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Research Article

A comparative study of DPPH radical scavenging property and antiproliferative potential on human hepatic cancer (HepG2) cells of methanolic extracts from different parts of *Ludwigia hyssopifolia* and *Ludwigia adscendens*

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Abstract

Ludwigia hyssopifolia and *Ludwigia adscendens* are two indigenous and common plant species in Vietnam with hepatoprotective property mentioned in numerous articles published in international journals. Therefore, the current paper aimed to evaluate the physicochemical parameters, total polyphenolic and flavonoid contents of the methanolic extracts from 04 parts of *Ludwigia hyssopifolia* and *Ludwigia adscendens* in Mekong delta, Vietnam. Most importantly, antioxidant properties by DPPH radial scavenging assay and antiproliferative potentials on human hepatic cancer (HepG2) cells of these multiple extracts also were determined in the *in vitro* models. These results showed that physicochemical parameters were within allowable limits according to the literatures. Among the investigated samples, the leaf and whole plant extract from *Ludwigia hyssopifolia* had the highest values of total phenolic and flavonoid contents being 196.55 ± 5.05 mg GAE/g and 64.72 ± 4.63 mg QE/g respectively. Additionally, the methanol whole plant extract of *Ludwigia hyssopifolia* and the methanol leaf extract of *Ludwigia adscendens* show the greatest antioxidant properties in DPPH scavenging assay with IC_{50} values of 44.48 ± 4.29 µg/mL and 26.11 ± 0.37 µg/mL respectively. Furthermore, the methanolic extract from *Ludwigia hyssopifolia* leaves displays the most potential anti-HepG2 activity among all surveyed samples. Therefore, the most promising extracts need further evaluation for potential herbal product developments.

Keywords: *Ludwigia hyssopifolia, Ludwigia adscendens, physicochemical, DPPH, HepG2.*

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1. INTRODUCTION

Ludwigia hyssopifolia (G. Don) Exell belongs to genus *Ludwigia of* family Onagraceae commonly known as Linear Leaf Water Primrose and "Rau mương thon" in Vietnam, which is extensively grown in many Asian nations. In India, the plant is conventionally used in the treatment of jaundice [1]. *Ludwigia adscendens* ("Rau dừa nước" in Vietnamese languages) is a popular vegetable food in Mekong delta, Vietnam and a perennial floating aquatic herbaceous plant, also in the genus *Ludwigia* of the family Onagraceae, which is commonly known as Water Dragon [2].

In terms of phytoconstituents, *L. hyssopifolia* consisted of various active phytochemicals which include flavonoids, steroids, triterpenoids, phenolics, and coumarics [3]. Meanwhile, using high-performance liquid chromatography (HPLC), several bioactive compounds have been identified from *L. adscendens*, including coumaric acid, gallic acid, myricetin, saponins, triterpenoids, flavonoids, and oligosaccharides [4]. Pharmacological studies have shown that *L. hyssopifolia* exhibits a variety of biological activities including anticancer, antibacterial, antidiarrheal, and anti-ulcer and hepatoprotective activities [5] while these studies further showed that *L. adscendens* have a broad spectrum of biological functions, such as anti-diabetic, hepatoprotective, cytotoxic, anti-oxidative, iron chelating, anti-bacterial, anti-lipoxygenase and anti-glucosidase activities [6].

When it comes to herbal remedies, *L. hyssopifolia* is a heat-clearing, detoxicating, and cooling blood flora [7]. Similarly, in the 'Flora of China', *L. adscendens* has been traditionally used in heat clearance and detoxification, diuretic detumescence, and can also treat snake bites [2]. According to traditional Chinese medicine theory, the accumulation of heat and toxins plays a key role in the occurrence and development of cancers, including liver cancers. Multiple evidences have shown that decoction or products originated of heat-clearing and detoxicating herbs exhibited favorable anticancer effects directly or through enhancing the activities of chemotherapeutic drugs [8]. However, there are few studies on the hepatoprotective effect of these medicinal plants in Vietnam. For the aforementioned reasons, this study aimed to estimate the total polyphenol and flavonoid contents, evaluate on the *in vitro* antioxidant properties, and anti-proliferative potetials against HepG2 cells of the methanolic extracts from various parts (whole plants, leaves, stems, and roots) from *Ludwigia hyssopifolia* and *Ludwigia adscendens* grown in Mekong delta, Vietnam.

2. MATERIALS AND METHODS

2.1. Plant material collection

Four various parts (whole plants, leaves, stems, and roots) of *L. hyssopifolia* and *L. adscendens* were collected in December 2022 in Tan Thanh commune, Thanh Binh district, Dong Thap province, Mekong delta, Vietnam (10.582002°N, 105.454175°E). The samples authenticated by the botanists at Department of Biology Education, Can Tho Univeristy, Vietnam as described in the references [9] and combined with DNA barcoding as a species identification method. Identification of DNA barcode sequence was conducted at Phu Sa Genomics Company, Vinh Long province, Vietnam. The leaf samples were dried in silica gel and stored at room temperature until usage.

DNA barcoding for species identification:

DNA was extracted with CTAB method (cetyl trimethyl ammonium bromide) as described by Doyle and Doyle [10]. PCR reaction for rbcL regions was amplified using the composition as follows: 20 µL 2X Mytaq Mix (Bioline, UK), 20 µL DNA, 1 µL primer rbcLF 0.4 µM, 1 µL rbcLR 0.4 µM , and PCR water (SigmaAldrich, USA) to final volume of 50 µL. The PCR reaction conditions were as follows: initial denaturation at 94°C for 4 min; then 35 cycles of 30 s at 94°C, 30 s at 55°C, and 1 min at 72°C. Finally, an additional of 10 min was continued at 72°C to complete the reaction. DNA after being extracted and purified will be checked by electrophoresis on 1% agarose gel. The PCR results were subjected to electrophoresis and subsequently purified using the Wizard SV Gel kit and PCR Clean-up System (Promega) following the Sanger technique [11]. The sequencing findings were processed using the most recent version 7.0.5 of the BioEdit program [12]. Subsequently, the BLAST algorithm was employed on the NCBI gene bank system, which is administered by the National Center for Biotechnology Information, for the purpose of species identification.

Then all plant materials were air-dried under shade, ground to a fine powder using a laboratory mill and kept in zipper bag with appropriate label before its usage. The powder moisture content determined by an Kern DAB 100-3 moisture analyzer (Germany). The amount of medicinal herbs used was 1.0 g, temperature at 105-110°C, duration of 30 min.

2.2. Extraction procedure

Herbal powders of four various parts were prepared by reflux extraction system with methanol solvent as the following method.

Preparation of methanolic extracts:

The methanolic extract was prepared by packing separately the powder in a reflux process and extracted at 60°C for 60 min using methanol (solid to solvent ratio of 1:20). This process was repeated until the solvent flows out of the extraction system leaving no residue. The extract was filtered using a funnel and filter paper. The filtrate obtained were concentrated in a rotary evaporator to obtain methanolic extracts. All methanolic extracts were covered with silver foil and stored at 4°C for 48 h before further use.

Methanol has been observed to be generally more effective in extracting polyphenols with lower molecular weights [13]. Flavonoids, which are the primary constituents examined in this study, are low molecular weight polyphenolic phytochemicals secreted as a secondary metabolite in plants [14]. Therefore, methanol was used to create methanolic extracts from powdered plants.

2.3. Physicochemical parameters

Total ash and acid insoluble ash contents were carried out by procedures according to Vietnamese Pharmacopoeia (the fifth edition, Appendix 9.8 and 9.7) while the method for determination of water-soluble extractives was conducted according to Vietnamese Pharmacopoeia (fifth edition, Appendix 12.10, the hot extraction method), using water and ethanol as solvents [15].

Determination of total ash

Weigh accurately about 3 gram of the powdered drug in silica crucible. Incinerate the powdered drug by increasing the heat gradually until the sample was free from carbon and cool it keep it in a desiccator. The content of total ash was calculated in mg/g of air-dried material.

Determination of acid insoluble ash

To the crucible containing the total ash, 25 mL of hydrochloric acid was added, covered with a watch glass and boiled gently for 5 min. The watch glass was rinsed with 5 mL of hot water and this liquid was added to the crucible. The insoluble matter was collected on an ashless filter paper and washed with hot water until the filtrate was neutral. The insoluble matter left on the filter paper was transferred to the original crucible, dried on a hot plate and ignited to constant weight. The residue was allowed to cool in a suitable desiccator for 30 min, and then weighed without delay. The content of acid-insoluble ash was calculated in mg/g of air-dried material.

Determination of water-soluble substances and ethanol-soluble substances

Place about 4.0 g of coarsely powdered air-dried material, accurately weighed, in a glass-stoppered conical flask. Add 100 mL of water or ethanol and weigh to obtain the total weight including the flask. Shake well and allow to stand for 1 hour. Attach a reflux condenser to the flask and boil gently for 1 hour; cool and weigh. Readjust to the original total weight with the solvent specified in the test procedure for the plant material concerned. Shake well and filter rapidly through a dry filter. Transfer 25 mL of the filtrate to a tared flat-bottomed dish and evaporate to dryness on a water-bath. Dry at 105°C for 6 hours, cool in a desiccator for 30 min, then weigh without delay. Calculate the content of extractable matter in mg per g of air-dried material.

2.4. Total phenolic and total flavonoid content

The total phenolic content was measured according to the method of Singleton *et al.* (1999) [16]. Slowly, 0.5 mL of sample was added to 4.5 mL of distilled water and was mixed with 0.2 mL of the Folin–Ciocalteu phenol reagent and 0.5 mL saturated solution of Na_2CO_3. Finally, 4.3 mL of distilled water was added to the solution. The reaction mixtures were incubated for 60 min in the dark at room temperature and then, the absorbances were measured at 765 nm. Total phenolic content was expressed as mg of gallic acid equivalents (GAE) per gram of dry sample (mg GAE/g).

The total flavonoid content was measured by a colorimetric assay of Zhishen *et al.* (1999) [17]. One hundred microliters of extract was added to 4 mL of distilled water. Then, 0.3 mL 5% sodium nitrite was added. After 5 min, 0.3 mL of 10% aluminum chloride was added. In 6 min, 2 mL of 1 M sodium hydroxide was added to the mixture. Immediately, the mixture was diluted by the addition of 3.3 mL distilled water and mixed thoroughly. The absorbance was determined at 510 nm versus a blank. Quercetin was used as standard for the calibration curve. Total flavonoids content of the extract was expressed as mg quercetin equivalents per gram of sample (mg QE/g).

2.5. DPPH radical scavenging assay

The experiment was conducted at Laboratory of Medicinal Chemistry, CTU High-tech Building, Can Tho University. Antioxidant activity of the methanolic extracts was tested using a 1,1-diphenyl-2-picryl hydrazyl (DPPH) assay as described previously [18]. The samples were dissolved in MeOH, and the solutions were dispensed into wells of a 96-well microplate with an appropriate volume. Briefly, 40 µL of DPPH solution (1000 µg/mL, in methanol) was incubated with varying concentrations of the different extracts. The final concentrations of tested samples in the mixtures ranged from 256 to 8 µg/mL. The reaction mixture was shaken well and incubated for 30 min in the dark at ambient temperature. The absorbance of the resulting solutions was measured at λ = 520 nm. Pure MeOH was used as a negative control and milk thistle extract (National Institute of Drug Quality Control, Vietnam) was used as a positive control. All data on antioxidant activity are the average of triplicate experiments. The radical scavenging activity of the extracts was expressed as IC_{50} (the concentration of the sample required to inhibit 50% of the DPPH concentration). The IC_{50} values (µg/mL) were calculated using a linear regression of plots. According to Marjoni et.al (2017), levels of antioxidant activity by DPPH assay are classified into 5 groups by IC_{50} values, including highly active (< 50 µg/mL), active (50-100 µg/mL), moderate (101-250 µg/mL), weak (250-500 µg/mL) and inactive (> 500 µg/mL) [19].

2.6. Anti-Hepatocellular Carcinoma HepG2 Activity

The experiment was conducted with support from Laboratory of Applied Biochemistry, Institute of Chemistry, Vietnam Academy Of Science And Technology. Methanolic extracts were screened on anti-HepG2 activity based on the MTT assay [20, 21].

Human hepatocellular carcinoma HepG2 (HB 8065™) was purchased from American Type Culture Collection (ATCC, USA). HepG2 cells were cultured in Dulbeccos Modified Eagle Medium (D-MEM, Sigma), supplemented with 10% fetal calf serum (Sigma) and additional necessary components. The cells were cultured under standard conditions (37°C, 5% CO_2 and 98% relative humidity). The test sample was dissolved in a dimethyl sulfoxide (DMSO) solvent to form the stock solution with a concentration of 20 mg/mL. A 2-fold serial dilution was conducted on a 96-well plate, resulting in concentration ranges of 2564, 640, 160, 40, and 10 µg/mL. The concentrations of the test in the plate were 128, 32, 8, 2, and 0.5 µg/mL. Briefly, 190 µL HepG2 cells (3×10^3 cells/well) were maintained in 96 well-culture plates in the presence of 10 µL of methanolic extract at the various concentrations for 72 h in a standard condition. After incubation, 10 µL MTT reagent (5 mg/mL) was added, and cell cultures were incubated for 4 hours. A quantity of 100 µL DMSO 100% was added to dissolve the resultant formazan crystals after the medium was removed. The absorbance (Abs) of the wells was measured in a microplate reader (BioTek Instruments, Inc., Winooski, VT, USA) at a wavelength of 540 nm. Wells without cells serve as a negative control, and their absorbance has to be subtracted from the other results. Untreated cells are the control. The growth inhibition (%) of HepG2 cells are calculated as the percentage of viability in relation to the untreated cells as described in equation (1).

$$\%\text{Inhibition} = \frac{(\text{Abs}_{\text{control }(+)} - \text{Abs}_{\text{test}})}{(\text{Abs}_{\text{control}(+)} - \text{Abs}_{\text{control}(-)})} \text{x}100 \qquad (1)$$

The IC_{50} value (50% of cytotoxicity inhibition) was determined through the %Inhibition of growth and Rawdata software as described in equation (2).

$$IC_{50} = High_{Conc} - \frac{(High_{Inh\%} - 50)x\ (High_{Conc} - Low_{Conc})}{High_{Inh\%} - Low_{Inh\%}} \quad (2)$$

$High_{Conc}$/Low_{Conc}: Tested samples at high concentration/Tested samples at low concentration.

$High_{Inh\%}$/$Low_{Inh\%}$: %Inhibition at high concentration/%Inhibition at low concentration.

2.7. Statistical analysis

Excel 2021 software (Microsoft Corporation, USA) was used for the statistical analysis. Data are presented as mean ± SD.

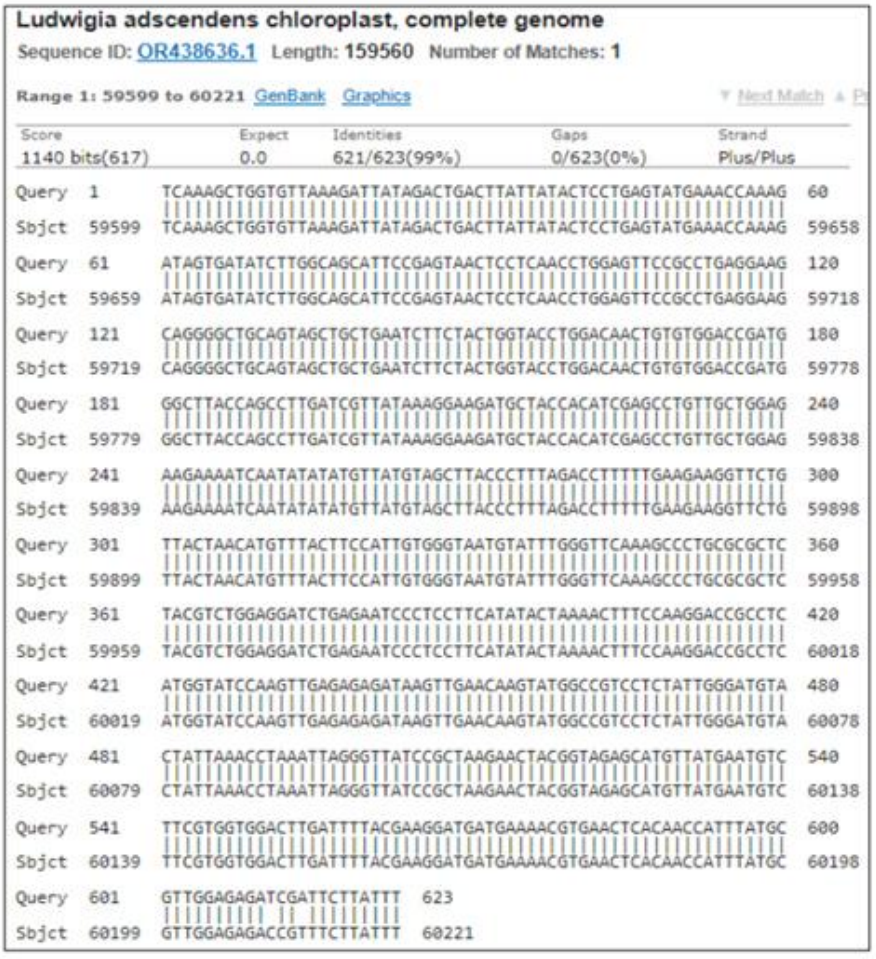
3. RESULTS AND DISCUSSION

3.1. Species identification of DNA barcode sequence

From the previous article, the results showed the percentage of identity of 100% with the species *Ludwigia hyssopifolia* [22]. Next, sequences of the *Ludwigia adscendens* sample compared with NCBI genebank were presented in Table 1 and Figure 1. The results showed that the sample was similar to the sequence of the species *Ludwigia adscendens* on genbank with query cover of 100% and percent of identity of 99.68%.

Table 1. *Sequences of the Ludwigia adscendens sample*

Description	Scientific Name	Max Score	Total Score	Query Cover	E Value	Percent identity	Acc. Length	Accession
Ludwigia adscendens chloroplast, complete genome	*Ludwigia adscendens*	1140	1140	100%	0.0	99.68%	159560	OR438636.1
Ludwigia adscendens chloroplast, complete genome	*Ludwigia adscendens*	1140	1140	100%	0.0	99.68%	159592	NC 081012.1
Ludwigia peploides ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) mRNA, complete genome	*Ludwigia peploides*	1127	1127	100%	0.0	99.20%	1428	L10222.1
Ludwigia octovalvis voucher 2014GH96 chloroplast, complete genome	*Ludwigia octovalvis*	1123	1123	100%	0.0	99.20%	159396	NC 031385.1
Ludwigia octovalvis voucher Zhu S.S.304 ribulose-1,5-bisphosphate carboxylase large subunit (rbcL) gene	*Ludwigia octovalvis*	1118	1118	100%	0.0	99.04%	717	MH050042.1



Ludwigia adscendens chloroplast, complete genome

Sequence ID: OR438636.1 Length: 159560 Number of Matches: 1

Range 1: 59599 to 60221 GenBank Graphics

Score	Expect	Identities	Gaps	Strand
1140 bits(617)	0.0	621/623(99%)	0/623(0%)	Plus/Plus

```
Query  1      TCAAAGCTGGTGTTAAAGATTATAGACTGACTTATTATACTCCTGAGTATGAAACCAAAG  60
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  59599  TCAAAGCTGGTGTTAAAGATTATAGACTGACTTATTATACTCCTGAGTATGAAACCAAAG  59658

Query  61     ATAGTGATATCTTGGCAGCATTCCGAGTAACTCCTCAACCTGGAGTTCCGCCTGAGGAAG  120
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  59659  ATAGTGATATCTTGGCAGCATTCCGAGTAACTCCTCAACCTGGAGTTCCGCCTGAGGAAG  59718

Query  121    CAGGGGCTGCAGTAGCTGCTGAATCTTCTACTGGTACCTGGACAACTGTGTGGACCGATG  180
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  59719  CAGGGGCTGCAGTAGCTGCTGAATCTTCTACTGGTACCTGGACAACTGTGTGGACCGATG  59778

Query  181    GGCTTACCAGCCTTGATCGTTATAAAGGAAGATGCTACCACATCGAGCCTGTTGCTGGAG  240
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  59779  GGCTTACCAGCCTTGATCGTTATAAAGGAAGATGCTACCACATCGAGCCTGTTGCTGGAG  59838

Query  241    AAGAAAATCAATATATATGTTATGTAGCTTACCCTTTAGACCTTTTTGAAGAAGGTTCTG  300
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  59839  AAGAAAATCAATATATATGTTATGTAGCTTACCCTTTAGACCTTTTTGAAGAAGGTTCTG  59898

Query  301    TTACTAACATGTTTACTTCCATTGTGGGTAATGTATTTGGGTTCAAAGCCCTGCGCGCTC  360
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  59899  TTACTAACATGTTTACTTCCATTGTGGGTAATGTATTTGGGTTCAAAGCCCTGCGCGCTC  59958

Query  361    TACGTCTGGAGGATCTGAGAATCCCTCCTTCATATACTAAAACTTTCCAAGGACCGCCTC  420
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  59959  TACGTCTGGAGGATCTGAGAATCCCTCCTTCATATACTAAAACTTTCCAAGGACCGCCTC  60018

Query  421    ATGGTATCCAAGTTGAGAGAGATAAGTTGAACAAGTATGGCCGTCCTCTATTGGGATGTA  480
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  60019  ATGGTATCCAAGTTGAGAGAGATAAGTTGAACAAGTATGGCCGTCCTCTATTGGGATGTA  60078

Query  481    CTATTAAACCTAAATTAGGGTTATCCGCTAAGAACTACGGTAGAGCATGTTATGAATGTC  540
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  60079  CTATTAAACCTAAATTAGGGTTATCCGCTAAGAACTACGGTAGAGCATGTTATGAATGTC  60138

Query  541    TTCGTGGTGGACTTGATTTTACGAAGGATGATGAAAACGTGAACTCACAACCATTTATGC  600
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  60139  TTCGTGGTGGACTTGATTTTACGAAGGATGATGAAAACGTGAACTCACAACCATTTATGC  60198

Query  601    GTTGGAGAGATCGATTCTTATTT  623
              ||||||||| || |||||||||
Sbjct  60199  GTTGGAGAGACCGTTTCTTATTT  60221
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Figure 1. *Comparison of the rbcL gene sequence of Ludwigia adscenden with database*

3.2. Physicochemical parameters

The total ash method is designed to measure the total amount of material remaining after ignition. This includes both "physiological ash", which is derived from the plant tissue itself, and "non-physiological" ash, which is the residue of the extraneous matter (e.g. sand and soil) adhering to the plant surface. Acid-insoluble ash is the residue obtained after boiling the total ash with dilute hydrochloric acid, and igniting the remaining insoluble matter. This measures the amount of silica present, especially as sand and siliceous earth. Total ash and acid insoluble ash are important parameters in the evaluation of purity of drugs [23]. Characterizations of dried whole plants powder were shown in Table 2. The results displayed all samples were within the 14% maximum limit for total ash value and below 2% maximum limit for acid insoluble ash in powdered medicinal plants [23]. The moisture content of the medicinal plants at the end of the herbs drying must be 10–14% and shows an ideal range for minimum bacteria as well as for fungal growth [24]. All physicochemical characteristics were within allowable limits, which contributed to quality control of herbal materials. Notably, water-soluble extractive values of Ludwigia hyssopifolia was higher than that of Ludwigia adscendens while ethanol-soluble substances in the two samples were not significantly different.

Table 2. *Characterizations of dried whole plants powder*

Parameters	***Ludwigia hyssopifolia***	***Ludwigia adscendens***
Total ash value (%)	9.69	10.06
Acid insoluble ash (%)	0.54	0.39
Moisture content (%)	12.06	8.43
Water-soluble substances (%)	25.90	20.02
Ethanol-soluble substances (%)	16.44	16.21

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

The equations for the standard curve of gallic acid ($y = 0.0417x – 0.0303$, $R^2 = 0.9937$) and quercetin ($y = 0.0417x – 0.0669$, $R^2 = 0.9963$) were shown in Figure 2.

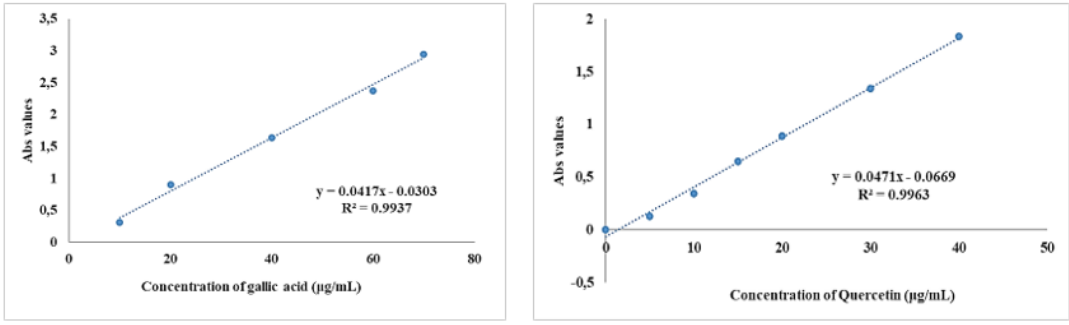

Figure 2. *The equations for the standard curve of gallic acid and quercetin*

Table 3 presents data on the total phenolic and total flavonoid contents extracted from Ludwigia hyssopifolia and Ludwigia adscendens using methanol extraction. In Ludwigia hyssopifolia, the leaves demonstrated the highest levels of total phenolic content, measuring 196.55 ± 5.05 mg GAE/g, while the total flavonoid content of the leaves was 50.25 ± 12.64 mg QE/g. Additionally, the whole plants exhibited the highest total flavonoid content at 64.72 ± 4.63 mg QE/g, while the total phenolic content of the whole plant samples was 122.61 ± 15.6 mg GAE/g. The results revealed that stems contained the lowest total phenolic content at 53.73 ± 4.34 mg GAE/g, while the total flavonoid content of the stems was 34.11 ± 1.44 mg QE/g. Roots showed values of 95.90 ± 4.57 mg GAE/g for total phenolic and the lowest for total flavonoid contents at 28.98 ± 7.27 mg QE/g. The total phenolic content of the leaves was more than 3.66 times that of the stem, the total flavonoid content of the whole plants was more than 2.23 times that of the roots. Concerning Ludwigia adscendens, leaves also displayed the highest levels of total phenolic content, measuring 193.48 ± 12.84 mg GAE/g, total flavonoid content at 46.77 ± 4.96 mg QE/g. The whole plants exhibited the highest total flavonoid content at 56.25 ± 7.21 mg QE/g, while the total phenolic content was 144.84 ± 5.78 mg GAE/g. Stems contained 123.32 ± 8.14 mg GAE/g of total phenolic and 38.06 ± 2.25 mg QE/g of total flavonoid contents, whereas roots showed values of 129.98 ± 16.36 mg GAE/g for total phenolic and 28.51 ± 9.48 mg QE/g for total flavonoid contents. Stems had the lowest data for total phenolic content, less than the leaves by more than 1.57 times, and roots displayed the lowest data for total flavonoid content, less than the whole plants by 1.97 times.

Both plants exhibited the highest quantity of flavonoids in the whole plant extract in comparison to the leaf and stem extracts. The root extract exhibited the most minimal flavonoid concentration. This pattern was similarly reported in multiple additional studies [25, 26]. Variations in the levels of flavonoids are likely attributed to climatic circumstances. Wind speed, ambient temperature, and annual sunlight duration were identified as the principal ecological factors influencing flavonoid content [27]. The intensity of ultraviolet light influences the transpiration rate of different plant components. Consequently, it is likely that the radiation may also affect the flavonoids [28].

Table 3. *Total phenolic and total flavonoid contents of methanolic extracts*

Tested samples		**Total polyphenol content (mg GAE/g)**	**Total flavonoid content (mg QE/g)**
Ludwigia hyssopifolia	Whole plants	122.61 ± 15.6	64.72 ± 4.63
	Leaves	196.55 ± 5.05	50.25 ± 12.64
	Stems	53.73 ± 4.34	34.11 ± 1.44
	Roots	95.90 ± 4.57	28.98 ± 7.27
Ludwigia adscendens	Whole plants	144.84 ± 5.78	56.25 ± 7.21
	Leaves	193.48 ± 12.84	46.77 ± 4.96
	Stems	123.32 ± 8.14	38.06 ± 2.25
	Roots	129.98 ± 16.36	28.51 ± 9.48

Values are represented as mean of 3 replicates ± SD.

3.4. DPPH radical scavenging assay

Antioxidant properties of methanolic extracts were carried out using the DPPH radical scavenging assay with IC_{50} values presented in Table 4. When it comes to *Ludwigia hyssopifolia*, the results showed that stems have the highest IC_{50} value, with 61.95 ± 4.47 µg/mL, followed by leaves and roots with the value of IC_{50} at 53.49 ± 5.66 and 52.38 ± 2.27 µg/mL, respectively. These parts presented the ability of antioxidant activity at an active level while the whole plants' samples had the strongest ability to antioxidant, with IC_{50} value at 44.48 ± 4.29 µg/mL. According to the results of *Ludwigia adscendens* samples, both leaves and roots demonstrated a highly active level of antioxidant activity, with the IC_{50} value at 26.11 ± 0.37 and 45.05 ± 0.65 µg/mL respectively. It is also noticed that 50.29 ± 0.78 µg/mL is the IC_{50} value of the whole plants' samples, with an active level of antioxidant ability. Stems' parts had the same level of antioxidant activity as the samples above, with 50.56 ± 0.95 µg/mL. On the other hand, positive control using milk thistle extract as the tested samples, and it depicted a highly active antioxidant activity, with IC_{50} 27.44 ± 5.64 µg/mL as given.

In general, the methanolic extract from *Ludwigia hyssopifolia's* whole plants had the strongest antioxidant capitacy and IC_{50} value was higher approximately 1.62 times than that of the milk thistle extract. Meanwhile, the antioxidant activity of *Ludwigia adscendens*

leaves methanolic extract was the most potential with the IC_{50} value that was not significantly different from the positive control.

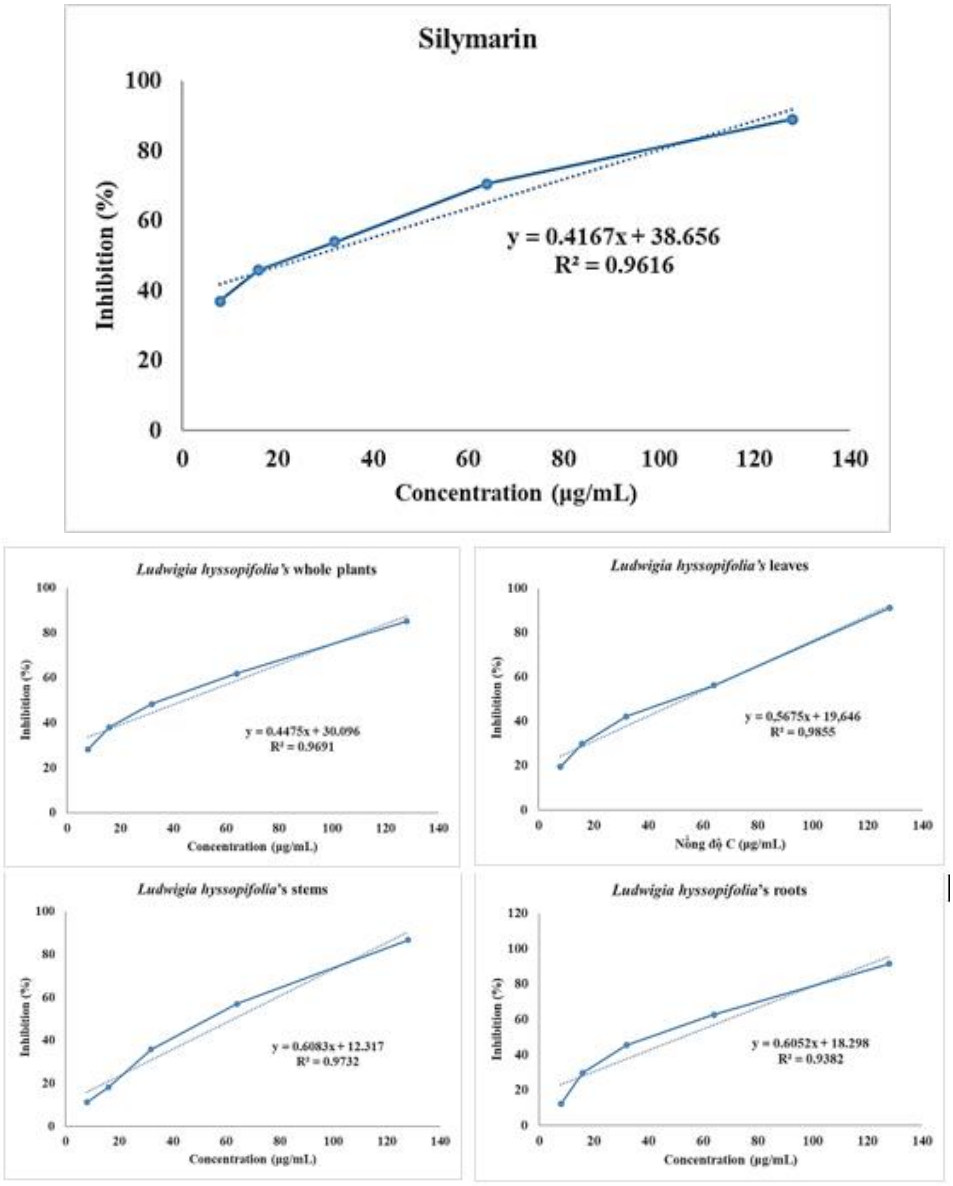

Figure 3. *Linear regression graph of DPPH radical scavenging test of Ludwigia hyssopifolia and silymarin*

Furthermore, the methanol extract of the *Ludwigia hyssopifolia* species in this current investigation shown greater antioxidant activity (IC_{50} = 44.48 µg/mL) in comparison to the aqueous (IC_{50} = 46.00 µg/mL) and ethanol extracts (IC_{50} = 69.75 µg/mL) obtained from the whole plant, as reported in our prior publications on *Ludwigia hyssopifolia* in Vietnam [22, 29]. Besides, a study conducted by Pallerla Praneetha *et al.* (2018) indicated that the methanol extract of *Ludwigia hyssopifolia*'s aerial parts in India shown antioxidant activity, with an IC_{50} value of 48.3 ± 1.6 µg/mL [30]. Nevertheless, the IC_{50} value obtained in this investigation was 1.09 times more than the IC_{50} value of the MeOH whole plant extract from our investigation, which was 44.48 ± 4.29 µg/mL.

Meanwhile, the crude methanolic extract of *Ludwigia adscendens*'s aerial parts in Bangladesh showed more potential antioxidant activity in DPPH assay with IC_{50} value of 11.5 µg/mL [31].

Milk thistle, also known as *Silybum marianum* (L.) Gaertn., family Asteraceae, is an important herbal medicine. The plant is primarily cultivated for the purpose of extracting its active compound, silymarin. The hepatoprotective effect of silymarin is attributed to its antioxidant and free radical scavenging activities. Several research have documented the biological assessment of silymarin, particularly in the areas of cancer chemoprevention and hepatoprotection. Silymarin possesses the capacity to eliminate free radicals. It has been observed to enhance the synthesis of glutathione in liver cells and the function of superoxide dismutase in red blood cells [32]. On that basis, silymarin was chosen as the positive control for the antioxidant assay.

Table 4. *IC_{50} values of methanolic extracts of herbs on DPPH assay*

Tested samples		**IC_{50} (µg/mL)**	**Levels of antioxidant activity**
Ludwigia hyssopifolia	Whole plants	44.48 ± 4.29	Highly active
	Leaves	53.49 ± 5.66	Active
	Stems	61.95 ± 4.47	Active
	Roots	52.38 ± 2.27	Active
Ludwigia adscendens	Whole plants	50.29 ± 0.78	Active
	Leaves	26.11 ± 0.37	Highly active
	Stems	50.56 ± 0.95	Active
	Roots	45.05 ± 0.65	Highly active
Positive control	Milk thistle extract	27.44 ± 5.64	Highly active

Values are represented as mean of 3 replicates ± SD.

3.5. Anti-Hepatocellular Carcinoma HepG2 Activity

IC_{50} values of methanolic extracts on anti-Hepatocellular Carcinoma HepG2 assay were shown in Table 5. In *Ludwigia hyssopifolia*, leaves reached the lowest IC_{50} value, with 123.0 ± 1.86 µg/mL while the remaining three tested samples have the IC_{50} value all above 256 µg/mL. Turning to the other, the results of *Ludwigia adscendens* samples witnessing the IC_{50} value of methanolic extracts of stems demonstrate at higher 256 µg/mL, followed by whole plants' parts and leaves with the IC_{50} value at 226.50 ± 5.50 µg/mL and 198.66 ± 5.31 µg/mL, respectively. Roots were the lowest samples of the *Ludwiga adscendens*, with the value of IC_{50} at only 156.9 ± 2.26 µg/mL. Regarding the positive control, milk thistle extracts are the tested samples, and it depict the IC_{50} value of about 179.2 ± 4.18 µg/mL as given. In a nutshell, the methanolic extracts of leaves from *Ludwigia hyssopifolia* have the most potential ability to anti-HepG2, compared with all of the tested samples that were surveyed.

The methanol extract from Ludwigia hyssopifolia leaves exhibited better inhibitory potential against HepG2 cells, with an IC50 of 123.0 ± 1.86 µg/mL, in contrast to the whole plant ethanol extract (IC50 140.82 ± 1.67 µg/mL) and the whole plant aqueous extract (IC50

176.3 ± 5.12 µg/mL). The aforementioned extracts were deemed the most effective samples if ethanol and water served as extraction solvents. Furthermore, the methanol extract of Ludwigia hyssopifolia leaf samples demonstrated a markedly lower IC50 value (IC50 123.0 ± 1.86 µg/mL) compared to the leaf aqueous extract (IC50 228.5 ± 4.03 µg/mL) in its capacity to inhibit HepG2 cells, according to data in our previous findings [22, 29]. This comparison analysis revealed that methanol extracts offered greater outcomes compared to both ethanol and water extracts for the Ludwigia hyssopifolia species. In addition, the methanolic extract obtained from the aerial parts of L. hyssopifolia displayed an IC50 value of 1870.45 ± 8.31 µg/mL when tested on the HepG2 cell line [30]. The findings of our investigation demonstrated a higher level of potential with an IC50 value of 123.0 ± 1.86 µg/mL obtained from the methanolic extracts of the L. hyssopifolia leaves.

The whole plant of *Ludwigia adscendens* has been reported for its emetic, laxative, anthelmintic, antidysenteric, anti-inflammatory, antioxidant, and antimicrobial properties [33]. To the best of our knowledge, there is no available evidence about the inhibitory effect of methanol extracts from *Ludwigia adscendens* on HepG2 cells.

The main constituent of milk thistle extract is silymarin, which is found in the leaves, seeds, and fruits. Silymarin is a mixture of seven flavonolignans silybin A, silybin B, isosilybin A, isosilybin B, silychristin, isosilychristin, silydianin and one flavonoid, taxifolin. Silymarin affects liver cancer by several mechanisms. It inhibits the population growth of HepG2, human hepatocellular cancer cells, which results in a rise in the concentration of apoptotic cells [34]. It is now used in Europe as complementary protection in patients receiving medication known to cause liver problems [35].

Table 5. *IC_{50} values of methanolic extracts on anti-HepG2 assay*

Tested samples		**IC_{50} (µg/mL)**
Ludwigia hyssopifolia	Whole plants	> 256
	Leaves	123.0 ± 1.86
	Stems	> 256
	Roots	> 256
Ludwigia adscendens	Whole plants	226.50 ± 5.50
	Leaves	198.66 ± 5.31
	Stems	> 256
	Roots	156.9 ± 2.26
Positive control	Milk thistle extract	179.2 ± 4.18

Values are represented *as mean of 3 replicates ± SD.*

Low-molecular-weight phenolic compounds constitute a diverse category of molecules characterized by a common phenolic backbone, encompassing a variety of (poly)phenols, including flavonoids and non-flavonoids such as lignans, tannins, stilbenes, ellagic acid, and phenolic acids, among others [36, 37]. Low molecular weight phenolic compounds (LMWPC) are highly reactive with antioxidants [38] and anticarcinogenic [39] properties. Furthermore, phenolic compounds are crucial in providing natural antibacterial

and anti-inflammatory properties, as well as in the management of disorders such as obesity, cancer, and diabetes [40]. Low-molecular-weight metabolites generated from polyphenols have garnered attention as modulators for alleviating neuroinflammation [37].

4. CONCLUSIONS

The present study provides the useful information about physicochemical parameters, total polyphenolic and flavonoid contents of the methanolic extracts from 04 parts of *Ludwigia hyssopifolia* and *Ludwigia adscendens* in Vietnam. Given the results obtained in this study, it can be concluded that the methanol whole plant extract of *Ludwigia hyssopifolia* and the methanol leaf extract of *Ludwigia adscendens* show the greatest antioxidant properties in the DPPH radical scavenging assay. Besides, the methanolic extract of *Ludwigia hyssopifolia* leaves displays the most potential anti-HepG2 activity in all surveyed samples. Further research is needed to obtain data on quality, efficacy, and toxicity so that medicinal plants can be called rational herbal medicines.

CONFLICTS OF INTEREST

The authors declare that they have no conflict of interest.

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