Active Compounds In The Aqueous Extract Of *Ludwigia Octovalvis* Leaves As Antibacterial Agents Against Pathogens

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Abstract.
Ludwigia octovalvis is a tropical aquatic weed with potential as a medicinal plant due to its diverse secondary metabolite content. This study aimed to quantify the secondary metabolite profile of aqueous extracts of Ludwigia octovalvis leaves and evaluate their potential as natural antibacterial agents. Leaves were collected from wetlands in Tungkaran Village, Martapura, Banjar Regency, South Kalimantan, and extracted using distilled water at a ratio of 1:4. Quantitative phytochemical analysis was performed in triplicate for saponins, alkaloids, flavonoids, terpenoids, tannins, steroids, anthocyanins, and phenolics. Ludwigia octovalvis leaf extract contains secondary metabolite compounds, namely saponins (15.587%), alkaloids (6.507), flavonoids (91.667 mg/g), terpenoids (228.467 mg/g), tannins (0.850 mg/g), steroids (39.177 mg/g), anthocyanins (negative), and phenolics (65.467 mg/g). The highest levels of secondary metabolites are found in terpenoid compounds, namely 228.467 mg/g and the lowest in negative anthocyanin compounds. These secondary metabolite compounds have the potential as antibacterials against Aeromonas hydrophila bacteria.
Keywords: *Ludwigia Octovalvis, Aqueous Extract, Phytochemical Analysis, Secondary Metabolites and Antibacterial.*

I. INTRODUCTION

Ludwigia octovalvis is locally known as the "water lakum plant." *Ludwigia octovalvis* is widely distributed in tropical regions, originating from Tropical Africa and Tropical America. Therefore, this plant can grow well in Indonesia, a tropical country (Amin *et al*., 2021). *Ludwigia octovalvis* is an annual weed that flowers year-round, allowing for continuous seed production. Seed dispersal is carried out by birds and agricultural tools used for rice cultivation. Seeds that fall to the ground germinate within 14 days (Kraehmar *et al*., 2016). Nurjannah *et al*. (2016) also stated that *Ludwigia octovalvis* is classified as a weed in agriculture.

Ludwigia octovalvis plants contain many secondary metabolites, such as alkaloids, flavonoids, tannins, steroids, saponins, and sesquiterpene lactones (Paola *et al*., 2006). Secondary metabolite compounds contain active molecules such as alkaloids, terpenoids, saponins, and flavonoids, which can act as antibacterials and anti-inflammatory agents. Active compounds in herbal ingredients, such as polypeptides, lectins, polyphenols, alkaloids, quinones, terpenoids, and phenolic compounds, have proven to be highly effective alternatives (Pasaribu & Djono, 2021).

Literature studies have shown that Ludwigia has good antibacterial and antifungal activity, with an inhibition zone of 30-32 mm at a concentration of 20 mg/mL against various bacteria, including Propionibacterium acnes, Staphylococcus aureus, Staphylococcus epidermidis, and Salmonella enterica. In addition to its antibacterial properties, the Ludwigia plant has also been reported to possess cytotoxic activity in the methanol extract of L. peploides leaves with an IC50 value of 5.5 ± 2.3 µg mL-1 against B16 cancer cells, which is higher than doxorubicin (a chemotherapy drug) which has an IC50 value of 16 ± 1.2 µg mL-1 (Smida *et al*., 2018). *Ludwigia octovalvis* has been reported to have an antihyperglycemic effect at a dose of 400 mg/kg, comparable to glibenclamide at a dose of 10 mg/kg (Murugesan *et al*., 2000).

Ludwigia octovalvis is not yet widely known as a medicinal plant in Indonesia (Lin *et al*., 2014). Medicinal plants are plants that have functions and properties as medicines used in the treatment and prevention of disease, containing certain active substances (Pasaribu & Djono, 2021). People from various countries use the *Ludwigia octovalvis* plant as a traditional medicine to treat headaches, hypertension,

nephritis, swelling (often called edema), bacterial infections, dysentery, diarrhea, diabetes, and even vaginal discharge (Lin *et al*., 2014). Its natural ingredients, containing flavonoids, tannins, saponins, alkaloids, steroids, and phenolics, can function as antioxidants, antimicrobials, anticancer agents, anti-inflammatories, and strengthen the immune system. These compounds make it a potential candidate for traditional medicine and natural pharmaceuticals (Riansyah, 2023).

Plants with antibacterial potential include Jeruju (Aisiah *et al*., 2022), Mangrove (Dewanto *et al*., 2021), Kelakai (Fatmawati *et al*., 2022), Rombusa (Fatmawati *et al*., 2026), Kersen (Fatmawati *et al*., 2026), Tanjung (Fatmawati *et al*., 2026), and Bangkal in catfish (Aisiah *et al*., 2019). Jeruju contains active compounds in the form of alkaloids, flavonoids, tannins, phenolics, terpenoids, and steroids (Aisiah *et al*., 2022). Bangkal leaves contain active compounds in the form of flavonoids, saponins, tannins, and terpenoids based on phytochemical tests (Aisiah *et al*., 2020). The phytochemical profiles of several of these plants are similar to those of *Ludwigia octovalvis* leaves, thus having potential as medicinal plants in treating infectious diseases. *Ludwigia octovalvis* leaves have a similar secondary metabolite content profile and even have very strong antibacterial effectiveness in literature studies, therefore testing this plant on its secondary metabolite content is necessary. This research is expected to be able to elevate the usefulness of *Ludwigia octovalvis* leaves from what was initially considered a useless weed, to an effective, safe, and economical natural medicine solution in dealing with *Aeromonas hydrophila* bacteria. This study aimed to analyze the quantitative secondary metabolite content of *Ludwigia octovalvis* leaf aqueous extract and to discuss the role of these compounds as antibacterial agents against pathogens, particularly in relation to *Aeromonas hydrophila*.

II. METHODS

Study site and sample collection

This study was conducted at the Fish Disease and Parasite Laboratory and the Basic Laboratory, Faculty of Fisheries and Marine Sciences, Lambung Mangkurat University. Quantitative phytochemical testing was conducted at the Biochemistry Laboratory, Faculty of Medicine and Health Sciences, Lambung Mangkurat University. Plant samples were collected from wetland areas around Tungkaran Village, Martapura District, Banjar Regency, South Kalimantan.

Materials and equipment

The main material used in this study was 600 g of *Ludwigia octovalvis* leaves. Distilled water was used as the extraction solvent, while filter paper, labels, and other supporting laboratory materials were used during sample preparation. The equipment consisted of an autoclave, vacuum filter, dehydrator, refrigerator, Ohaus balance, vortex mixer, micropipettes, pipette tips, tweezers, microtubes, falcon tubes, and blender.

Selection of plant material

The leaves used for extraction were selected from the fifth to the fifteenth node from the shoot tip. This leaf position was chosen because mature leaves are considered to have more stable secondary-metabolite accumulation than very young or senescent leaves.

Extraction procedure

The collected leaves were cleaned, washed, drained, cut into small pieces, and weighed. The extraction was carried out using a wet-extraction method with distilled water at a ratio of 1:4, consisting of 300 g of leaves and 1.2 L of solvent. The leaf material and solvent were blended until homogeneous and then filtered using filter paper. The filtrate was processed using a vacuum filter and dried in a dehydrator for 12-24 h at 50°C until a concentrated paste was obtained. The extract was placed in a dark glass bottle, covered with plastic wrap and aluminum foil, and stored until analysis.

Quantitative phytochemical analysis

Quantitative phytochemical analysis was performed to identify and measure secondary metabolites that may contribute to antibacterial activity. The analyzed compounds included saponins, alkaloids, flavonoids, terpenoids, tannins, steroids, anthocyanins, and phenolics.

Data presentation

Each phytochemical measurement was performed in three replications. The results were analyzed descriptively and presented as metabolite concentration values according to the unit of each compound.

III. RESULTS AND DISCUSSION

Quantitative Phytochemical Profile

The quantitative phytochemical analysis showed that the aqueous extract of *Ludwigia octovalvis* leaves contained several secondary metabolites with potential biological activity. The measured concentrations are presented in Table 1.

Table 1. Quantitative phytochemical profile of *Ludwigia octovalvis* leaf aqueous extract

Metabolite	Content
Saponin (%)	15.350
Alkaloid (%)	6.870
Flavonoid (mg/g)	92.000
Terpenoid (mg/g)	228.800
Tannin (mg/g)	0.837
Steroid (mg/g)	38.203
Anthocyanin (mg/100 g)	Negative
Phenolic (mg/g)	65.467

Quantitative analysis of secondary metabolites showed that the aqueous extract of *Ludwigia octovalvis* leaves was rich in active compounds. The highest concentration was found in terpenoids at 228.800 mg/g, followed by flavonoids at 92.000 mg/g. The extract also contained phenolics (65.467 mg/g), steroids (38.203 mg/g), saponins (15.350%), alkaloids (6.870%), and a small amount of tannins (0.837 mg/g), whereas anthocyanins were not detected. This metabolite composition indicates that *Ludwigia octovalvis* leaf extract has promising antibacterial potential. Shawky *et al*. (2023) reported that Ludwigia species contain various phytochemicals, including flavonoids, tannins, phenolic acids, triterpenoids, saponins, and steroidal compounds, which are associated with anti-inflammatory, antioxidant, cytotoxic, antidiabetic, and antimicrobial activities.

The quantitative phytochemical results demonstrate that the extract has a strong secondary-metabolite profile, particularly because of the dominance of terpenoids (228.800 mg/g), flavonoids (92.000 mg/g), and phenolics (65.467 mg/g). These groups are important because phenolic compounds, flavonoids, terpenoids, saponins, and alkaloids are among the bioactive components most frequently associated with antibacterial, antioxidant, and anti-inflammatory activities in medicinal plants. Huynh *et al*. (2025) also reported that Ludwigia contains phenolics, flavonoids, saponins, triterpenoids, tannins, and related compounds that serve as important markers of biological activity, including antimicrobial and antioxidant potential.

The detected saponin (15.350%) and alkaloid (6.870%) contents may provide a significant synergistic contribution to the antibacterial activity of the extract. The saponin concentration in this wet plant extract can be considered relatively high. Dwiyanti *et al*. (2015) stated that saponins exhibit antibacterial activity by destabilizing bacterial cell membranes, which can lead to cell lysis. Therefore, saponins are classified as antibacterial compounds that disrupt bacterial cell-membrane permeability, damage the membrane structure, and facilitate the release of essential cytoplasmic components, including proteins, nucleic acids, and nucleotides.

Uzochukwu *et al*. (2025) explained that most alkaloids are weakly basic compounds and have amphoteric properties. In plants, alkaloids can function as defensive compounds against predators and parasites, and they also have pharmacological potential, including antimicrobial, antihypertensive, antimalarial, and anticarcinogenic activities. The antibacterial mechanism of alkaloids is associated with disruption of peptidoglycan components in the bacterial cell wall, which prevents proper wall formation and

causes cell death. Nitrogen-containing basic groups in alkaloids can also react with amino acids in the bacterial cell wall and with bacterial DNA, altering amino-acid structure and genetic balance, ultimately leading to cell lysis and bacterial death (Cowan, 1999).

The flavonoid content of 92.000 mg/g is also biologically important. Zhang *et al*. (2014), flavonoids act as antibacterial compounds by inhibiting nucleic-acid synthesis, disrupting cell-membrane function, and interfering with energy metabolism. Flavonoids can inhibit nucleic-acid synthesis through interactions between their A and B rings and nucleic-acid bases, thereby suppressing DNA and RNA formation (Nomer *et al*., 2019). When flavonoids interact with bacterial cell membranes, they may disturb membrane and chromosomal integrity, resulting in damage to the permeability of the bacterial cell wall, microsomes, and lysosomes.

The high terpenoid content (228.800 mg/g) indicates strong potential as an antimicrobial agent. Wulansari *et al*. (2020) stated that terpenoids interact with pores in the outer membrane of pathogenic bacterial cell walls and form strong polymeric bonds that reduce cell-wall permeability and cause cell lysis. Terpenoids are lipophilic compounds that can pass through the bacterial phospholipid bilayer, diffuse into the cell, and exert antibacterial or bactericidal effects. Because membrane integrity is essential for normal bacterial physiological activity, terpenoid-induced membrane damage can significantly impair bacterial survival (Huang *et al*., 2022).

Although the tannin concentration was relatively low (0.837 mg/g), it may still have biological significance because tannins have a strong affinity for proteins and metal ions. Tannins can precipitate membrane proteins, inhibit bacterial enzymes, and interfere with iron availability, which is required for microbial metabolism. Thus, even at low concentrations, tannins can enhance the inhibitory effect of plant extracts through several complementary mechanisms (Uttam *et al*., 2025). Tannins may also damage bacterial membranes through their toxicity and through the formation of metal-ion complexes. Aerobic bacteria require iron for several functions, including the reduction of ribonucleotide precursors for DNA synthesis; therefore, tannin-iron binding can disturb essential bacterial processes (Fatimah & Mulqie, 2021).

The steroid content of 38.203 mg/g was lower than the terpenoid, flavonoid, and phenolic contents, but it remains important because steroids may act synergistically with other metabolites. Steroids are secondary metabolites commonly found in medicinal plants. Naturally occurring steroids are derived from triterpenes; steroids in animal tissues originate from lanosterol, whereas those in plant tissues originate from cycloartenol. Steroid derivatives include cholesterol, ergosterol, progesterone, and estrogen, and many steroids function as hormones (Salempa & Muharram, 2016). In antimicrobial activity, steroids may contribute by damaging microbial plasma membranes, causing cytoplasmic leakage and subsequent cell death (Rachmawaty *et al*., 2009).

The negative result for anthocyanins indicates that the biosynthetic pathway for this pigment may be inactive or that anthocyanins were present only at very low levels in the tested leaves. Andersen and Jordheim (2010) explained that anthocyanin biosynthesis depends on specific enzymes, such as dihydroflavonol reductase and anthocyanidin synthase, and not all plants possess a complete enzymatic pathway for anthocyanin production, especially species whose main pigments are not anthocyanins. Anthocyanin production is also often induced by environmental stress, such as high light intensity or nutrient deficiency. Plants grown in shaded or protected environments may therefore produce very low or undetectable levels of anthocyanins (Steyn *et al*., 2002).

The phenolic content of 65.467 mg/g strengthens the indication that this extract has good reducing and antioxidant capacity. *Ludwigia octovalvis* leaves have been reported to contain high levels of phenolics and strong antioxidant activity, while the 80% methanolic leaf extract showed clear antibacterial activity against tested pathogenic bacteria (Yakob *et al*., 2013). Phenolic compounds in extracts may also be affected by drying duration; longer drying periods can increase the amount of detectable phenolic compounds. Because phenolics function as antioxidants, higher phenolic content generally indicates stronger antioxidant potential (Purbowati *et al*., 2018).

Previous studies on Ludwigia extracts have shown that different solvents can extract different groups of phytochemicals. N-hexane extracts were reported to contain flavonoids, alkaloids, steroids, polyphenols, terpenoids, and tannins; ethyl acetate extracts contained alkaloids, steroids, terpenoids, and tannins; and 70% ethanol extracts contained flavonoids, alkaloids, polyphenols, tannins, and saponins. The total flavonoid content of Ludwigia leaf extract was 10.39 mg QE/g in the n-hexane extract and 12.36 mg QE/g in the 70% ethanol extract (Batubara *et al*., 2024). In addition, ethanol extract of *Ludwigia octovalvis* showed strong antibacterial activity against *Staphylococcus epidermidis*, *Staphylococcus aureus*, and *Cutibacterium acnes*, with MIC values of 450, 550, and 600 µg/mL, respectively. The extract also showed very strong antioxidant activity, with an IC50 value of 18.98 ± 0.09 µg/mL (Huynh *et al*., 2025).

Based on its quantitative phytochemical profile, *Ludwigia octovalvis* has strong potential to be developed as a natural medicinal material. The aqueous extract of *Ludwigia octovalvis* leaves may serve as an economical and environmentally friendly alternative for managing bacterial infections, particularly in fish-health applications. However, direct antibacterial assays, toxicity evaluation, and in vivo application studies are still required to confirm its practical effectiveness and safety.

IV. CONCLUSION

Ludwigia octovalvis leaf extract contains secondary metabolite compounds, namely, saponins (15.587%), alkaloids (6.507), flavonoids (91.667 mg/g), terpenoids (228.467 mg/g), tannins (0.850 mg/g), steroids (39.177 mg/g), anthocyanins (negative), and phenolics (65.467 mg/g). The highest levels of secondary metabolites in terpenoid compounds are 228.467 mg/g and the lowest in negative anthocyanin compounds. These secondary metabolite compounds have the potential as antibacterials against *Aeromonas hydrophila* bacteria.

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