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Morphological, anatomical and phytochemical properties of (*Ludwigia hyssopifolia* (G. Don) Exell) at Can Tho province, Vietnam

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Abstract
The objectives of the study were to investigate the botanical characteristics and determinate the natural compounds in aerial part and root extracts of *Ludwigia hyssopifolia* (G. Don) Exell in CanTho province. Double staining with carmine-iodine green was used for anatomical examination, hypo-osmotic shock method was used to estimate chromosome numbers, and the phytochemical components were determined by using reagents and standards. The results showed the morphological and anatomical characteristics of *L. hyssopifolia* leaf, stem, and root. The chromosome number were estimated (2n = 16). Aerial part and root extracts showed the presence of saponins, flavonoids, tannins, glycosides, alkaloids, steroids, phenolics, coumarins, and terpenoids. No terpenoids was detected at both aerial part, and root extracts. At aerial part, the total phenolic, tannins, and flavonoid contents were respectively 126.8±28 mg gallic acid /g dry weight of the extract, 116.2 ± 18 mg gallic acid/g dry extract, and 242.3 ± 23 mg quercetin/g dry extract. At root, the total phenolic, tannins, and flavonoid contents were 45.5 ± 11 mg gallic acid/g dry extract, 54.8 ± 23 g gallic acid/g dry extract, and 58.6 ± 2.4 mg quercetin/g dry extract respectively.

Keywords: *Ludwigia hyssopifolia* (G. Don) exell, methanol extraction, ethanol extraction, phytochemical

Introduction
Ludwigia hyssopifolia (G. Don) Exell belonging to *Oragraceae* family is native to tropical America. The plant grows wild in humid areas of the tropics, often in standing water, rice fields, stream banks, and swamps, as well as along roadside and wet fields. *L. hyssopifolia* is widely distributed in Bangladesh, Bhutan, Burma, Sri Lanka, India, Nepal, Colombia, Ghana, Iraq, New Guinea, Nigeria, Laos, Cambodia, Hong Kong, Australia, Samao [8]. *L. hyssopifolia* are considered as a "serious" weed in rice fields of many countries [3]. However, leaf extract of *L. hyssopifolia* has potential as a bioherbicide by inhibiting the shoot growth and biomass accumulation of weeds [13]. *L. hyssopifolia* is valuable as green manure improve soil structure [9].

L. hyssopifolia has used as a traditional herbal medicine in many countries. It has a light sweet taste, cool properties, clearing heat, and detoxifying. *L. hyssopifolia* is used for treatment of rheumatism, emphysema, diarrhea and dysentery, hemostasis, and inflammation. *L. hyssopifolia* can also treat mouth ulcers, eczema, flatulence, leukemia, and hemolysis [8]. *L. hyssopifolia* holds the promise of being utilized in developing herbal medicines [7]. Many important phytochemicals and pharmaceuticals have been studied in *L. hyssopifolia*, including piperine, vitexin, isovitexin, orientin, isoorietin, and sitosterol [8]. However, not many published on the anatomical and morphological characteristics of *L. hyssopifolia* have been found. The aim of this study was to investigate the morphological and anatomical properties, estimate chromosome numbers: and determinate qualitatively and quantitatively phytochemical components of *L. hyssopifolia* (G. Don) Exell in CanTho provine, Vietnam.

Materials and Methods
Collection of plant materials
L. hyssopifolia were collected in CanTho city, Mekong Delta, Vietnam. Mature plants are harvested, washed to remove dust, and dried in the laboratory.

Moisture content
Plant materials were divided into roots, and aerial part. 5g each fresh samples was placed in a hot-air oven at 65ºC for 5 minutes until the dry mass remained constant. Moisture was measured in percentages. Moisture (%) = (Fresh weight - Dry weight) / Fresh weight [12].

Morphological and anatomical characters
Physical examination and physiological measurements of fresh *L. hyssopifolia* samples were to assess morphological characters. Mature roots and stems were horizontally sliced. Leaf samples were cut through both vein and leaf blade. All fresh sliced samples were double-stained with carmine alum laque-iodin green dye. Cells with cellulose cell walls were stained pink with carmine, while cells with lignin- impregnated cell walls were stained green with iodide. Olympus microscope connected to a digital camera were used to observe and capture samples [20].

Estimate chromosome numbers
Young leaves were collected and hypotonic-shocked with 0, 1% sodium citrate for 45 minutes before being fixed with Carnoy solution for 4 hours. Samples were stored in 70º ethylic alcohol at 4-5ºC. Samples were stained for 60 minutes with 1% aceto-carmine solution. Make preparation and count chromosome

numbers of at pro-metaphase plate. Estimate the chromosome number by using a sample T test at a confidence level value of 1 - α = 95% [20].

Qualitative analysis of the phytochemicals

The mature plant was divided into aerial part and root. Samples were cut in small pieces and dried at 50ºC until the dry weight remained constant. The dried samples were ground to a fine powder for identifying organic compounds. 10 g of each powder sample were soak with 100 ml of methanol and incubated in the dark for 24 h at room temperature. The samples were sonicated for the first time, and then incubated for another 24 h. The samples were subjected to a second ultrasonic wave. The extracts were filtered by filter paper, and evaporated to dryness under reduced pressure by using a rotary evaporator at 50ºC. The extracts then stored at 4 - 5ºC. Preliminary phytochemical screening was investigated with reagents with some modifications [1, 16, 19].

Test for steroids

To 0.5 ml of extract, add 0.25 ml of chloroform solution into a test tube. Add 0.25 ml of concentrated H_2SO_4 from the sides of the test tube. The solution turning dark red/blue/purple-blue indicated the presence of steroids.

Test for saponins

Pipet 1 ml extract in a test tube and add 3 ml of distilled water. Cover the test tube and shake it vertically for 15 minutes. A column of foam about 1 cm high that was stable for 15 min showed positive results.

Test for coumarins

To 50 µl of extract into a test tube, add 750 µl 10% NaOH. If the extract contains compounds of coumarins, the solution appeared yellow.

Test for flavonoids

To 0.5 ml of extract into a test tube, add 1.25 ml of dilute ammonia solution, and slowly add a few drops of concentrated H_2SO_4 from the sides of the test tube. The appearance of yellow proved compounds of flavonoids.

Test for tannins

Add to 0.5 grams of medicinal powder with 20 ml of water, and boil on the stove for about 10 minutes, stirred well while boiling. Filter to get clear filtrate. Pipet 1 ml of the extract in a test tube, add a few drops of 5% $FeCl_3$. A blue-black precipitate demonstrated the presence of tannins.

Test for terpenoids

1 ml of extract was mixed with 0.2 ml of chloroform, and slowly add 0.6 ml of concentrated H_2SO_4. The solution appeared a reddish brown ring between the chloroform layer and H_2SO_4, indicating the presence of terpenoids.

Test for alkaloids

0.5 ml of extract solution was added into a test tube. Add 0.75 ml of reagent. If the extract contains alkaloids, the brown precipitation appeared in solution. The precipitate was soluble in the reagent excess.

Test for glycosides

Pipet 0.25 ml of extract in a test tube. Add 1 ml of a mixture of Fehling A ($CuSO_4.5H_2O$ 10%) and Fehling B ($KNaC_4H_4O_6.4H_2O$) solutions in a 1:1 ratio to the test tube and mix well. Place the test tube in a cup of hot water and boil for 3-4 minutes. A brick red precipitate appears on the bottom of the test tube, indicating that the extract contains glycosides.

Test for phenolics

50 µl of extract solution was added into a test tube. Add 500 µl of distilled water, and 2 - 3 drops of 0.1% $FeCl_3$. Blue-black precipitation indicated the presence of phenolic compounds.

Quantitative determination

The aerial part and root extracts of *L. hyssopifolia* were used to estimated the total phenolics, tannins, and flavonoids contents.

Determination of total phenolic content

The total phenolic content was determined by the Follin - Ciocalteu method [21]. Prepare a calibration curve by adding 0.5 ml of gallic acid solution (concentrations 20, 40, 60, 80 and 100 µg/ml) to 2.5 ml of 10% Follin-Ciocalteu reagent. Keep for 5 minutes, then add 2 ml of 7.5% Na_2CO_3. For sample test, extractions were performed in the same manner as gallic acid. Add 0,5 ml of extract to 2,5 ml of 10% Follin-Ciocalteu reagent, and keep for 5 minutes before adding 2 ml Na_2CO_3 7,5%.

The absorbance at 765 nm was measured after 30 minutes of reaction at room temperature for both standard and sample solution. The experiment was carried out three times.

Determination of total flavonoid content

The total flavonoid content was determined by forming a yellow complex with $AlCl_3$ when reacting flavonoid compounds [4]. Prepare a calibration curve by adding each concentration of quercetin in ethanol (20, 40, 60, 80, 100 µg/ml) to test tube containing 1 ml of quercetin in ethanol, 1 ml of distilled water. Add 0.2 ml of 5% $NaNO_2$, and keep for 5 minutes. Then, add 0.2 ml of 10% $AlCl_3$, and leave to react for 5 minutes. The mixture was added to 2 ml of 0.8 M NaOH, and 0.6 ml of distilled water, and shaken well. For sample test, extractions with quercetin were carried out in the same manner. Mix 1 ml of extract and 1 ml of distilled water in a test tube. Then add 0.2 ml of 5% $NaNO_2$ and leave to react for 5 minutes. Add 0.2 ml of 10% $AlCl_3$ and let it react for 5 minutes. Finally, the mixture was added to 2 ml of 0.8M NaOH and 0, 6 ml of distilled water, and shaken well.

The absorbance at 510 nm was measured using a spectrophotometer for both standard and sample solutions. The experiment was carried out three times.

Determination of total tannin content

Total tannin content was determined by the method Follin-Ciocalteu [21]. A calibration curve was created by adding 1 ml of gallic acid solution (concentration 20, 40, 60, 80, 100 µg/ml) to 7.5 ml of distilled water, followed by 0.5 ml of 10% Follin reagent-Ciocalteu and 1 ml of 35% Na_2CO_3. Keep 30 minutes of reaction at room temperature. For sample test, extractions were carried out in the same manner with gallic acid. Add 1 ml of extract to 7.5 ml of distilled water. Then add 0.5 ml of 10% Follin-Ciocalteu reagent, and 1 ml of 35% Na_2CO_3. Keep 30 min of reaction at room temperature.

Absorbance was determined by spectrophotometer at 725 nm for both standard and sample solution. The experiment was repeated 3 times.

Results and Discussion

Moisture content

Moisture content of *L. hyssopifolia* was 85 ± 0, 92% (at aerial part**)** and 71, 33 ± 4,04% (at root).

Morphological characters

L. hyssopifolia was a perennial herbaceous plant that grew wild along the edge of canals, ponds, rice fields, and lawns. Under shade and good nutrition, this plant throve with dark colored leaves. In arid conditions, the plant grew poorly with reduced height: reduced new young leaves small: thin, pale colored leaves: and sparse flowers.

Roots were in the form of piles with many well-developed auxiliary roots. Young roots could grow above or below ground. Roots were early woody.

Stems were approximately 60.9 ± 21.3 cm tall, multi-branched, and upright. Stems and branches were hairless, and 4 - 5 obtuse angle-shaped.

Leaves were iternate and simple, spear-shaped, oblong to petioles, and pointed to the tip, margin was entire. Mature leaves were approximately 6.39 ± 1.52 cm long and 1.44 ± 0.31 cm wide.

Sessile inflorescence was cymes, and arrived in leaf axils. Flowers are yellow. Flowers were small and solitary. Floral formulary of *L. hyssopifolia was* $K_{(4)}C_4A_{4+4}G_{(4)}$. Each flower had 4 green sepals: 4 yellow petals: 8 stamens, roughly equal filaments, anthers opening by slits: ovary with 4 chambers, axile placentation, one ovule in each locule.

Fruits were cylindrical, smooth, slightly swollen at the top. The fruits were 15 - 18 mm long, 2.5 mm wide, and containing many oval seeds. Old fruits were darken with 4 persistent sepals, and divided into 4 boxes. Seeds were arranged in two rows in each box (Figure 1).

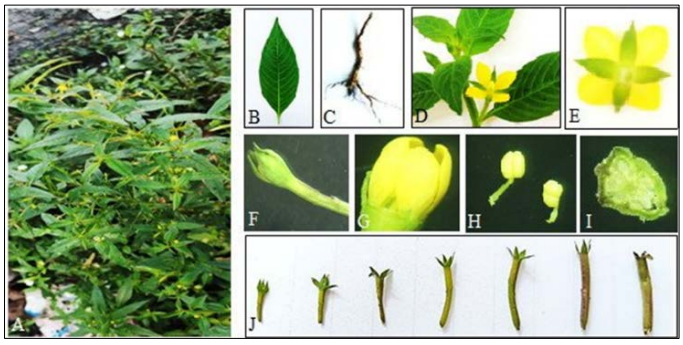

Fig 1: *Ludwigia hyssopifolia* (G. Don) Exell

A. Aerial part, B. Leaf, C. Root, D. Branch with flower, E, F G. Flower, H. Stamen, I. Ovary cross section, J. Fruits

Anatomical characteristics

Root

Transverse section of root was nearly circle in shape and consisted of thin cortex and thick stele. The outermost layers of bar were layers of dead cork cells. Deep to the cork cell were a layer of living cork cambium. Beneath cork cambium layers were cork parenchyma. Vascular bundles were located at stele region of root. Phloem was the outer narrow layer and deep to this layer was vascular cambium. Phloem layer was difficult to distinguish because it is mixed with parenchyma cells. Meta-xylems varied in diameter and were accompanied by medullary rays and parenchyma cells. Proto-xylem and phloem were alternatively located at the center of root (Figure 2).

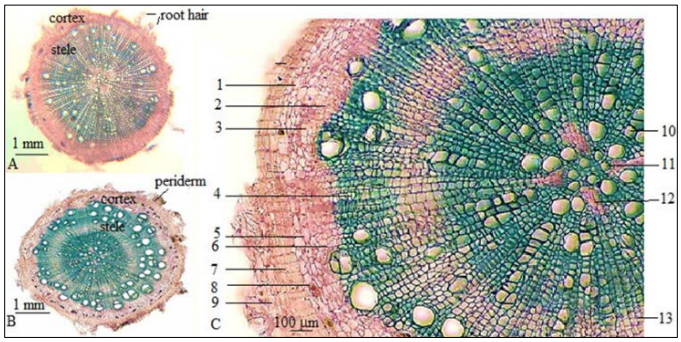

Fig 2: Tranverse section of *Ludwigia hyssopifolia* root.

A. Primary growh, B-C. Secondary growth
1. Parenchyma, 2. Secondary phloem, 3. Primary phloem, 4. Medullary ray, 5. Endodermis, 6. Pericycle, 7. Cork cambium, 8. Druse, 9. Cork parenchyma, 10. Metaxylem, 11. Protoxylem 12. Parenchyma, 13. Cambium

Stem

Stem transverse section were showed polygonal shape with small edges, thin cortex, and thick stele. The outermost layers of cortical region was a layer of epidermal cells that made up of compactly arranged parenchyma cells. Below of epidermis, collenchyma cell bundles were located nearby the edges of stem. Secondary aerenchyma were less prominent. 5 - 7 layers of polygonal parenchyma cells closed intercellular space in cortex region. Bundles of sclerenchyma cells were distributed through the cortex. Endodermis was an innermost layer of the cortex. At stele region, pericycle was lied above vascular bundles. Many bundles of phloem and xylem were arranged in concentric circles behind pericycle. Pith were made up of closely spaced polygonal or nearly circular parenchymal cells with small interspaces. Secretory vesicles were small cells arranged closely together and forming nearly circular cross sections (Figure 3).

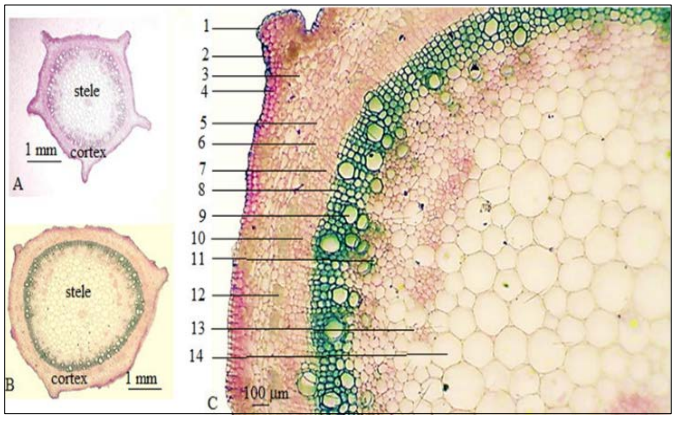

Fig 3: Transverse section of *L. hyssopifolia* stem. A. Young stem, B-C. Mature stem

1. Cuticle, 2. Epidermis, 3. Parenchyma, 4. Collenchyma, 5. Endodermis, 6. Pericycle, 7. Cambium, 8. Medullary, 9. Meta-xylem, 10. Phloem, 11. Proto-xylem, 12. Sclerenchyma, 13. Parenchyma

Leaf blade

Leaf blade transverse section included lamina and venation (or main vein). Venation section was nearly triangle-shaped. The adaxial surface was little convex while it is semi-circular on the abaxial surface. The outermost layer of venation was a single layer of epidermis that consisted of cuticle impregnated cells. Under epidermis was 1 - 2 layers of collenchyma cells. At the center of venation, parenchyma cells were showed the difference in size and shape. Vascular tissue was U-shape that made up of bundles of xylem above and phloem below with medullary rays. Lower epidermis was the layer of cuticle impregnated cells that were interlaced with stomata. Leaf blade was made up of an upper epidermis, followed by 2 - 3 layers of palisade parenchyma cells, bellowed by 2 - 4 layers of spongy parenchyma cells, and an layer of lower epidermis. Druse crystals were revealed in parenchyma cells of both venation and leaf blade (Figure 4).

Petiole

Petiole cross section included main vein and two very short or absent leaf blades. Vascular tissue was U-shape. Lower epidermis was impregnated cuticle, interlaced with stomata and short trichomes (Figure 4).

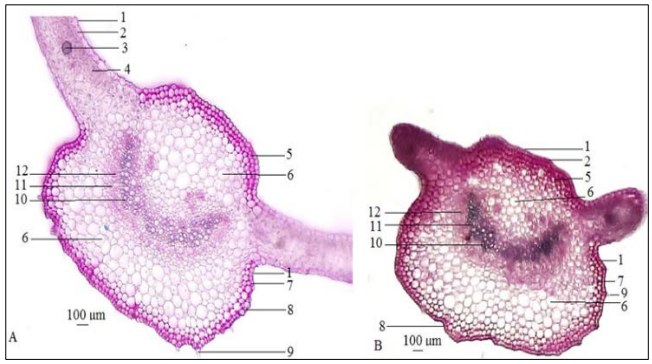

Fig 4: Transverse section of *L. hyssopifolia* leaf

A. Leaf blade, B. Petiole

1. Cuticle, 2. Upper epidermis, 3. Druse, 4. Palisade mesophyll, 5. Collenchyma, 6. Parenchyma, 7. Lower epidermis, 8. Trichome, 9. Medullary ray, 10. Phloem, 11. Xylem, 12. Spongy mesophyll

Medicinal powder characteristics

The medicinal powder of aerial part were light green, while that of roots were brown. Both medicinal powder were fragrant. Under optical microscope, calcium oxalate crystals, stomata, cork fragments, and veins as point, helix, and spiral vessels were revealed (Figure 5).

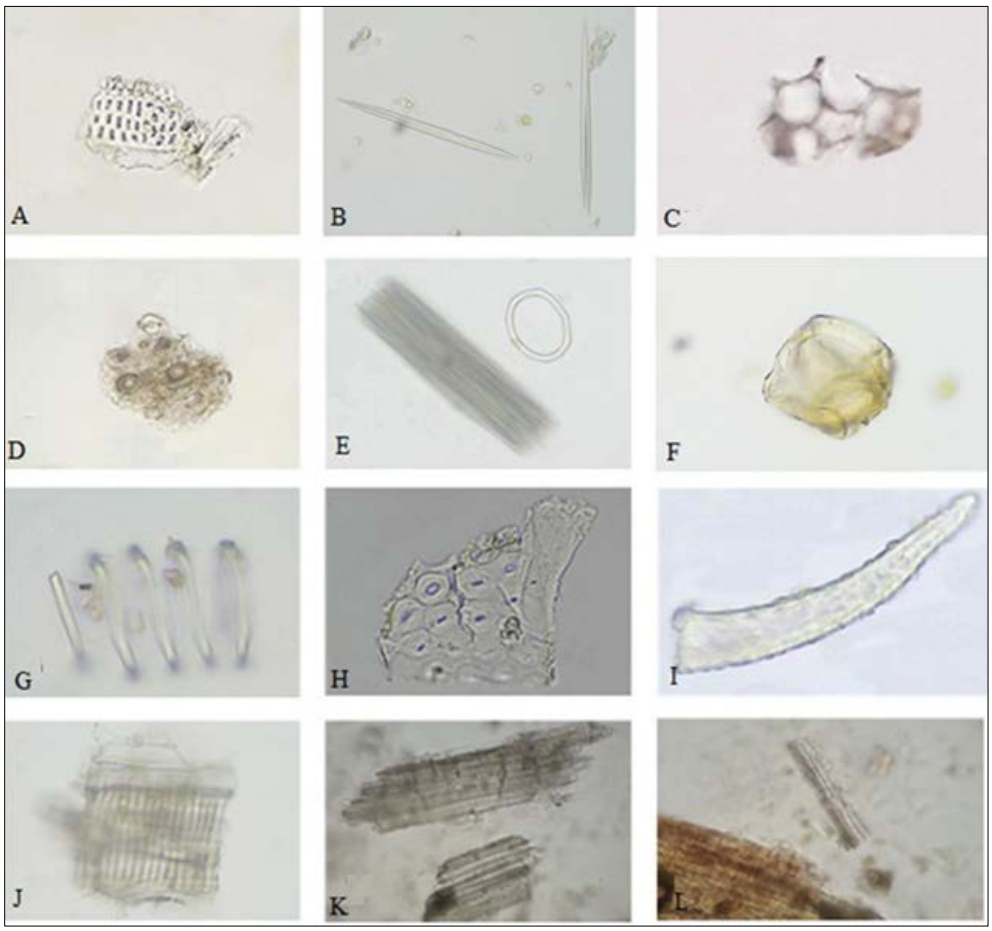

Fig 5: Medicinal powder of *L. hyssopifolia*

A. Pit xylem vessel, B. Calcium oxalate crystal (Raphide), C. Parenchyma cells, D. Sclerenchyma cells, E. Calcium oxalate crystal (Raphide bundle) and annular vessel, F. Calcium oxalate crystal (Prism), G. Spiral xylem vessel, H. Bordered pits, I. Trichome, J. Scalariform xylem vessel, K. Xylem fibers, L. Cork fragnent

Chromosome estimation

The number of chromosomes in young leave was estimated 2n = 16 ± 0,83045 (p> 0,05). Therefore, chromosome numbers of *L. hyssopifolia* were 2n = 16.

Qualitative phytochemical screening

The extracts of aerial part, and roots showed the presence of phenolic compounds, flavonoids, glycosides, tannins, steroids, alkaloids, coumarins and saponins. No terpenoids were detected at both aerial part, and root extracts (Table 1).

Table 1: Phytochemical screening of *L. hyssopifolia*

Compounds	Aerial part	Root
Phenolics	+	+
Tannins	+	+
Flavonoids	+	+
Steroids	+	+
Alkaloids	+	+
Coumarins	+	+
Terpenoids	-	-
Saponins	+	+
Glycosides	+	+

Negative: -, Postive:+

Phytochemical quantitative assays

The extraction efficiency obtained from the aerial part and the root was 6.6% and 8.7%, respectively.

To determinate the total phenolic content, the equation of the absorbance was measured at 765 nm was y = 0.0118x + 0.0538 (R^2 = 0,9924). For determination of the total tannins content, the absorbance was measured at 725 nm with the equation as y = 0.0074x + 0.0333 (R^2 = 0.9897). The absorbance was measured at 510 nm with the equation y = 0.0016x + 0.0079 (R^2 = 0.9907) for total tannins content.

Phytochemicals that was quantitatively determinated in aerial part and root extracts were reported on Table 2.

Table 2: Total phenolics, tannins, and flavonoids content of *L. Hyssopifolia*

Phytochemicals	Arial part	Root
Total phenolics (mg gallic acid/g extract)	126.8 ± 2.8	45.5 ± 1.1
Total tannins (mg gallic acid/g extract)	116.2 ± 1.8	54.8 ± 2.3
Total flavonoids (mg quercetin/g extract)	242.3 ± 2.3	58.6 ± 2.4

Discussion

The *Ludwigia* genus contains about 75 species of aquatic plants with a global distribution but mainly in the tropics. Therefore, the indentification of species was very important. Leaf epidermal characteristics were used to were compared about the species of *Ludwigia* in West African [11]. Anatomical characters of leaf and stem were used in the identification of species of Ludwigia [9].

The morpho-anatomical adaptation of *Ludwigia* root and stem to aquatic habit was studied by Bedoya *et al*. (2014) [2]. *Ludwigia hyssopifolia* was reviewed about morphological and anatomical properties, habitate, and phytopharmacological activities [8]. This present study characterized the morphological and anatomical features of *L. hyssopifolia* and found that *L. hyssopifolia* was essentially similar to those described in the above studies. Due to the plant grown in the edge of wet areas such as canals, ponds, rice fields, and lawns, roots of *Ludwigia hyssopifolia* gave rise to many young roots and secondary growth was strongly developed. Transverse sections of the mature root showed more woody. At both root and stem, aerenchyma tissue was not found. The pith of stem was made up of parenchymal cells with small intercellular spaces. At old stem, cortex parenchyma cells showed larger intercellular spaces. Lower epidermis was interlaced with more stomata than upper epidermis. Calcium oxalate crystals found in forms of druses, raphide bundles and prisms.

The chromosome number of *L. hyssopifolia* was estimated as 2n = 16. This result was similar to the studied results of Gadella *et al*. (1968) [10] and Matoba & Hiroshi (2009) [13]. The basic chromosome number of genus *Ludwigia* was x = 8 with extensive polyploidy (2n = 24, 32, 48) [18].

This study revealed that both aerial part and root extracts of *L. hyssopifolia* showed positivity for phenols, flavonoids, glycosides, tannins, steroids, alkaloids, coumarins and saponins, with the exception of terpenoids. The total polyphenolic, tannin and flavonoid content was also determinated. According to Mangao *et al*. (2020) [13], leaf aqueous extract of *L. hyssopifolia* contained phenols, tannins, flavonoids, terpenoids, saponins and coumarins. For the whole plant of *L. hyssopifolia*, terpenoids were present in both hexane and methanol extracts, while flavonoids and alkaloids were present in the ethanol extracts [7].

The total phenol content of *L. hyssopifolia* methanol extract was determined as 29.6 mg/g by using Follin - Ciocalteu reagent [5]. However, at *Ludwigia octovalvis*, the leaf and stem methanol extracts contained amount of the total phenolic content as 0.23 GAE mg/g, and 239.05 GAE mg/g dry weight [22]. At whole plant extracts of *Ludwigia octovalvis*, the total phenolic and total flavonoid contents were calculated as 5.15 GAE mg/g and 43.9 QE mg/g respectively [17]. The different results of qualitative and quantitative phytochemicals may be due to diffences in species, extract solvents, growth and development conditions.

L. hyssopifolia extracts possessed anti-tumour, antibacterial, anti-inflammatory, cytotoxic, analgesic, hepatoprotective, antioxidant activities [8, 6]. Alkaloids, flavonoids, tannins, coumarins, glycosides, phenolics, steroids, saponins were well known to possess biological and pharmacological activity against various chronic diseases such as cancer and cardiovascular and gastrointestinal disorders [15]. Therefore, *L. hyssopifolia* has potential to be used in pharmacology to provide an important source of medicinal herbs.

Conclusion

This study showed the anatomical and morphological characteristics of the roots, stems, and leaves of *L. hyssopifolia*. The aerial part and root extracts of *L. hyssopifolia* showed qualitatively chemical compositions as phenolic compounds, tannins, flavonoids, steroids, coumarins, saponins, glycosides, alkaloids. Terpenoids were absent from both root, stem and leaf extracts. Total phenolics, total tannins, and total flavonoids were quantitatively derterminated in aerial part and root extracts.

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