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Antibacterial and Antioxidant Activities of *Ludwigia* Species: Potential Applications in Acne Treatment

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ABSTRACT

Acne vulgaris is a current issue of concern due to its negative impact on the patient's life, highlighting the urgent need for safer natural treatments with fewer side effects. Current efforts focus on the exploration of natural medicinal sources to identify bioactive compounds with antimicrobial and antioxidant properties. The genus *Ludwigia* (Onagraceae), traditionally used to treat hormonal imbalances, remains poorly studied in terms of bioactivities, phytochemistry, and pharmaceutical applications, particularly in Vietnam. In this study, ethanol extracts from 5 *Ludwigia* species (*L. octovalvis, L. adscendens, L. hyssopifolia, L. prostrata,* and *L. peruviana*) were evaluated for antibacterial activity against *Staphylococcus epidermidis, Staphylococcus aureus,* and *Cutibacterium acnes*. Extracts were prepared using ultrasound-assisted extraction, and antibacterial effects were assessed by agar diffusion and agar dilution methods to determine inhibition zones and minimum inhibitory concentrations (MICs). The results showed that the ethanol extract of *L. octovalvis* (LOE) exhibited the highest antibacterial efficacy against the three tested bacterial strains, including *S. epidermidis, S. aureus,* and *C. acnes*, with MIC values of 450 µg/mL, 550 µg/mL, and 600 µg/mL, respectively. This extract also showed high antioxidant activity with an IC_{50} of 18.98 ± 0.09 µg/mL, as determined by the 2,2 diphenyl - 1 - picrylhydrazy (DPPH) assay. Preliminary phytochemical screening confirmed the presence of phenolics, flavonoids, saponins, triterpenoids, tannins, glycosides, carotenoids, fixed oils, amino acids, and proteins. The quantitative phytochemical analysis displayed a total phenolic content of 305.94 ± 1.46 mg GAE/g and a total flavonoid content of 51.42 ± 0.27 mg QE/g. Our report considers the first to investigate the *in vitro* anti-acne and potential application of *L. octovalvis* in producing natural skin care products with antimicrobial and antioxidant capabilities.

Keywords: antibacterial, antioxidant, *Ludwigia*, acne, *L. octovalvis*

INTRODUCTION

The genus *Ludwigia* belongs to the Onagraceae family consisting of about 87 species of herbaceous plants or shrubs, which grow wild and have a strong vitality. Some *Ludiwigia* species are used as a traditional remedy for diseases such as diarrhea, dysentery, gastrointestinal complaints, and diabetes in some South East Asian countries such as Indonesia (Eliza et al., 2023), Malaysia (Yakob et al., 2012), and Vietnam (Nguyen et al., 2020). Previously conducted research has globally revealed numerous biological benefits associated with this genus, including its antibacterial (Nanda et al., 2008), antioxidant (Barman et al., 2018), anti-cancer (Das et al., 2007) and anti-inflammatory properties (Bhuiyan et al., 2023). The plant contains also many bioactive components such as tannins, phenolic acids, flavonoids and

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triterpenoids (Shawky et al., 2021). The *Ludwigia* genus in the Mekong Delta region of Vietnam has a large amount of biomass incorporated with their potential for biological activity, which is a fortunate condition for us to research and develop scientifically on the application of the *Ludwigia* genus to natural treatment products that are safer for human health.

Acne vulgaris is a skin disease related to inflammation of the sebum glands frequently in face and chest and negatively affects the quality of life and self-image, making people feel imperfect and increasing the rate of depression (Eichenfield et al., 2021). Currently, there are many causes of acne vulgaris, including stress, environmental pollution, cosmetics misuse, excessive secretion of oils and especially inflammation caused by microorganisms (Lim et al., 2021). Three common gram-positive bacteria were found on the *Cutibacterium acnes*, *Staphylococcus aureus*, and *Staphylococcus epidermidis*. These bacteria exist in equilibrium and play a protective role by protecting the skin from external harmful agents However, some previous studies showed when the equilibrium of this microbial community is destroyed that the main cause of acne (Claudel et al., 2019), (Dréno et al., 2020). Although acne is frequently treated with antibiotics, such treatment may increase the resistance of bacteria, on average about 50% of *C. acnes* strains were reported to be antibiotic-resistant (Weber et al., 2019). As an effort to address this issue, exploring and analyzing the bioactivity of naturally occurring plants were consistently developed as potential alternatives or complement to acne treatment (Proença et al., 2022).

In this study, we evaluated the antibacterial effect of five ethanol extracts of five *Ludwigia* species on three strains of *S. aureus*, *S. epidermidis*, *C. acnes* bacteria to provide a preliminary view and a comparison of their biological activity. On the basis of these results, the species with promising potential for treatment was identified, opening new opportunities for further evaluation of the antioxidant capacity of the plant. The purpose of this study is to discover new therapeutic candidates for skin care based on natural products isolated from Vietnamese plants.

MATERIALS AND METHODS

The aerial parts of *Ludwigia octovalvis* (Jacq.) Raven, *Ludwigia adscendens* (L.) Hara, *Ludwigia hyssopifolia* (G. Don) Exell, *Ludwigia prostrata* Roxb and *Ludwigia peruviana* (L.) Hara were collected in An Giang province (10°40'17.2"N 105°09'37.1"E) and Can Tho city (10°04'51.1"N 105°43'05.6"E), Viet Nam from December 2023 to January 2024. We conducted species identification based on external morphological characteristics and anatomical observations (Figure 1). To ensure the most accurate results, we employed DNA analysis methods to identify the samples. This analysis was conducted at the Faculty of Agriculture, Can Tho University.

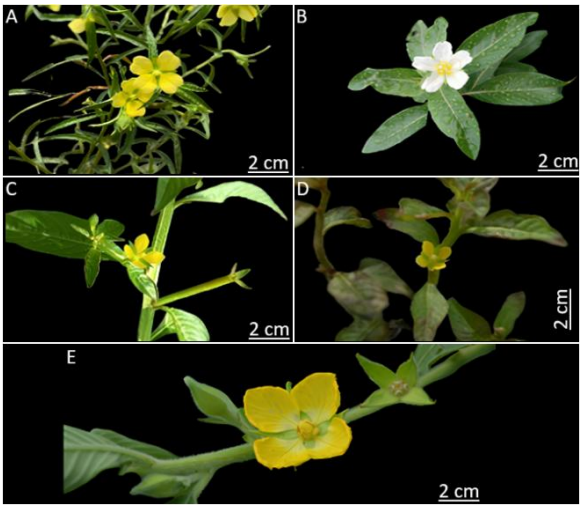

Figure 1. Morphological habits of the *Ludwigia* studied in the Mekong Delta
(A). *Ludwigia octovalvis* (Jacq.) Raven; (B). *Ludwigia adscendens* (L.) Hara; (C). *Ludwigia hyssopifolia* (G. Don) Exell; (D). *Ludwigia prostrata* Roxb; (E). *Ludwigia peruviana* (L.) Hara.

Ethanol (purity ≥ 99.0%) was the only solvent used for extraction and was purchased from Chemsol. 2,2 diphenyl - 1 - picrylhydrazyl (DPPH) was provided by Sigma Aldrich, and DPPH was stored in a freezer at temperatures ranging from 2-8°C. Ascorbic acid (Vitamin C) was provided by Duchefa and stored at room temperature. Tryptic Soy Agar (TSA) and Mueller-Hinton (MH) media were produced by Oxoid (Thermo Fisher Scientific, UK).

The bacterial strains *Staphylococcus aureus* ATCC ® 29213, *Staphylococcus epidermidis* ATCC ® 14990 and *Cutibacterium acnes* ATCC ® 6919 were provided by the Inspection & Analysis Center For Import Export Products Vietnam. Bacterial cultures were maintained in Luria-Bertani (LB) agar and stored at 4°C. These three bacterial strains have been reported to be present in acne lesions (Huang

et al., 2022) and are associated with the pathogenesis of acne (Kim et al., 2024).

Preparation of ethanol extracts

To obtain the crude extracts, 20 g of ground, air-dried material from each *Ludwigia* species was successively and exhaustively extracted using ultrasonic-assisted extraction (UAE) (Nguyen et al., 2020) with 500 mL of ethanol. The extracts were then filtered using Whatman® qualitative filter paper by pressure reducing devide. The filtrates were concentrated using a rotary evaporator under reduced pressure (Heidolph™ Hei-VAP, Germany) with the following conditions: bath temperature of 40°C, pressure of 175 mbar, and speed of 50 rounds/min to remove the solvent. The final extracts were stored at 2–8°C for further use.

Screening of antibacterial activity

Three gram-positive bacteria (*S. aureus, S. epidermidis, C. acnes*) were employed to evaluate the antibacterial activity of five samples including *L. octovalvis* extract (LOE), *L. adscendens* extract (LAE), *L. hyssopifolia* extract (LHE), *L. prostrata* extract (LPrE) and *L. peruviana* extract (LPE). The preliminary screening of antibacterial activity was carried out by agar plate diffusion method (Tran et al., 2022), using Vancomycin (30 µg/mL) as positive control and DMSO as negative control (Teo et al., 2021). The bacteria were cultured on Tryptic Soy Agar (TSA), then a bacterial suspension (10^8 CFU/mL, 100 µL) was evenly spread on the surface of Mueller-Hinton (MH) agar plates to evaluate antibacterial activity. Subsequently, 60 µL of the test sample at a concentration of 5000 µg/mL for *S. aureus* and 2000 µg/mL for *S. epidermidis* and *C. acnes* was added to a well with a diameter of 9 mm. The diameter of the zone of inhibition was measured after 24 h of incubation at 37 °C.

Determination of Minimum Inhibitory Concentration (MIC)

The MIC value was determined for the potential sample using the agar dilution method, as described by Kowalska-Krochmal & Dudek-Wicher, (2021). In this method, the extract was diluted and added to each medium plate MHA for *S. aureus, S. epidermidis,* and TSA for *C. acnes* at concentrations ranging from 100 µg/mL to 1000 µg/mL. Vancomycin (30 µg/mL) was used as the positive control. The bacterial concentration was cultured at 10^8 CFU/mL. The MIC was the lowest concentration of the test substance, and it completely inhibits the growth of bacteria (no colony formation) on the plate.

Phytochemical constituents screening

Test for Alkaloids

Dragendroff's Test (sodium iodide + bismuth carbonate). The addition of few drops of Dragendroff's reagent into the extract indicated a red colour precipitate. This result showed the presence of alkaloids (Silva et al., 2017).

Mayer's Test (mercuric chloride + potassium iodide + distilled water). A few drops of Mayer's reagent are added extract. A white creamy precipitate showed the presence of alkaloids (Silva et al., 2017).

Test for Phenolics

To an ethanol extract (2 mL) was added a few drops of 10% aqueous ferric chloride. The presence of a green precipitate forming indicated the presence of phenolic compounds (Audu et al., 2007).

Test for Flavonoids (Lead acetate Test)

An extract (1 mL) was added and mixed widrops of aqueous solution of lead (II) acetate. The presence of a yellow colour precipitate was an indication of flavonoids (Silva et al., 2017).

Test for Saponins (Foam test)

To 0.5 gram of extract was shaken with water (2 mL) for 15 minutes. The appearance of 2 cm thick foam indicated presence of saponins (Tiwari et al., 2011).

Test for Triterpenoids (Salkowski's Test)

Salkowski's Test (filtrate + few drops of conc. H_2SO_4). The extract was mixed with 0.5 mL of chloroform and filtered. Then the solution was added with few drops of concentrated H_2SO_4 acid. The appearance of golden yellow layer at the bottom indicated presence of triterpenes (Singh & Kumar, 2017).

Test for Tannis (Gelatin Test)

To the extract, 1% gelatine solution containing sodium chloride was added. A white colour precipitate indicated the presence of tannin (Pandey & Tripathi, 2014).

Test for Carbohydrates (Fehling's Test)

Fehling's Test (copper sulphate + potassium sodium tartarate + NaOH + distilled water). The 100 mg of extract is dissolved in 5 mL of distilled water and filtered. The filtrate (2 mL) mixed with 1 mL each of Fehling's solution A & B was added and boiled in water bath. A red colour precipitate showed a possible presence of carbohydrates (Silva et al., 2017).

Test for Glycosides

Liebermann's Test (acetic anhydride +conc. H_2SO_4). The aqueous crude extract was combined with acetic acid (2 mL) and chloroform (2 mL). After cooling, concentrated H_2SO_4 was added. The green colour showed the entity of aglycone, a steroidal part of glycosides (Gul et al., 2017).

Salkowski's Test (filtrate + few drops of conc. H_2SO_4). The extract was mixed with concentrated H_2SO_4 (2 mL). A reddish brown colour formed which showed the presence of steroidal aglycone part of the glycosides (Gul et al., 2017).

Test for Carotenoids

The extract (10 mL) was transferred to a test tube and evaporated to dryness using a water bath. The resulting residue was treated with 2–3 drops of a saturated solution of antimony trichloride ($SbCl_3$) in chloroform ($CHCl_3$). The appearance of a blue-green colour, which gradually transitioned to red, indicated the presence of carotenoids (Jagessar, 2017).

Test for Fixed oil

The test solution absorbed on the filter paper developed a transparent appearance, indicating the presence of volatile oils and resins (Tyagi, 2017).

Test for Amino acids and proteins

The plant extract was mixed with a small amount of concentrated nitric acid. If a yellow colour appeared, it meaned the present of amino acids and protein (Tiwari et al., 2011).

Determination of antioxidant activity

DPPH scavenging activity was evaluated according to the method of (Iordănescu et al., 2021). The various concentrations of 0.1 mL sample extracts were mixed with 0.1 mL DPPH 0.5 mM. The reduction of the DPPH radical was determined by reading absorbance at 517 nm using a spectrophotometer (Inoue et al., 2005). Ascorbic acid concentrations of 1 µg/mL to 7 µg/mL were used as a positive control. The percentage of DPPH radical scavenging was calculated as follows:

$$\%\ Inhibition = \frac{Ac - As}{Ac} \times 100$$

Where A_c is the absorbance of the blank (without the sample), and A_s is the absorbance of the sample. Inhibitory concentration IC_{50} values (µg/mL) were determined as inhibitory concentration of the extract necessary to decrease the initial DPPH radical concentration by 50%. Lower IC_{50} value indicated higher DPPH radical scavenging activity.

Determination of Total Phenolic Content (TPC)

The total phenolic content (TPC) of a potential extract was assessed using the Folin-Ciocalteu reagent method as described by Matić et al., (2017). A solution mixture was prepared by combining 20 µL of the extract (50 µg/mL), 1580 µL of distilled water, 100 µL of Folin-Ciocalteu reagent, and 300 µL of an aqueous sodium carbonate solution (200 g/L), followed by incubation at 40 °C for 30 min in a water bath. Absorbance was measured at 765 nm against a blank solution (20 µL distilled water instead of the extract). A linear equation was derived from the calibration curve formulated using a gallic acid working stock solution. The TPC was measured as mg gallic acid equivalent (GAE) per gram dry extract weight (mg GAE/g).

Determination of Total Flavonoid Content (TFC)

The total flavonoid content (TFC) was determined using a spectrophotometric method that involved complex formation with $AlCl_3$ (Pękal & Pyrzynska, 2014). A 1000 µL extract and then combined with 500 µL of 10% $AlCl_3$ and 500 µL of distilled water. Following a ten minutes incubation at room temperature, the absorbance was measured at 428 nm. The blank solution contained the same amount of water instead of $AlCl_3$. A linear equation was derived from the calibration curve using quercetin standard. The TFC was measured as mg quercetin equivalent (QE) per gram dry extract weight (mg QE/g).

Statistical analysis

All tests were one in triplicate, and the averaged results were used. The data were assessed through both qualitative and quantitative methods. Analysis was conducted using Minitab 6, with a One-way analysis of variance (ANOVA) and the Tukey post-hoc test employed for statistical evaluation. A 95% confidence level was applied, with a significance criterion set at $p < 0.05$. Results are presented as mean ± standard deviation (SD).

RESULTS AND DISCUSSION

Screening of antibacterial activity

In investigating the antibacterial activity of five ethanol extracts derived from five *Ludwigia* species through the diameter inhibitory zone, we obtained preliminary insights that could facilitate the evaluation of the biological activity potential of these species. The results showed that the extract of the species displayed different strong and weak antibacterial activity for each bacterial strain.

Table I. Antibacterial activity of *Ludwigia* extracts against skin bacterial strains

	Diameter of inhibition zone (mm)		
	S. aureus	***S. epidermidis***	***C. acnes***
LOE	$12.50^{b} \pm 1.00$	$15.00^{a} \pm 0.71$	$15.13^{b} \pm 0.85$
LAE	$11.00^{cd} \pm 0.41$	$13.38^{b} \pm 0.95$	$13.13^{c} \pm 1.03$
LHE	$11.25^{c} \pm 0.29$	$14.25^{ab} \pm 0.96$	nd
LPrE	$10.38^{de} \pm 0.48$	$13.63^{b} \pm 0.75$	nd
LPE	$10.00^{e} \pm 0.41$	$9.50^{c} \pm 1.00$	nd
Vancomycin	$16.25^{a} \pm 0.29$	$15.00^{a} \pm 1.00$	$18.25^{a} \pm 0.87$

Means followed by different letters in the same column ($p < 0.05$) showed a significant difference based on one-way ANOVA; nd: no detected; LOE: L. octovalvis extract*, LAE: L. adscendens* extract*, LHE: L. hyssopifolia* extract*, LPrE: L. prostrata* extract*, LPE: L. peruviana* extract

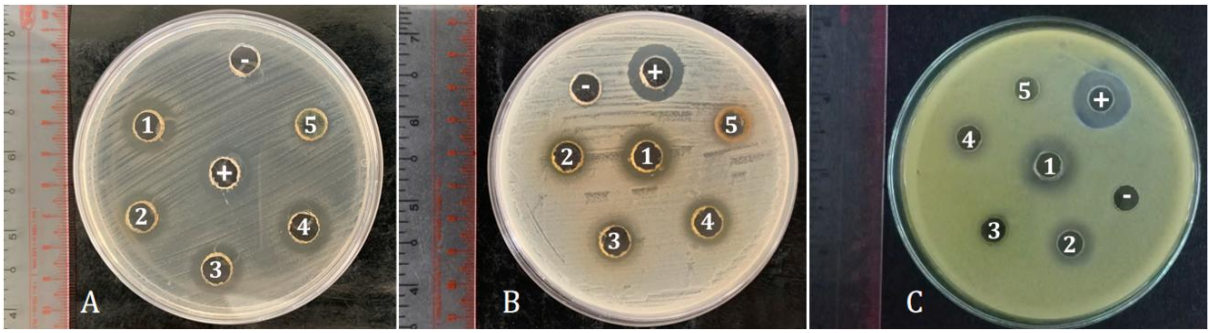

Figure 2. Diameter of the inhibition zone of five *Ludwigia* species extracts against skin bacterial strains (1)LOE; (2) LAE; (3) LHE; (4) LprE; (5) LPE at a concentration of 5000µg/mL for *S. aureus* and at 2000 µg/mL for *S. epidermidis and C. acnes*; (+) Vancomycin (30 µg/mL); (-) DMSO 10%; (A) *S. aureus*; (B) *S. epidermidis;* (C) *C. acnes.*

Most species could against *S. aureus* and *S. epidermidis*. In particular, LAE and LOE were resistant to *C. acnes* as the main subjects of this study. LOE demonstrated the most effective antibacterial activity against all three bacterial strains, with inhibition zones ranging from 12.50 to 15.13 mm (Table I), (Figure 2).

Determination of Minimum Inhibitory Concentration (MIC)

LOE exhibited the most effective antibacterial activity among the tested *Ludwigia* species, as indicated by the largest inhibition zone diameter. Based on this result, LOE was selected for further MIC determination. The sample was conducted on all three bacterial strains, using a concentration range from 100 µg/mL to 1000 µg/mL. The results demonstrated promise, MIC of 450 µg/mL for *S. epidermidis*, 550 µg/mL for *S. aureus* and 600 µg/mL for *C. acnes* (Figure 3). Following Aligiannis et al., (2001), the antibacterial activity of plant extracts was considered strong when the MIC is less than 500 µg/mL, moderate when it ranges from 500 µg/mL to 1500 µg/mL, and weak when it exceeds 1500 µg/mL. Compared to our study findings, the LOE demonstrated strong efficacy against *S. epidermidis* and moderate efficacy against *S. aureus* and *C. acnes*.

The obtained results showed that LOE extract was a potential agent for a targeted antibacterial solution to address acne. Our research indicated that *L. octovalvis* demonstrates greater efficacy than certain medicinal plant species in combating acne-causing bacteria. For instance, in a study by Hanphanphoom & Krajangsang, (2016), the ethanol extract of *Chromolaena odorata* leaves had MIC values of 810 µg/mL against *S. aureus*, 6250 µg/mL against *S. epidermidis* and 12500 µg/mL against *C. acnes*.

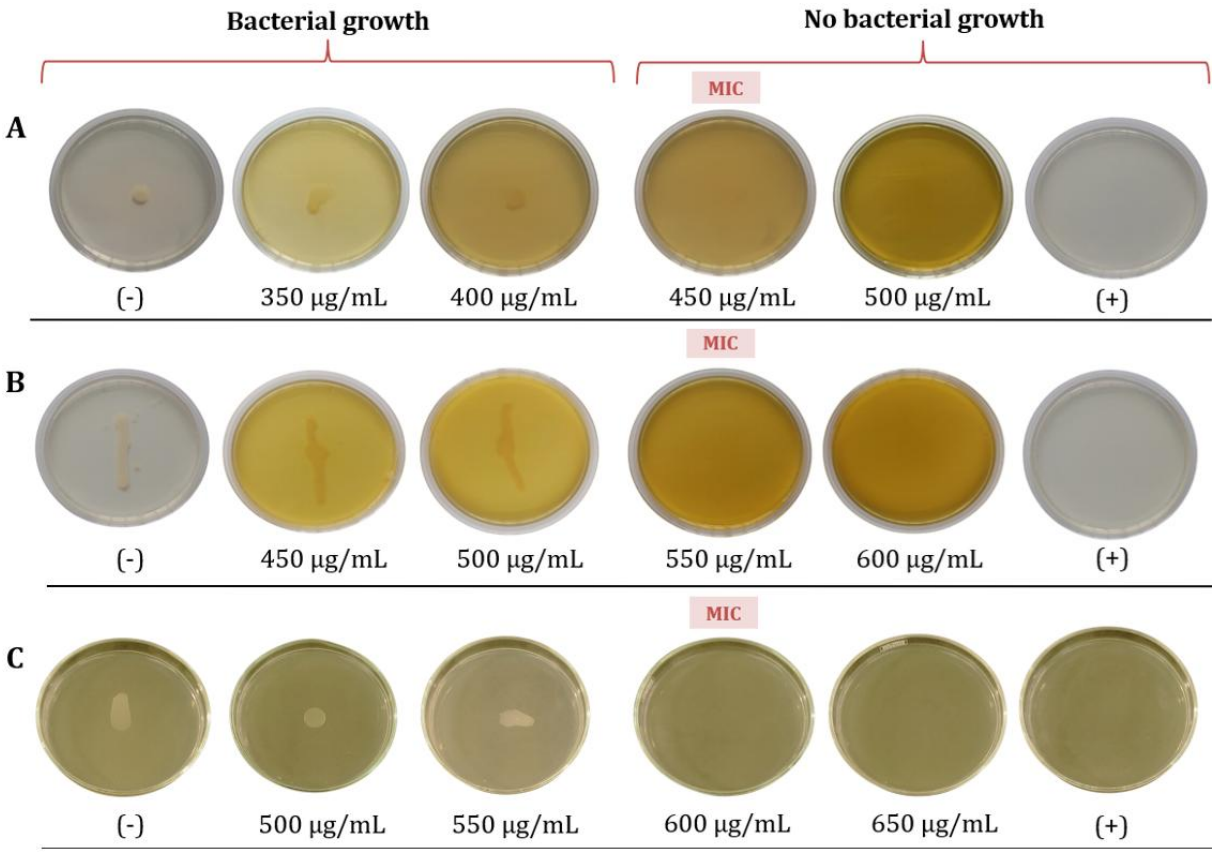

Figure 3. MIC determination of LOE of 450 µg/mL against *S. epidermidis* (panel A), 550 µg/mL against *S. aureus* (panel B), and 600 µg/mL against *C. acnes* (panel); (+) Vancomycin (30 µg/mL); (-) DMSO 10%.

The study by Chomnawang et al., (2005) investigated the antibacterial activity against *C. acnes* and *S. epidermidis* of 19 traditional plants in Thailand, where 15 of these plants exhibited MIC values ranging from 650 µg/mL to 5000 µg/mL. In addition, Yakob et al., (2012) reported that the methanol extract of *L. octovalvis* exhibited varying antibacterial activity depending on the plant part used. The leaf extract showed a MIC of 500 µg/mL against *S. aureus* and 250 µg/mL against *S. epidermidis*. Meanwhile, the stem extract demonstrated a MIC of 1000 µg/mL against *S. aureus* and 120 µg/mL against *S. epidermidis.* In comparison to our findings, there were notable differences, which may be attributed to differences in soil conditions, harvest periods, and different solvents used during extraction, all of which could affect the biological activity of the extracts (Teffo et al., 2024).

Ethanol was selected for this study based on previous reports indicating that extracts obtained with this solvent were capable of producing high concentrations of flavonoids and polyphenols, which significantly contribute to antibacterial and antioxidant activities (Rafińska et al., 2019). At the same time, the study using ethanol extraction was beneficial for future applications of therapeutic support products. From another perspective, extracting each organ separately and extracting multiple plant organs together showed different antibacterial results, suggesting the interaction between the bioactive components present in the various organs of *L. octovalvis* in terms of synergistic effects (Elhaj et al., 2021).

Phytochemical constituents screening

The chemical analysis of the LOE showed the existence of phenolics, flavonoids, saponins,

triterpenoids, tannins, glycosides, carotenoids, fixed oil, amino acids and proteins. These components likely contribute to the extract's significant biological activity (Vitale et al., 2022). Flavonoids and phenolics have been well-documented for their diverse bioactive properties, including antioxidant, anti-inflammatory, and antibacterial effects (Teffo et al., 2024). These findings served as the foundation for a quantitative study of the presence of these two kinds of chemical components. Furthermore, our results were compatible with previous reports on the phytochemicals of *Ludwigia* species (Shawky et al., 2023).

Table II. Phytochemical screening of the LOE

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

(-): negative; (+): positive

Determination of antioxidant activity

Upon consideration of the antibacterial properties of the LOE, we proceeded to conduct further exploration into the antioxidant capabilities of the species. In this assay, we determined the antioxidant activity of LOE at various concentrations using the DPPH method (Table III). The LOE extract displayed significant antioxidant activity with IC_{50} of 18.98 ± 0.09 µg/mL using the DPPH radical scavenging test. In assessing the strength of antioxidant activity, it is commonly classified as very strong when the IC_{50} value is less than 50 µg/mL, strong when the IC_{50} falls within the range of 50–100 µg/mL, moderate when such value ranges from 101–150 µg/mL, weak when the IC_{50} falls within the 151–200 µg/mL range, and finally very weak when the IC_{50} is greater than 200 µg/mL (Nurmazela et al., 2022). Based on our findings, the LOE exhibited very strong antioxidant activity. In comparison to previous studies, such as those by Sukweenadhi et al., (2020), which investigated the antioxidant activity of seven herbal species from Indonesia using the DPPH radical method, with IC_{50} values ranging from 102 to 2221 µg/mL. Another study by Smida et al., (2018) assessing the antioxidant capacity of *Ludwigia peploides* leaf extract revealed an IC_{50} of 58 µg/mL.

Table III. Percentage inhibition and antioxidant activity of LOE

LOE		Ascorbic acid	
Concentration (µg/ml)	**% Inhibition**	**Concentration (µg/ml)**	**% Inhibition**
5	16.09±0.03	1	11.67±1.53
10	29.93±0.94	2	22.71±0.97
15	40.80±0.48	3	31.97±0.44
25	59.29±1.18	5	48.38±0.12
35	93.43±0.92	7	63.87±0.68
IC_{50}	**18.98±0.09**	**IC_{50}**	**5.31±0.06**

Our result on LOE demonstrated significantly superior antioxidant activity. The antibacterial and antioxidant activities were closely related to the amount of polyphenolic compounds in the extract (Kozłowska et al., 2022). The reality that they exhibited effective biological activity indicates that a significant amount of polyphenolic compounds was present in the LOE (Martelli & Giacomini, 2018). Polyphenolic compounds were also known for other notable biological activities, including anti-inflammatory effects and the capacity to reduce sebum production on the skin (Saric et al., 2017). Our research findings provided compelling evidence for the potential of LOE as a valuable component in cosmetic formulations. This supports its efficacy in treating acne and protecting the skin, attributed to its potent antibacterial and antioxidant properties.

Total phenolic content (TPC)

The total phenolic content (TPC) of the LOE was determined using the Folin-Ciocalteu method. The results were calculated through a linear equation that we constructed from the calibration curve of gallic acid (2-20 µg/mL) $y = 0.0677x - 0.0625$, $R^2 = 0.9943$ (Figure 4). Results showed that the TPC in the LOE reached 305.94 ± 1.46 mg GAE/g. The high TPC in LOE indicates a significant presence of phenolic compounds, which have been associated with antibacterial and antioxidant activities (Kozłowska et al., 2022). However, further analysis is required to confirm their specific role in LOE's antibacterial effects. Previous studies had shown that phenolic compounds were one of the main compounds with biological effects of plants. In terms of antibacterial capabilities, they

were associated with their ability to destroy bacterial membranes, inhibit virulent elements such as enzymes and toxins, and prevent the formation of bacterial biofilms (Koch et al., 2024).

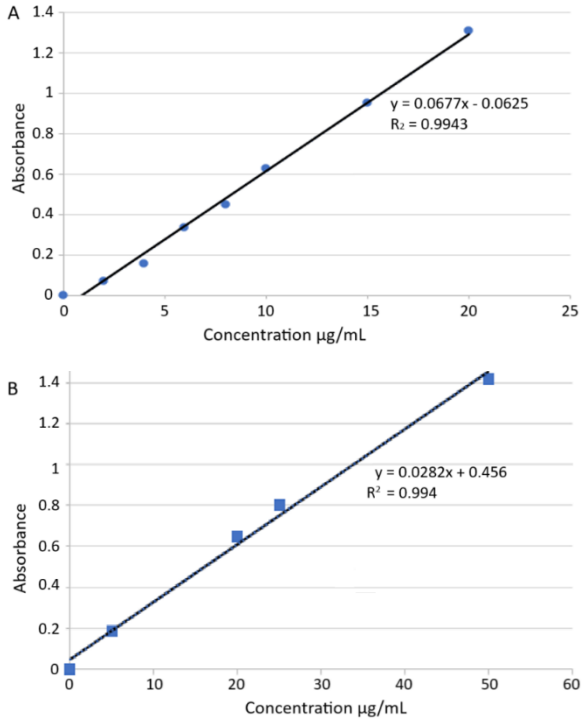

Figure 4. Graph of the standard curve of Gallic acid (panel A), and Quecertin (panel B)

In addition, they were used as an antioxidant with the ability to provide hydrogen atoms or electricity to free radicals, due to their composition containing many hydroxyl functional groups (Ouamnina et al., 2024). A comparison with a study by Pukumpuang et al., (2012) total phenolic quantification of ethanol extracts from 10 plants in Thailand with resistance to *C. Acnes* ranged from 10.67 ± 0.24 to 50.80 ± 0.42 mg GAE/g. Similarly for the study of Muddathir & Mitsunaga, (2013), the survey of the TPC of some traditional plants in Sudan believed thought to have acne treatment potential ranging from 34.98 ± 2.83 to 47.89 ± 4.99 mg GAE/g. Thereby, LOE had more potential than many other plant species to replace the application in acne treatment. Compared to previous reports of *L. octovalvis*, the TPC of leaf methanol extract was 264.76 ± 0.23 mg GAE/g in accordance with Yakob et al., (2012). In the study by Calonico & Rosa-Millan, (2023), *L. octovalvis* water extracts yielded TPC of 652.96 ± 10.58 mg GAE/g. Our study showed somewhat different results from the above studies which may been due to the nutrient, time of harvest, differences in extraction solvents, and extraction methods (Hartanti et al., 2023).

Total flavonoid content (TFC)

The total flavonoid content (TFC) was measured by complexing with $AlCl_3$. The results were calculated according to a linear equation constructed from the calibration curve of quecertin (5-100 µg/mL) $y = 0.0282x + 0.0456$, $R^2 = 0.994$ (Figure 4). Our study showed that the achieved TFC of LOE was 51.42 ± 0.27 mg QE/g. In the study by Abu-Qatouseh et al., (2019) the TFC of some plants in Jordan could treat acne ranged from 12.36 ± 0.84 to 114.17 ± 0.63 mg QE/g. Specifically, the LOE had a higher TFC than *Quercus calliprinos, Arum palaestinum,* and *Utricadioica* species with the contents of 18.45 ± 0.19 mg QE/g, 12.36 ± 0.84 mg QE/g, 41.25 ± 1.51 mg QE/g, respectively. In the study of Aryal et al., (2019), the determination of TFC content from eight tree species in Nepal resulted in a range from 6.61 ± 0.42 to 39.38 ± 0.57 mg QE/g. Hence, it could be inferred that the TFC of the LOE demonstrates noteworthy biological potential and concurrently exhibited a greater potential for application compared to certain other plant species. Compared with the report of Pandey et al., (2021) on the detection of TFC of hydrogen-alcoholic extract of *L. octovalvis* of 43.9 mg QE/g, our study is somewhat better. The reason may come from soil nutrition, time of harvest, and extraction solvent (Aryal et al., 2019). Previous studies had demonstrated that flavonoid compounds were associated with antioxidant capacity from plants. Plants with high levels of TFC acted as a stabilizer of ROS (Reactive oxygen species), enhancing the reaction efficiency of antioxidant compounds (Mehmood et al., 2022).

CONCLUSION

Our study investigated five *Ludwigia* species in the Mekong Delta, identifying *L. octovalvis* as having significant antibacterial activity against three strains of acne-causing bacteria, particularly *C. acnes*. Additionally, it exhibited strong antioxidant properties. The LOE showed significant potential as a novel natural source for acne treatment, addressing antibiotic resistance in multiple bacterial strains.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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