors to antioxidant capacity and other related biological activities, as previously reported for medicinal plants and members of the Onagraceae family (Tiwari et al., 2011; Vitale et al., 2022; Teffo et al., 2024).

4.2. Antioxidant activity

The ethanolic extract of *L. peruviana* showed weak antioxidant activity in the DPPH assay (IC_{50} = 229.96 μg/mL), which is classified as "very weak" (Nurmazela et al., 2022) and was markedly lower than that reported for other *Ludwigia* species, commonly associated with higher phenolic and flavonoid contents. The low activity observed may be partly attributed to the use of absolute ethanol, as hydroalcoholic solvents have been reported to extract phenolic compounds more efficiently, as well as to the exclusive use of aerial parts, which may contain lower levels of antioxidant constituents. Moreover, since the DPPH assay reflects only hydrogen-donating ability, the weak scavenging activity does not preclude the presence of bioactive compounds acting through other antioxidant mechanisms, thereby warranting further investigation using alternative extraction conditions and complementary assays. Moreover, since the DPPH assay reflects only hydrogen-donating ability, the weak scavenging activity does not preclude the presence of bioactive compounds acting through other antioxidant mechanisms, thereby warranting further investigation using alternative extraction conditions and complementary assays. Although the antioxidant activity was classified as very weak according to the DPPH model, the considerable phenolic and flavonoid contents observed in the extract suggest that *L. peruviana* may still represent a potential source of bioactive compounds. Therefore, this species could be further investigated for other pharmacological properties, including anti-inflammatory, antimicrobial, and cytoprotective activities. A limitation of this study is that antioxidant activity was evaluated using only the DPPH assay. Therefore, additional antioxidant models such as ABTS, FRAP, and CUPRAC should be employed in future studies to provide a more comprehensive assessment of the antioxidant potential of *L. peruviana*.

4.3. Total phenolic content (TPC) and total flavonoid content (TFC)

The ethanolic extract of *L. peruviana* contained 125.81 ± 7.99 mg GAE/g extract of total phenolics and 39.20 ± 0.49 mg QE/g extract of total flavonoids, indicating a considerable polyphenolic content. However, the extract exhibited weak DPPH radical scavenging activity, suggesting that antioxidant capacity depends not only on the total quantity of phenolic compounds but also on their structural characteristics and reactivity. Compared with previously reported *Ludwigia* species, the TPC and TFC values of *L. peruviana* were lower than those observed in *L. hyssopifolia* and *L. octovalvis*, which are known to exhibit stronger antioxidant activities. Variations in polyphenolic contents among *Ludwigia* species may result from differences in plant species, geographical origin, environmental conditions, extraction solvents, and plant parts used for extraction. In particular, hydroethanolic solvents have frequently been reported to provide better recovery of phenolic compounds than absolute ethanol.

Phenolic compounds possessing ortho-dihydroxy groups generally exhibit stronger radical scavenging activity due to enhanced hydrogen atom transfer (HAT) and electron transfer (ET) capacities. In contrast, glycosylated flavonoids or polymerized phenolics may display reduced accessibility to DPPH radicals, resulting in lower apparent antioxidant activity despite relatively high total phenolic content. These findings suggest that the antioxidant potential of *L. peruviana* may depend on the composition and structural characteristics of individual phenolic constituents rather than solely on total phenolic concentration.

5. Conclusion

This study represents the first comprehensive investigation of the phytochemical composition, antioxidant activity, and polyphenolic content of the ethanolic extract obtained from the aerial parts of *L. peruviana*. Phytochemical screening revealed a diverse profile of secondary metabolites, with phenolic and flavonoid compounds being predominant. The extract exhibited weak DPPH radical-scavenging activity, with an IC_{50} value of 229.96 µg/mL. Quantitative analysis showed relatively high levels of total phenolic and flavonoid contents, reaching 125.81 ± 7.99 mg GAE/g extract and 39.20 ± 0.49 mg QE/g extract, respectively. Although the antioxidant activity was limited in the DPPH assay, the notable polyphenolic content suggests that *L. peruviana* may exert biological effects through alternative antioxidant mechanisms or other pharmacological pathways, thereby warranting further phytochemical and biological investigations. These findings contribute new phytochemical and antioxidant-related data for this Ludwigia in Vietnam and provide a scientific basis for future studies on the isolation of bioactive compounds and evaluation of other pharmacological activities, including anti-inflammatory and antimicrobial effects.

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