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# Morphology, microscopic anatomy and bacterial inhibition of extracts of *Ludwigia adscendens* (L.) H. Hara plants growing in Lang Sen Wetland Reserve, Long An province, Vietnam

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## Abstract

The study aimed to supplement data on a plant that was considered a folk medicine of Vietnam. Morphological and microscopic anatomical characteristics of this species which were adapted to the frequently flooded habitat of the reserve was studied and analyzed. Alcoholic extracts of each stem, leaf and root part were recovered using a rotary evaporator. The inhibitory ability on bacterial strains including *Bacillus cereus, B. subtilis* and *Escherichia coli* was tested through agar plate diffusion method. The result showed that the stem and leaf extracts were effective against all three bacterial strains, while the root extracts had no effect against *E. coli*.

**Keywords:** Bacterial inhibition; Folk medicine; Lang Sen Wetland Reserve; *Ludwigia adscendens* (L) H Hara; Microscopic anatomy; Plant morphology

## 1. Introduction

Lang Sen Wetland Reserve was located upstream of the Dong Thap Muoi region, covering an area of 5,030 hectares in Tan Hung district, Long An province, Vietnam. This was the place where many types of habitats were found suitable for water-loving plants and animals, especially waterfowl [1]. The reserve had 4 types of vegetation, in which "vegetation on canals" was the most typical habitat of the Dong Thap Muoi region. This habitat consisted of a natural system of rivers and canals with many dominant species, one of which was the *Ludwigia adscendens* [1].

*Ludwigia adscendens* (L.) H. Hara, common name water primrose, was a species of herbaceous perennial in the family Onagraceae. It was reported on their wide adaptability to water conditions ranging from swampy to dry [2], but given the special hydrological conditions of the reserve, it was likely that the plants there had developed some adaptive traits. Although considered a pest due to its ability to invade crops, *L. adscendens* was a 'wild' vegetable and folk medicine in some Southeast Asian and African countries. In Vietnam, the use of this plant in the treatment of diseases was recorded by folk such as fever, cystitis, painful urination. The whole plant was also pounded, used outside to treat skin diseases, mammary gland abscesses, parotid gland inflammation, eczema, zona, animal bites [3-5]. Existing works in Vietnam included research on the distribution or microscopic anatomy of this plant [6, 7]. Some studies from other countries included classification, anatomy or antibacterial potential [8-10]. To supplement the morphological and anatomical data of *L. adscendens* plants under the specific waterlogging conditions of the Lang Sen Wetland Reserve, furthermore, on the antibacterial properties of these plant extracts, this study was conducted.

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## 2. Material and methods

### 2.1. Samples collection and preparation

The geographical coordinates of Lang Sen Wetland Reserve and sampling location (Sub- zone 11) were from 10°45'00'' to 11°50'00'' North latitude and from 105°45'00'' to 105°50'00'' East longitude and 105°42'20'' East longitude and 10°46'31'' North latitude, respectively (Figure 1).


**Figure 1** The map of Lang Sen Wetland Reserve and sampling location (star shape) [6, 11]

Sampling time included 2 batches, February 2021 and October 2021. Soil and water samples were taken according to the description in Vietnam National Standard TCVN 7538-2: 2005 and TCVN 5994: 1995 respectively [12]. Fifteen samples of *Ludwigia adscendens* was selected from different sites to analyze morphological and anatomical features. These samples were transferred into sterile plastic bags, sealed, labeled, refrigerated, and transported immediately to the laboratory. Plant samples were stored in a refrigerator at 5°C for use in morphological studies, and then preserved by immersion in 70°C ethanol solution for further use in anatomical studies.

### 2.2. Analyzing some physical and chemical criteria of soil

Soil texture particles and soil organic matter were analyzed according to the Vietnamese National Standards TCVN 8941-2011 and TCVN 8567-2010, respectively [12] by the Institute of Agricultural Science for Southern Vietnam, Ho Chi Minh City.

Soil salinity via electronic conductivity (EC) and soil acidity via $pH_{H2O}$ and $pH_{KCl}$ were determined according to the description of the Soil and Fertilizer Research Institute [13].

### 2.3. Analyzing morphological and anatomical characteristics of plant

Classification of *Ludwigia adscendens* based on some literatures [3-5]. Basic morphological and habitat characteristics of the species were recorded at the sampling area. The intermediate leaves ($3^{rd}$ and $4^{th}$ leaves from the top of tree), stems and roots were used for microscopic analysis. The process of staining samples was carried out according to the description of Tran Cong Khanh [14].

Select intermediate leaf from different plants to study stomata in terms of type and number by collodion film method [15]. The collodion films were observed under the microscope with help of a gridded glass slide.

### 2.4. Investigation of the antibacterial ability of crude plant extract

The plant was dried by parts of the stems, leaves, and roots and grounded into a fine powder. Alcohol extraction was performed by soaking and extracting as described by Nguyen Kim Phi Phung [16]. Solvent depletion was facilitated by a rotary evaporator. The concentrated extract was diluted in 70% v/v alcohol giving a concentration of 1000 mg/mL (the stock solution). A wide range of concentrations from 200 - 800 mg/mL were produced with a difference of 200 mg/mL.

The antibacterial activity was examined by well diffusion method [17]. The ability to inhibit bacteria (in mm) was calculated as the halo diameter (bacteriostatic zone on agar) minus the well diameter. The negative control was a 70% alcohol solution (that was, the concentration of the extract was 0). The positive controls were gentamicin 1 mg/mL (Gentamicin Sulfate - Gentamicin 80mg/2mL - DOPHARMA, Vietnam) and tetracycline 1 mg/mL (Tetracycline 500 mg - Mekophar, Vietnam).

### 2.5. Processing statistics

All quantitative data were analyzed for LSD by using IBM SPSS Statistics 26.0

## 3. Results and discussion

### 3.1. Characteristics of the soil and water in the sampling area and the relevance to adaptation of *Ludwigia adscendens*

In Lang Sen, *Ludwigia adscendens* plants were widely distributed in sub-zones 10, 11, 12 of the reserve, from dry to moist places, but were most abundant in the canal system of Sub-zone 11 (Figure 2)

**Figure 2** Habitat of *Ludwigia adscendens* species in Sub-zone 11 in dry season

The mechanical composition of the soil in the sampling area was sandy loam with the proportions of sand, slit and clay at 61%, 19%, and 20% respectively. Soil was nutrient-poor with 1.19% organic matter content. The soil was acidic with a pH that ranges between4.30 - 5.32, while water has a pH that ranges from 4.13 (acid) in dry season to 6.97 (neutral) in rainy season. The EC value of water samples in the rainy season (EC = 410.8 ± 4.21 mS/cm) was higher than in the dry season (EC = 141.77 ± 0.25 mS/cm) and this difference was statistically significant. This could be explained by the fact that in the rainy season, the water quality was better, the silt was relatively stable and the content of water-soluble substances was higher than in the dry season.

In particular, this species was also adapted to water level fluctuations between the two seasons, from 79.0 cm in the dry season to 125.7 cm in the rainy season. In deep water conditions, this species had the following adaptations. The straight stem length could be up to 10 feet with 1/3 of the stem above the water. There were two types of roots, one that connected the stem to the soil (standard) and the other that helped absorb oxygen from the atmosphere ("breathing roots"/ "air roots"). "Breathing roots" were white, spongy, fleshy roots with small air-filled pores bladders that grew upward and floated (Figure 3).

Fluctuations in water levels in wetlands were thought to disturb aquatic plants. A study of Chen et al. [18] showed that under simulated conditions, water flow fluctuated from 0 to 4 times within 60 days with an amplitude of ± 25 cm, all of which had an effect on the number of branches, shoot length, total biomass and the chlorophyll content of *L. adscendens* in a downward direction but had no significant effects on root shoot ratio or maximum PSII quantum efficiency (Fv/Fm). Tolerance and plasticity related to water level fluctuations were associated with the entry of introduced aquatic plant species. This was one of the concerns of countries that manage *L. adscendens* as a pest.

**Figure 3** “Breathing roots” of *Ludwigia adscendens*

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### ***3.2.*** **Morphological characteristics of *Ludwigia adscendens***

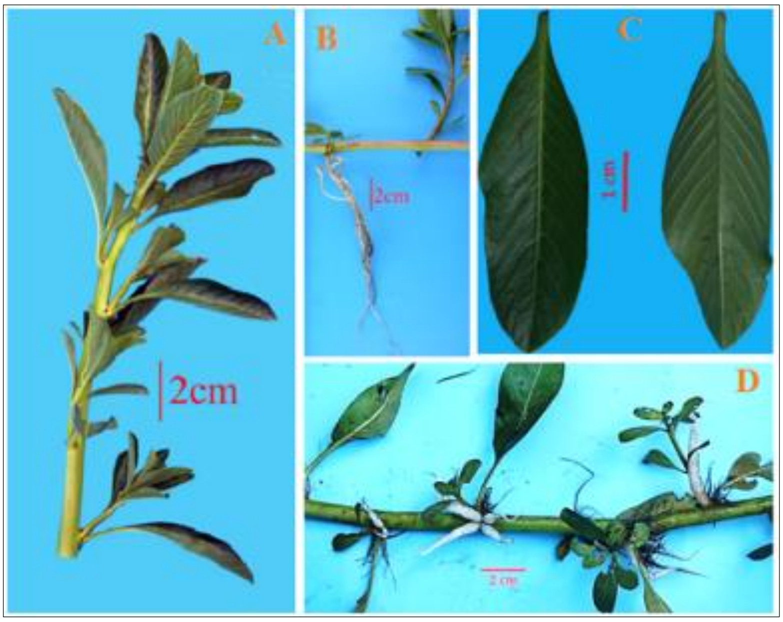



**Figure 4** Vegetative organs of *Ludwigia adscendens*

*Ludwigia adscendens* was an herbaceous, perennial aquatic creeper. They have the form of growth floating on the water or crawling in wet, muddy places. The stem was circular in cross-section, heavily branched, and branches grew from the leaf axils (Figure 4 A). "Standard" roots grew from inter-node to help the plant cling to moist soil (Figure 4 B), and “breathing" roots contained oxygen, which helped the plant to be submerged in water but still performed normal physiological functions (Figure 4 D). Foliage had alternately arranged leaves. Each leaf was obovate or oblanceolate, 4 -

8 cm long, had an entire margin and an acute or obtuse apex. The petiole was half as long as the leaf blade, the leaf blade was smooth, dark green on the upper side compared to the lower side. The venation was pinnate, with the midrib and veins both white, clearly prominent on the underside (Figure 4 C).

The color of the flowers of *Ludwigia adscendens* was described as creamy white, to pale yellow, bright yellow, or dark yellow but there the petals were white with their base were bright yellow (Figure 5). The bisexual, solitary, long stalked (about 1cm) flowers grew on the axils of upper leaves. The calyx was tubular, glabrous, 1.0 - 1.3 cm long, with 5 oblong lanceolate lobes attached to the ovary, each lobe 0.7 - 0.9 cm long. The corolla had 5 ovate, rounded and emarginate petals, 0.8 - 2 cm long and 0.8 - 1.2 cm wide. Stamens had 10 filaments, arranged in two rings that the outer shorter than the inner. Anthers were oblong, 0.7 – 1.0 mm long, yellowish in color. The compound pistil consisted an ovary with 5 carpels, a style with a globose stigma. *Ludwigia adscendens* flowered around October. Flowers bloomed only once a day. When fruiting, the ripe fruit was a capsule, cylindrical, with small hairs, 3 - 4 cm long, opening into 5 pieces. Seeds were small, numerous, rectangular. Seeds were dispersed by water.

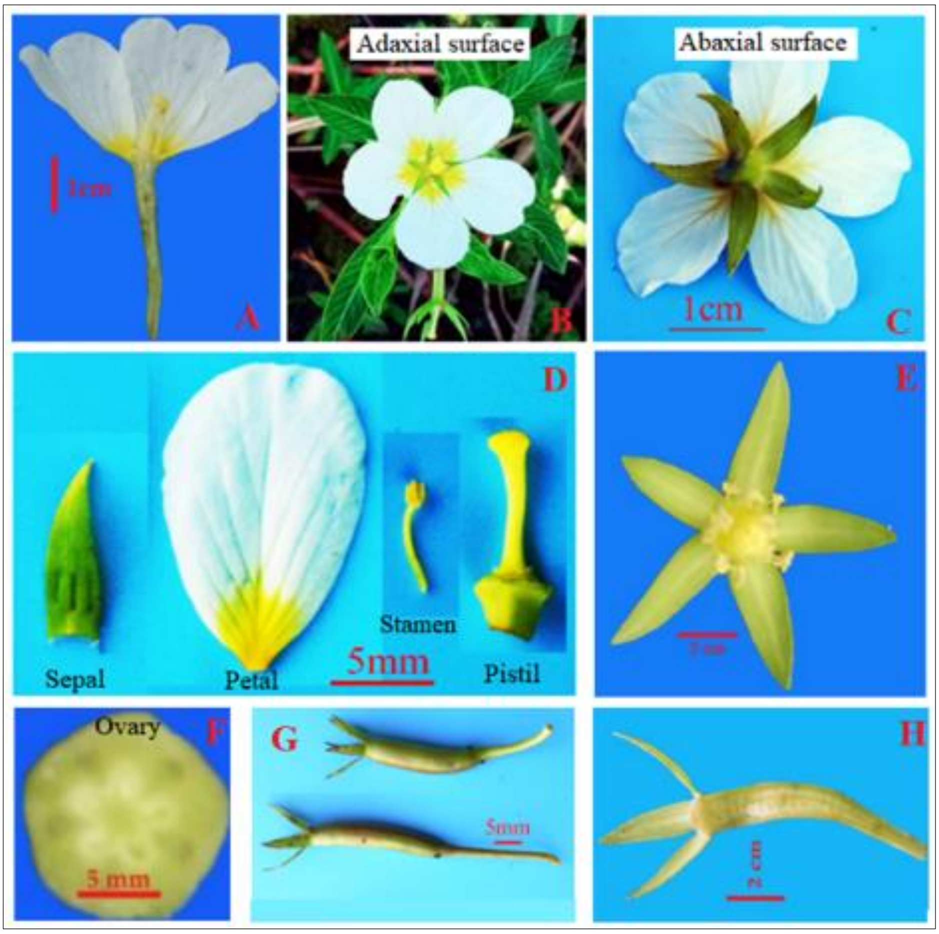



**Figure 5** Reproductive organs of *Ludwigia adscendens*

The description in documents [2], [3], [7], [8] showed that the flowers of the plants were white, while in document [4] plants were described with creamy white flowers. In addition, document [4] also stated that the underside of the leaves of the plants in dry conditions was covered with hairs (trichomes), but according to the observation of the plants in Lang Sen, only a few hairs were seen on the underside of the petiole, possibly because they were in a humid environment. All the morphological features were found in *Ludwigia adscendens* plants in Lang Sen and the descriptions in the above documents showed the diversity of morphology of this species.

### *3.3.* Anatomical characteristics of *Ludwigia adscendens*

#### *3.3.1. Anatomical characteristics of stems*

The transverse section of *Ludwigia adscendens* had a circular cross section, clearly distinguishing the cortex and the stele (Figure 6). Epidermis were small rectangular monolayer cells covered with cuticle. Inside the epidermis, there were 1 - 3 layers of angular collenchyma consisting of closely spaced polygonal cells, followed by many layers of spherical parenchyma cells of different sizes, which were characteristically arranged to form many large hollow cavities. The endodermis was a spherical layer of cells, slightly elongated in a tangential direction. Endodermis cells closely resembled cortex parenchyma cells, distinguished by soon turning into hard tissue in their secondary structure.

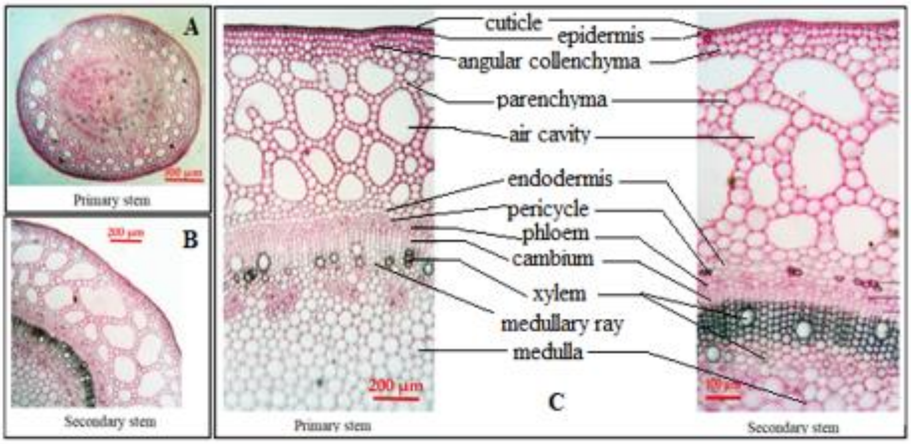



**Figure 6** Anatomy of primary and secondary stems of *Ludwigia adscendens*

The stele was started from a layer of cells of the pericycle with a small polygonal shape, which looked quite similar to phloem cells. The primary vascular tissue consisted phloem and xylem that separated by 3 - 4 layers of radial rectangular cells forming a continuous ring of cambium. The primary phloem was outside the cambium, consisted of living cells that were small, closely spaced, and centripetal division. Primary xylem consisted of 5 - 6 layers of thick-walled cells. The xylem bundles were separated by 3 - 4 layers of primary medullary ray cells, which were slightly elongated in the radial direction. Parenchymal cells of medulla occupied most of the cross section (45.6% of the radius of the stem), consisted of globular cells with small intercellular spaces. Dimensions of the components of the primary stems were presented in Table 1 below (Percentage on the radius of the slice).

**Table 1** Dimensions of components of primary stems (number samples = 15)

| Components | Thickness (µm) | Percentage (%) |
|---|---|---|
| Epidermis | 17.43 ± 1.96 | 1.26 |
| Collenchyma | 80.70 ± 9.62 | 5.81 |
| Cortex parenchyma | 420.90 ± 27.22 | 30.31 |
| Endodermis | 27.12 ± 1.46 | 1.95 |
| Pericycle | 17.14 ± 1.57 | 1.23 |
| Primary phloem | 38.54 ± 4.72 | 2.78 |
| Cambium | 71.85 ± 2.50 | 5.17 |
| Primary xylem | 89.13 ± 1.53 | 6.42 |
| Medullary parenchyma | 625.64 ± 26.59 | 45.60 |
| Total | 1243.44 ± 38.81 | 100 |

Primary stem structure of *Ludwigia adscendens* showed a large proportion of air cavities, which enhanced the circulation and exchange of gases in the stem, providing air to all parts of the plant, demonstrating the adaptation of the plant. *Ludwigia adscendens* grew in an oxygen-poor flooded environment. Beneath the epidermis were 1 - 3 layers of thick tissue responsible for supporting and increasing the stability of the stem. This results were consistent with other works also noted that the primary cortex has many air cavities [7]. These characteristics helped this species adapt to flooded conditions and environmental changes during the rainy season. However, the study of Nguyen Thi Thu Ha et al.

[7] and also of Folorunso et al. [9] did not record the characteristics of the secondary structure. The microscopic anatomy results of the present study showed that the secondary structure of the stem was underdeveloped (Figures 6B and C). Compared with the primary structure, the difference was that the cell layer of the pericycle had a few cells that had turned into hard tissue. Meta-xylem developed into a continuous ring with large cells and pushed proto-xylem inward. Proto-xylem cells were still active.

### *3.3.2. Anatomical characteristics of roots*

In primary roots, the document [7] described the epidermis as consisting of a closely spaced rectangular layer of cells, inside which were 13 - 14 layers of cortex parenchyma cells and a layer of cells of the endodermis with Casparian strip. However, the cross section in Figure 7A showed a fairly early transformation into the secondary structure of *Ludwigia adscenden* roots. Some parenchyma cell layers of the primary cortex sloughed away. A nearly continuous ring of secondary xylem appeared and the walls of medullary ray cells became thickened.

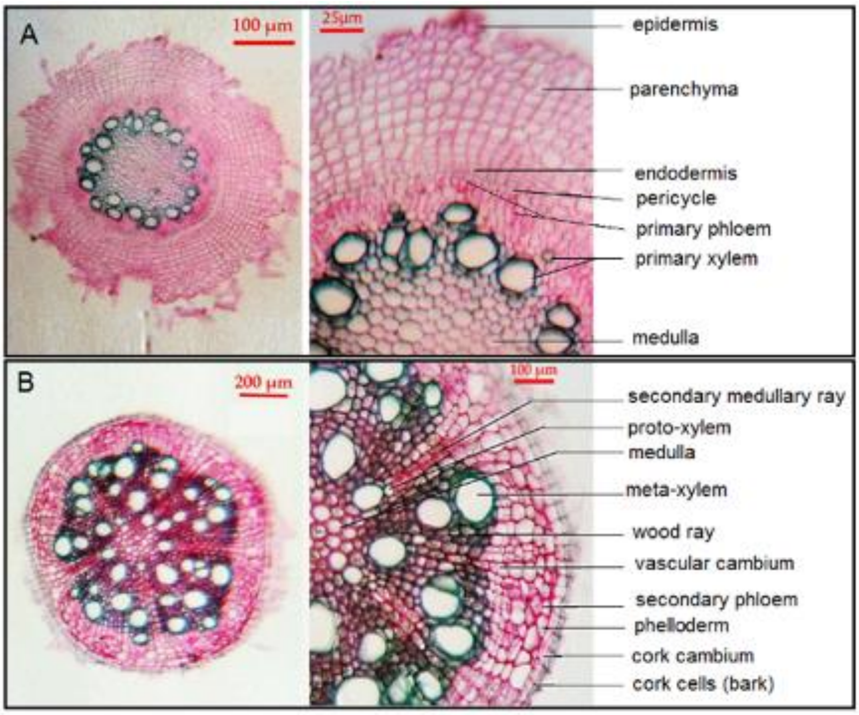



**Figure 7** Anatomy of primary and secondary roots of *Luwigia adscendens*

In addition, the microscopic anatomy results of the present study showed that the secondary structure of the roots of *Ludwigia adscenden* (Figure 7B), in which the secondary cortex was quite thin, and the presence of vascular cambium was difficult to distinguish. Five vascular bundles could be recognized based on the appearance of the wood bundles, separated by five radiologically arranged secondary medullary rays. The radius of the stele accounts for more than 75% of the radius of the slice. Dimensions of the components of the primary roots were presented in Table 2 below (Percentage on the radius of the slice).

**Table 2** Dimensions of components of the primary roots

| Components | Thickness (µm) | Percentage (%) |
|---|---|---|
| Epidermis | 13.29 ± 3.64 | 4.82 |
| Cortex parenchyma | 104.77 ± 22.86 | 37.96 |
| Endodermis | 10.30 ± 5.33 | 3.73 |
| Pericycle | 8.21 ± 4.22 | 2.98 |
| Phloem | 36.49 ± 10.49 | 13.22 |

| | | |
|---|---|---|
| Xylem | 54.72 ± 22.22 | 19.83 |
| Medullary parenchyma | 84.69 ± 12.03 | 30.69 |
| Total | 275.99 ± 35.36 | 100 |

*3.3.3. Anatomical characteristics of leaves*

The cross-section of the midrib of *Ludwigia adscendens* was elliptical. Most of the protrusion was on the abaxial side and the minor axis height was about 781 μm. In a bilateral comparison, the epidermis and collenchyma tissue of the adaxial side were thicker than those of the abaxial side, 13.9 and 67.7 μm versus 11.5 and 53.8 μm, respectively. These structures had the effect of supporting and protecting the leaves. The adaxial collenchyma was also more layered (2 - 3 layers) than the abaxial collenchyma (1 - 2 layers). In contrast, for parenchyma, the adaxial side had 4 - 5 layers of spherical cells arranged quite close together, about 145 μm thick while the abaxial side had 7 - 8 layers arranged in a characteristic pattern forming many large, about 210 mm thick. In terms of vascular tissue, cross section showed a semicircular structure with underlying curvature. In the upper part of this structure, there were only traces of a few groups of sclerenchyma pericycle cells about 60.9 μm thick. From the abaxial up, the sclerenchyma pericycle aggregated into a semicircle about 28.4 μm thick, followed by the phloem about 42.7 μm thick and the xylem about 47.9 μm thick. The medullary parenchyma was also semicircular with a underlying curved margin, about 100 μm thick (Figure 8 A, B).

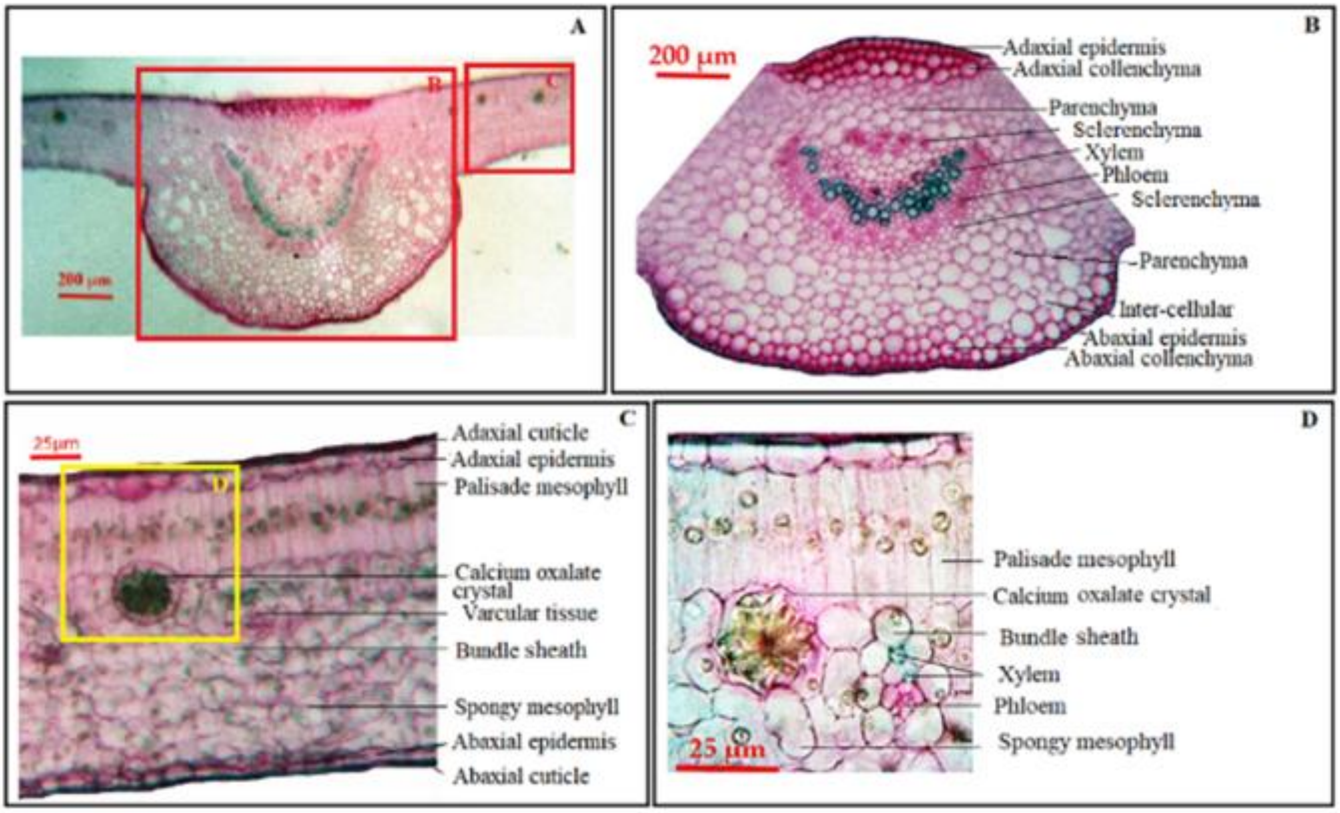



**Figure 8** The anatomical characteristics of midrib and lamina of *Ludwigia adscendens*

In terms of the lamina (leaf blade), from adaxial down and from abaxial up to the central layer consisted of the epidermis, mesophyll and vascular bundles of veins. The adaxial and abaxial epidermis were approximately 16.4 and 11.1 μm thick, respectively. The adaxial palisade mesophyll consisting of 1 - 2 cell layers was 44.1 μm thick while the abaxial spongy mesophyll consisting of 5 - 7 cell layers was 155.6 μm thick. In particular, in the spongy mesophyll, there were some spherical cells containing calcium oxalate crystals that increased the firmness of the leaf blade (Figure 8 C, D). In the leaf blade, there were also several small vascular bundles of veins, consisting of 5 - 7 cells of the bundle sheath on the outside, and inside with xylem above and phloem below (Figure 8 D). The present study also noted that the arrangement of the stomata was anomocytic pattern (Figure 9). Stomata were concentrated mainly on the adaxial side of leaves with the number per $mm^2$ about 393 while on the abaxial about 290. These records contributed to clarifying the structure of *Ludwigia adscendens* species, supplementing existing data in Vietnam such as documents [1], [3], [4], [7].

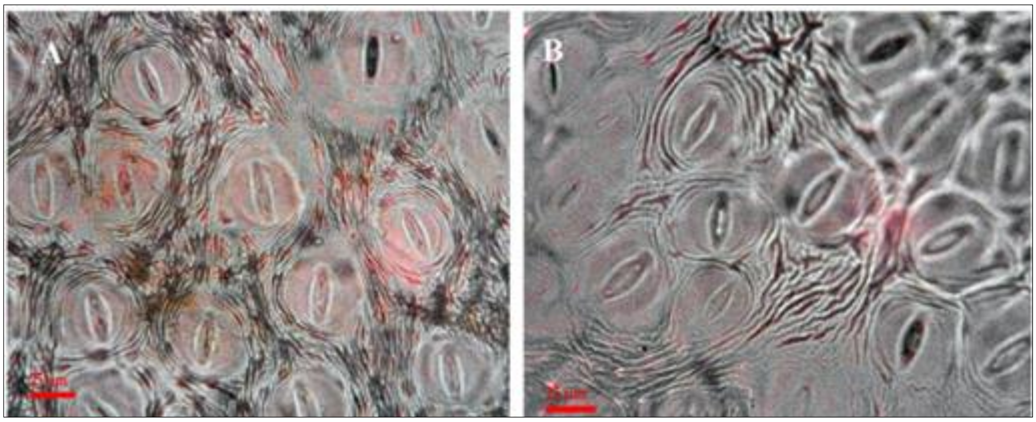
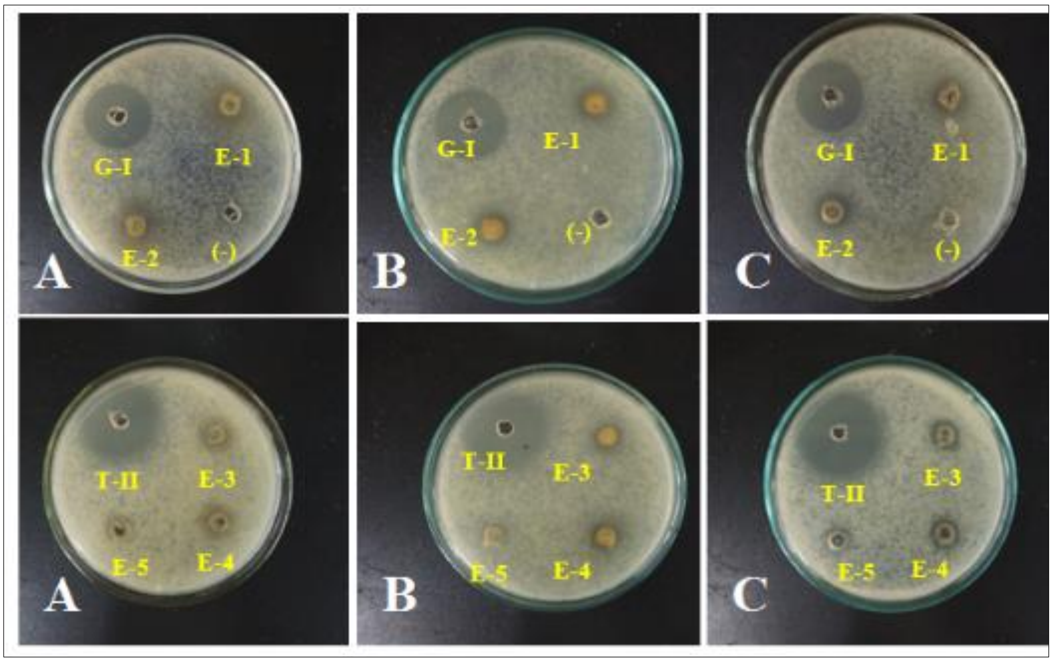



**Figure 9** Pattern of stomata on the adaxial (A) and adaxial (B) sides of *Ludwigia adscendens*

*3.3.4. Antibacterial ability of the plant extract*

The antibacterial ability of crude extract from *Luwigia adscendens* was shown in Table 3. There were differences in antibacterial activities of stem, leaf and root extracts. The leaf and stem extracts were effective on all the three strains (*Bacillus cereus*, *B. subtilis*, *and Escherichia coli*) while the root extracts were not observed antibacterial activities against *E. coli.* The data presented in the table were the highest data obtained when using the extract at a concentration of 1,000 mg/mL. Antibiotics were used at a concentration of 1 mg/mL.




(A) Leaf extract; (B) Stem extract; (C) Root extract. (E-1) 1,000 mg/mL; (E-2) 800 mg/mL; (E-3) 600mg/mL; (E-4) 400 mg/mL; (E-5) 200 mg/mL; (-) Ethanol 70%v/v; (G-I) Gentamicin; (T-II) Tetracycline.

**Figure 10** Zone of inhibition on *B. cereus* of *Luwigia adscendens* extracts

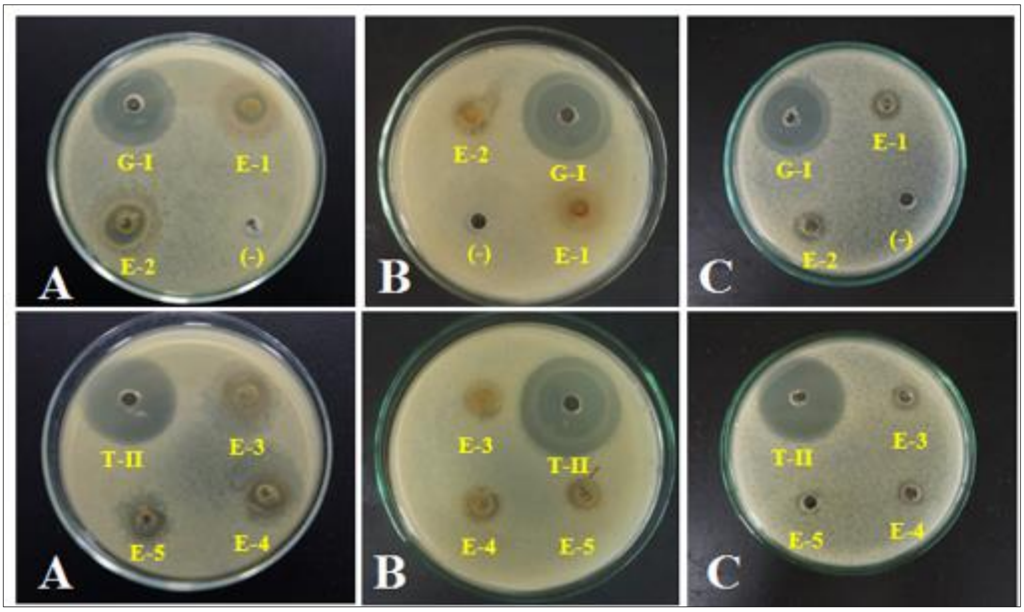
On *Bacillus subtilis*, extracts from stems, leaves, and roots all had antibacterial activity at the all tested concentrations, in which the leaf extract showed the largest inhibitory area (9.90 ± 0.27 mm). Particularly for *E. coli*, the stem extracts showed the highest efficiency (10.36 ± 0.27 mm) while the root extracts had absolutely no inhibitory effect on bacteria. A study of Ahmed et al. [19] also showed that the methanolic extracts of *Luwigia adscendens* plants (stems and leaves) was also resistant to *E. coli* and some other bacteria species, except *Stapylococcus aureus*.

**Table 3** Antibacterial abilities of crude extracts of *Luwigia adscendens*

| Test samples | Zone of bacterial inhibition (mm) | | |
|---|---|---|---|
| | ***B. cereus*** | ***B. subtilis*** | ***E. coli*** |
| Stem extract | 7.34 ± 0.12 | 8.95 ± 0.17 | 10.36 ± 0.27 |
| Leaf extract | 8.13 ± 0.63 | 9.90 ± 0.27 | 9.15 ± 0.11 |
| Root extract | 5.86 ± 0.14 | 5.82 ± 0.05 | - |
| Gentamicin (G-I) | 26.14 ± 0.84 | 25.85 ± 1.12 | - |
| Tetracycline (T-II) | 23.75 ± 0.18 | 28.36 ± 0.21 | 22.4 ± 0.62 |

(-): inefficient; number of sample = 3.

On *Bacillus cereus*, the root extracts from the of plant *Luwigia adscendens* exhibited antibacterial activity at all concentrations from 200 mg/mL to 1000 mg/mL, while the ethanol extracts from the stems and leaves at all concentrations 200 mg/mL showed no antimicrobial activity. However, Table 3 showed that the leaf extracts showed a better inhibitory effects on these bacteria (8.13 ± 0.63 mm) than the extracts from other parts of plant with the same concentration of action.




(A) Leaf extract; (B) Stem extract; (C) Root extract. (E-1) 1,000 mg/mL; (E-2) 800 mg/mL; (E-3) 600mg/mL; (E-4) 400 mg/mL; (E-5) 200 mg/mL; (-) Ethanol 70%v/v; (G-I) Gentamicin; (T-II) Tetracycline

**Figure 11** Zone of inhibition on *B. subtilis* of *Luwigia adscendens* extracts

## 4. Conclusion

The morphological and anatomical characteristics of *Luwigia adscendens* were suitable for the habitat conditions in the annual flooded area of Lang Sen Wetland Reserve. The leaf and stem extracts were effective on all the three strains (*Bacillus cereus*, *B. subtilis*, *and Escherichia coli*) while the root extracts were not observed antibacterial activities against *E. coli*.

## Compliance with ethical standards

### *Acknowledgments*

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*Disclosure of conflict of interest*

All authors declare no conflicts of interest regarding this article.

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