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Research Article

Larvicidal Activity and Midgut Histopathological Effects of *Urena lobata* Leaf Extract on *Aedes aegypti*

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ABSTRACT

The use of chemical larvicides to control *Aedes aegypti* mosquito populations poses significant environmental risks. As an alternative, researchers are investigating plant-derived natural larvicides, which are biodegradable and safe for humans and non-target organisms. *Urena lobata* leaf extract has demonstrated biolarvicidal effect by causing larval mortality and morphological changes. This study evaluated the efficacy of *U. lobata* leaf extract against *Ae. Aedes aegypti* larvae, their effects on midgut histopathology, and their bioactive compounds were identified by liquid chromatography-mass spectrometry (LC-MS). A post-test control design was implemented with extract concentrations of 0.3%, 0.5%, 0.7%, and 0% (control), each replicated six times using 20 larvae per treatment. Mortality of third-instar larvae increased with higher extract concentrations. The lethal concentration (LC_{50}) of *U. lobata* extract is 0.5% with a 95% confidence interval of 0.484-0.566. Histopathological analysis revealed structural damage in the midgut, including epithelial disorganization, vacuolization, swelling, and detachment of epithelial cells, loss of microvilli, and an irregular peritrophic membrane. Semi-quantitative histopathological scoring indicated that the 0.7% extract produced the most severe alterations. The bioactive compounds identified in the extract include flavonoids, saponins, triterpenoids, and tannins. In conclusion, these findings indicate that *U. lobata* leaf extract exhibits potential larvicidal activity targeting the midgut of *Ae. aegypti* larvae.

1. Introduction

Aedes aegypti mosquito is one of the most life-threatening insect species, serving as the primary vector of Dengue Haemorrhagic Fever (DHF) (Ambari and Suena 2019). This disease imposes a substantial burden due to frequent outbreaks in tropical and subtropical regions worldwide. Controlling mosquito populations is essential for mitigating the transmission of vector-borne diseases. Current strategies primarily target immature mosquito stages to reduce dengue and chikungunya infections (Arosteguí *et al.* 2017).

In Indonesia, chemical insecticides remain the predominant vector control method, encompassing pyrethroid-based fogging and temephos-containing larvicides (Supriyono *et al.* 2023). However, the extensive and prolonged use of chemical larvicides has led to significant environmental and public health concerns, including ecological pollution and toxicity to non-target organisms. These adverse effects stem from the accumulation of chemical residues in environmental matrices, including food, water, and soil. Furthermore, environmental contamination with residual insecticides has accelerated the development of resistance within *Ae. aegypti* populations, posing a critical challenge to disease management efforts (Norris *et al.* 2019). Resistance facilitates rapid vector population growth,

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thereby exacerbating the global prevalence of dengue fever (Amelia-Yap *et al.* 2018).

In response to these challenges, research has increasingly focused on developing botanical larvicides as environmentally sustainable alternatives to synthetic chemicals. Botanical derivatives offer a promising avenue due to their biodegradability, minimal environmental impact, and safety for humans and non-target organisms. Plants produce a diverse array of bioactive secondary metabolites, many of which exhibit potent larvicidal properties with reduced ecological risks (Wang *et al.* 2019; Walkowiak-Nowicka 2023). Numerous plant species have demonstrated larvicidal potential, including *Plectranthus barbatus* (de-Almeida *et al.* 2021), *Aegle marmelos* (Sari *et al.* 2023), and *Citrus hystrix* (Adrianto *et al.* 2024).

Insecticides can be categorized by their mechanisms of action, including physical toxicity, respiratory disruption, ingestion-based poisoning, protoplasmic disruption, neurotoxicity, and growth inhibition (Souto *et al.* 2021). According to Kadu (2021), the mosquito larval midgut is a critical target organ for larvicidal agents, leading to larval mortality. The midgut plays a pivotal role in digestive processes, enzyme production, ion transport, nutrient absorption, and osmoregulation (Adrianto *et al.* 2023). Several botanical species, including *Averrhoa bilimbi, Asarum heterotropoides, Annona squamosa, Anacardium occidentale, Brucea javanica, Magnolia denudata, Passiflora foetida*, and *Aegle marmelos*, have been documented to cause structural and functional damage to the midgut of mosquito larvae (Rohmah *et al.* 2020; Sari *et al.* 2023)

A previous study by Andani *et al.* (2023) identified *Urena lobata* leaf extract as a promising larvicidal agent against *Ae. aegypti*. The investigation primarily focused on determining effective concentrations that induce larval mortality and morphological abnormalities, including darkened exoskeletons, elongated cervical segments, damaged antennae, darkened siphons, and loss of seta hairs. Despite these findings, there is limited information regarding the physiological and histopathological impacts of botanical larvicides. Therefore, it is important to explore how *U. lobata* disrupts critical internal organs, such as the midgut, which is the main target of larvicidal agents.

Andani *et al.* (2023) did not characterize the chemical constituents of the extract. Consequently, the bioactive compounds responsible for the larvicidal effect remain unidentified. Liquid chromatography–mass spectrometry (LC-MS) profiling enables comprehensive identification of phytochemical constituents responsible for larvicidal activity. This study aims to evaluate the larvicidal efficacy of *U. lobata* leaf extract, assess its histopathological effects on the midgut of *Ae. aegypti* larvae, and identify its bioactive phytochemical constituents through LC-MS profiling. By integrating LC–MS profiling with larval bioassays and midgut histopathological observations, the present study provides a novel, mechanism-oriented approach to understanding the larvicidal action of *U. lobata*.

2. Materials and Methods

This study employed an experimental design with a post-test control group. The subjects were third-instar larvae of *Ae. aegypti* obtained from colonies maintained at the Parasitology Laboratory, Faculty of Medicine, Udayana University, Denpasar, Bali, Indonesia. Histological sections of the larval midgut were prepared at the Veterinary Center, Balai Besar Veteriner (BBVet), Denpasar, Bali, Indonesia. Ethical exemption for this research was granted by the Research Ethics Committee of the Faculty of Medicine, Udayana University, under approval number No: 2962/UN14.2.2.VII.14/LL/2022.

2.1. Plant Collection and Extraction

Urena lobata leaves were collected from Tegalalang Village, Gianyar Regency, Bali, Indonesia (8°32'39.6"S, 115°16'37.9"E). The leaves were separated from their stalks, washed under running water, and air-dried at room temperature until a constant weight was achieved. The dried leaves were then ground into a fine powder using a blender. The powdered leaves were macerated in 96% ethanol at a 1:10 ratio for 48 hours, with agitation twice daily. The resulting macerate was filtered, and the filtrate was evaporated using a rotary evaporator to produce a crude extract (Yunita *et al.* 2023).

2.2. Larvicide Test

The concentrations of the extract tested in this study were 0.3%, 0.5%, and 0.7%. Each concentration was repeated six times. The volume of well water used as a solvent for the tests was 200 mL per glass beaker. The control group contained only well water without the extract.

For each concentration and its corresponding repetition, 20 third-instar larvae were placed in each glass beaker. The use of six replicates with 20 larvae each (120 larvae per treatment) followed WHO Guidelines for Laboratory and Field Testing of Mosquito Larvicides

(WHO 2005) and the experimental design by Wahyuni *et al.* (2022). Larvicidal activity was observed over a 24-hour treatment period, with the room temperature maintained between 21-25°C and humidity ranging from 50-64%. The percentage of larval mortality was calculated using the following formula by Nelly *et al.* (2024).

$$\text{Mortality (\%)} = \frac{\text{Number of dead larvae}}{\text{Number of larvae introduced}} \times 100\%$$

2.3. Histological Preparation of Midgut Sections

Histological preparation was performed according to the method of Al-Mehmadi *et al.* (2010). After 24 hours, larvae were fixed in 10% buffered formaldehyde at room temperature for 24 hours. The midgut to be examined was cross-sectioned using a rotary microtome at a thickness of 4 µm. The sections were then prepared and stained with Hematoxylin-Eosin. All larvae were dissected, and histological slides were prepared; however, only slides with clear, readable tissue structures—approximately 50% of the total preparations—were selected for microscopic observation. Histological slides of the midgut were examined under a light microscope (Miconos, China) at 400x magnification. The midgut alterations were scored semi-quantitatively according to Meyerholz and Beck (2019).

2.4. Identification of Active Compounds in *Urena lobata* Leaf Extract by LC-MS

The identification of compounds was performed using Liquid Chromatography-Mass Spectrometry (LC-MS) on the *U. lobata* leaf extract. A total of 0.5 g of the 96% ethanol extract was dissolved in 50 mL of reagent-grade methanol. The solution was filtered through a 0.22 µ syringe filter, placed into a 2 mL vial, and then injected into the LC-MS system for analysis. Compound identification was carried out by comparing the obtained mass spectra with entries in the NIST Mass Spectral Library (NIST 17) using the NIST Mass Spectral Search Program version 2.3 and the MS Interpreter version 3.4.5. The identification threshold was determined based on the signal-to-noise ratio, with a limit of detection (LOD) set at S/N > 3.

2.5. Data Analysis

The differences in treatment effects were analysed using the Kruskal-Wallis test. The LC_{50} values were determined through probit analysis, with a 95% confidence interval. All data analysis was conducted using the statistical package for social sciences (SPSS version 21). Histological changes in the larvae's midgut were documented and analysed descriptively.

3. Results

3.1. Larvicide Test

The results indicated that as the extract concentration increased, the number of larvae exhibiting mortality also increased. This pattern was consistent across all concentration-replicating procedures (Table 1). No mortality was observed in the control group after 24 hours. The dead larvae were observed partially floating, while some were found at the bottom of the water, exhibiting morphological changes, such as a darkening of the body colour (Figure 1).

Statistical analysis using the Kruskal-Wallis test revealed a significant difference in mean larval mortality ($p<0.05$) between the control and treatment groups (Table 2). This non-parametric test was applied because the data were not normally distributed. Further analysis with the Mann-Whitney test showed significant differences ($P<0.05$) among the treatment groups (Table 3).

Based on the number of larval deaths following exposure, the LC_{50} value was calculated. The probit analysis indicated that a 0.5% concentration of *U. lobata* leaf extract induced death in 50% of the *Ae. aegypti* larval population (Table 4).

3.2. Phytochemical Analysis

The LC–MS analysis yielded numerous peaks corresponding to diverse phytochemicals in the

Table 1. Average mortality of *Aedes aegypti* mosquito larvae after 24 hours of exposure to *Urena lobata* leaf extract

Concentration (%)	Mortality of larvae on each replicatoin								
	R1	R2	R3	R4	R5	R6	Mean	Mortality (%)	Mean±SD
0	0	0	0	0	0	0	0	0	0.00 ± 0.00^{a}
0.3	4	4	6	4	4	4	4.3	21.7	4.33 ± 0.82^{b}
0.5	10	11	12	9	11	10	10.5	52.5	10.50 ± 1.05^{c}
0.7	19	18	18	18	17	17	18.0	90.0	18.00 ± 0.63^{d}

R1: replication 1, R2: replication 2, R3: replication 3, R4: replication 4, R5: replication 5, R6: replication 6. Values in the last column represent the mean ± standard deviation (SD) of six replications (R1–R6). Different superscript letters indicate significant differences among treatments (p<0.05)

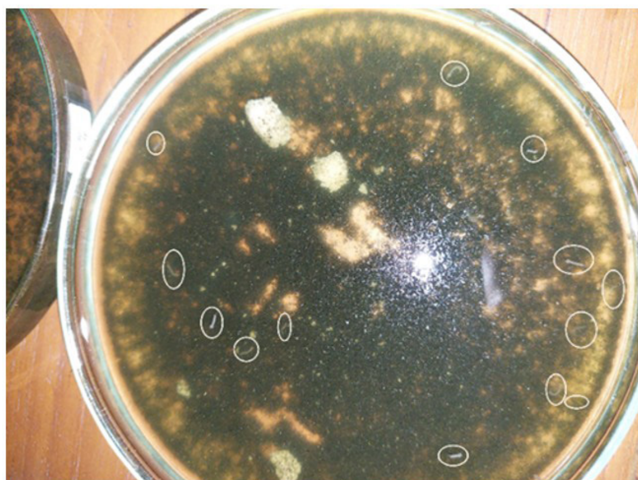

Figure 1. Larvae that died (indicated by white circles) after 24 hours of exposure to *U. lobata* leaf extract. The diameter of the petri dish is 12 cm

Table 2. Mean mortality of *Aedes aegypti* larvae analysed using the Kruskal-Wallis test

Variable	Concentration (%)	Rank mean	P
Total larval mortality	Control	3.50	0.000
	0.3	9.50	
	0.5	15.50	
	0.7	21.50	

extract. To focus on bioactive constituents potentially responsible for larvicidal activity, compound selection was performed based on structural classes commonly associated with biological activity, such as flavonoids, alkaloids, terpenoids, and phenolics. The LC-MS analysis of *U. lobata* leaf extract revealed 44 peaks corresponding to compounds suspected to be flavonoids, tannins, saponins, and terpenoids. The chromatogram of *U. lobata* leaf extract obtained using LC-MS is shown in Figure 2. The bioactive

Table 3. Mean mortality of *Aedes aegypti* larvae analysed using the Post-hoc Mann-Whitney test

Concentration	Mean mortality			
Control	-	-	-	-
0.3%	0.001*	-	-	-
0.5%	0.002*	0.003*	-	-
0.7%	0.002*	0.002*	0.003*	-

Asterisks (*) indicate significant differences between treatments

Table 4. Lethal concentration 50% (LC_{50}) of *U. lobata* leaf extract on the mortality of *Aedes aegypti* larvae

Probability	Estimate (%)	95% Confidence limits	
		Lower bound	Upper bound
LC_{50}	0.532	0.484	0.566

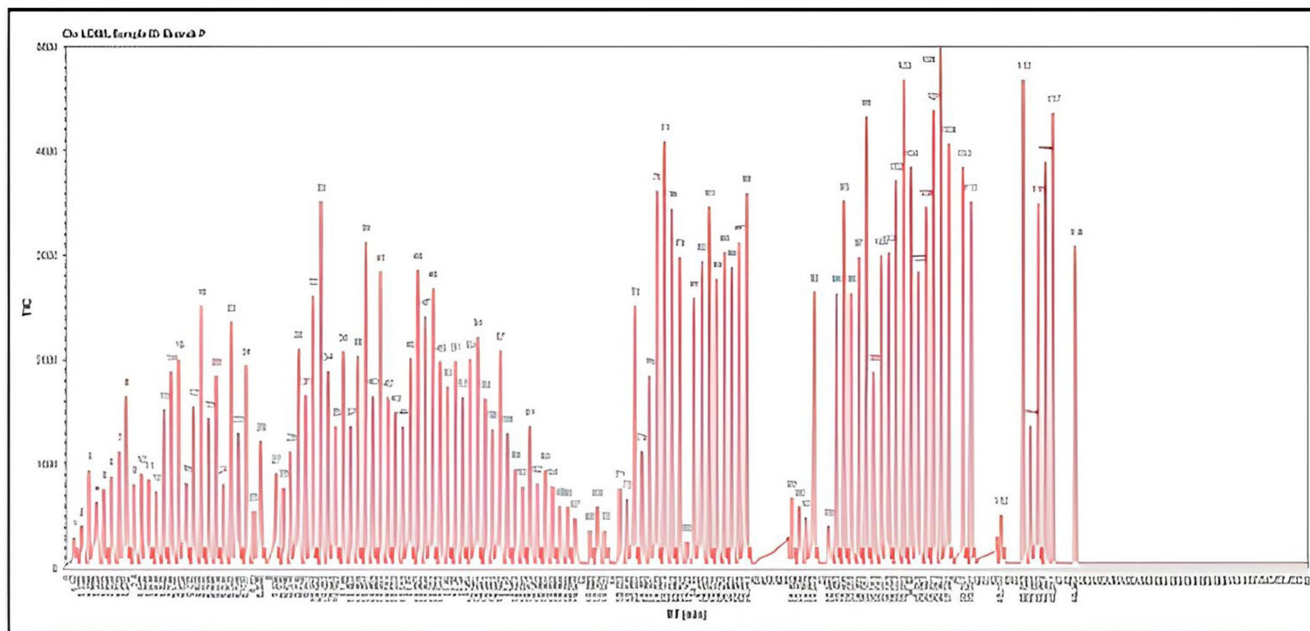

Figure 2. LC-MS chromatogram profile of *U. lobata* leaf extract. Peaks correspond to compounds identified. X axis: retention time (min), Y axis: intensity, the relative quantity of ions detected for that compound

components identified in *U. lobata* leaf extract using LC-MS are listed in Table 5.

3.3. Histopathology Test

Histological observations of the midgut of third-instar *Ae. aegypti* larvae in the control and treatment groups exposed to *U. lobata* leaf extract were examined using light microscopy. The observations revealed that in the control group, the peritrophic membrane (pm) of the midgut surrounded the contents of the midgut lumen (lu) and was separated from the outer surface by the midgut epithelial tissue, with peritrophic spaces (Figure 3A). The midgut of control larvae consisted of well-organized cuboidal epithelial cells that were

Table 5. Interpretation of the LC-MS chromatogram data of *U. lobata* leaf extract

Peak number	RT (min)	Composition (%)	Compound	Classification
32	10.265	1.10075	luteolin	Flavonoid
33	10.322	1.48107	kaempferol	Flavonoid
39	11.427	1.31700	quercetin	Flavonoid
41	11.503	1.19887	epigallocatechin	Flavonoid
46	11.561	1.20642	2'-hydroxy-5-methoxy-6,7- methylenedioxyisoflavone	Flavonoid
47	11.606	1.01736	5,7-dihydroxy-2',6- dimethoxyisoflavone	Flavonoid
48	11.613	1.13089	5,3'-dihydroxy-7,2'- dimethoxyflavanone	Flavonoid
68	17.048	0.15535	diosgenin	Saponin
73	20.093	1.05866	apigenin-7-O-glucoside	Flavonoid
76	21.436	1.52164	quercetin-3-arabinoside	Flavonoid
77	22.174	1.72053	quercetin-3-O-rhamnoside	Flavonoid
78	22.624	1.45174	kaempferol-7-O-glucoside	Flavonoid
79	22.628	1.25688	luteolin-7-glucoside	Flavonoid
81	24.020	1.09287	hyperoside	Flavonoid
82	24.026	1.24090	gossypetin-8-rhamnoside	Flavonoid
83	24.032	1.45057	isoquercetin	Flavonoid
84	24.733	1.16943	gypsogenin	Triterpenoid
85	25.835	1.27426	quercituron	Flavonoid
86	25.891	1.21563	gossypetin-3-gluside	Flavonoid
87	25.902	1.31657	gossypetin-8-gluside	Flavonoid
88	25.913	1.51565	myricetin-3-glucoside	Flavonoid
92	31.819	1.11800	quercetin-3-O-malonylglucoside	Flavonoid
94	33.483	1.11337	kaempferitrin	Flavonoid
95	33.496	1.48370	Procyanidin B2	Tannin
96	33.618	1.11269	apigenin 7-sophoroside	Flavonoid
97	34.002	1.25775	saponarin	Flavonoid
98	35.511	1.82191	quercetin-3-glucoside-7-rhamnoside	Flavonoid
100	35.517	1.26304	rutin	Flavonoid
101	35.518	1.27432	kaempferol 3-O-sophoroside	Flavonoid
102	35.519	1.56547	quercetin-3-robinoside	Flavonoid
103	36.833	1.97214	5,7,4’-trihydroxy-6-methoxyisoflavone7-O-glucoside-4’-O-glucoside	Flavonoid
104	36.850	1.62024	Isorhamnetin 3-O-robinobioside	Flavonoid
105	36.854	1.19774	Luteoayamenin	Flavonoid
106	36.857	1.45971	quercetin 3,4’-di-O-β-D-glucopyranoside	Flavonoid
107	36.871	1.84689	quercetin-3-diglucoside	Flavonoid
108	36.983	2.10019	Myriceric acid A	Triterpenoid
109	37.042	1.71409	Myriceric acid B	Triterpenoid
110	39.017	1.62024	Uncarinic acid A	Triterpenoid
111	39.021	1.48112	Uncarinic acid B	Triterpenoid
113	46.014	1.97231	quercetin 3-glucosyl-(1-2)-rhamnoside-7-glucoside	Flavonoid
115	46.565	1.47349	kaempferol 3-glucosyl-(1-2)-[glucosyl-(1-3)-rhamnoside]	Flavonoid
116	46.567	1.63924	kaempferol 3-rutinoside-7 glucoside	Flavonoid
117	46.911	1.83883	3-[(6-deoxy-2-O-β-D-glucopyranosyl-α-L-mannopyranosy)oxy]-2-(3,4-dihydroxyphenyl)-7-(β-D-glucopyranosyloxy)-5-hydroxy-4H-1-benzopyran-4-one	Flavonoid
118	49.883	1.29929	uncarinic acid C	Triterpenoid

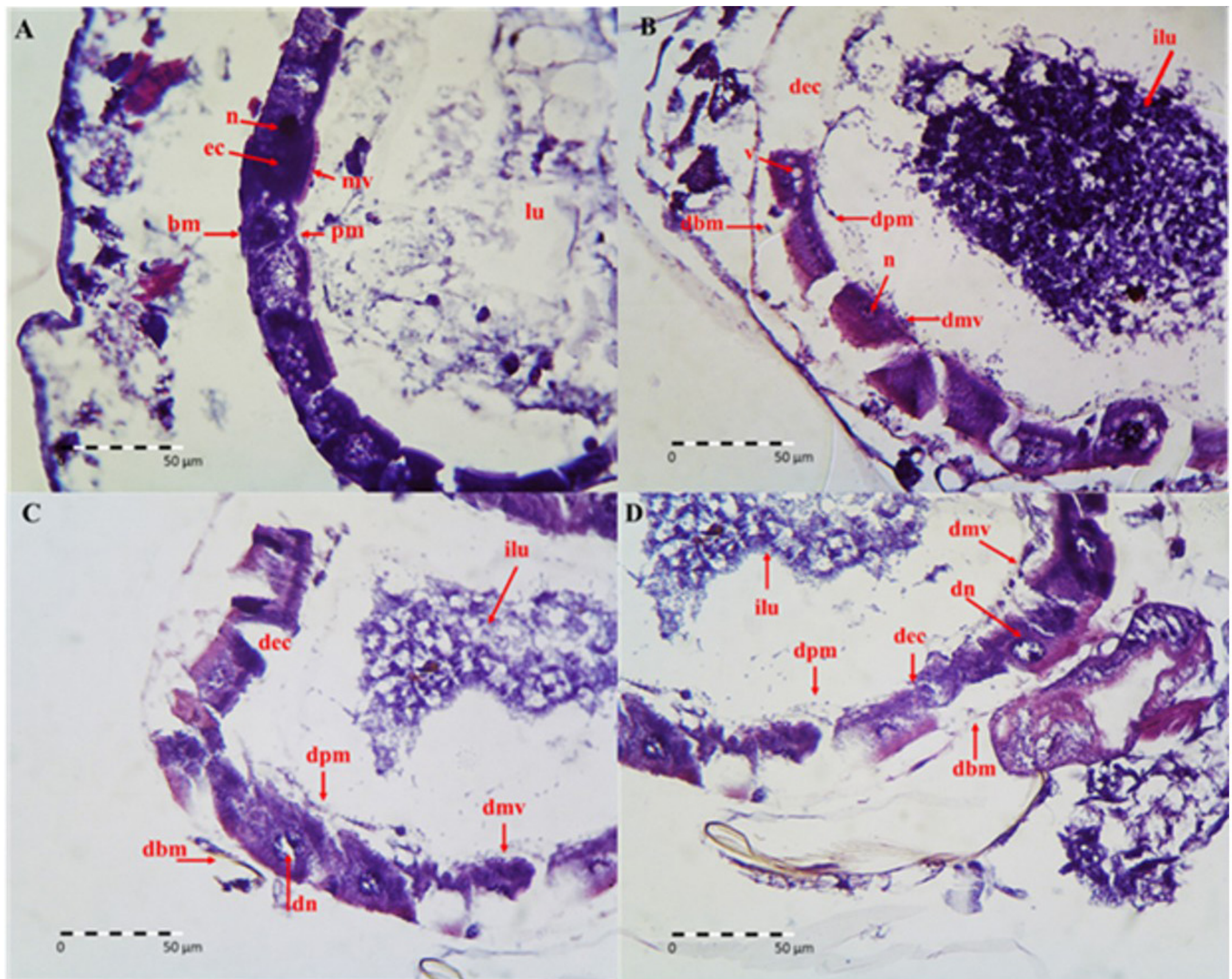

Figure 3. Cross-sectional view of the midgut of *Aedes aegypti* larvae (400x magnification). (A) Control, (B) the larva treated with 0.3% extract, (C) the larva treated with 0.5% extract, (D) the larva treated with 0.7% extract. (ec) epithelial cell, (dec) damaged epithelial cell, (n) nucleus, (dn) damaged nucleus, (mv) microvilli, (dmv) damaged microvilli, (bm) basal membrane, (dbm) damaged basal membrane, (pm) peritrophy membrane, (dpm) damaged peritrophy membrane, (lu) lumen, (ilu) inside lumen), and (v) vacuolization

tightly attached to the basal lamina. The nucleus was clearly visible, and small vacuoles were present in the cytoplasm. Microvilli were observed lining the apical surface of the cells in a regular pattern.

In contrast, larvae treated with *U. lobata* leaf extract exhibited similar damage to the midgut epithelial cells (Figure 3B, C, and D). Cytopathological changes included irregularly arranged epithelial cells, damaged microvilli, epithelial cell swelling, and detachment from the basal lamina. The cytoplasm of the epithelial cells showed large vacuoles, and the peritrophic membrane appeared irregular, lining the lumen.

Histological examination showed that midgut damage in *Ae. Aedes aegypti* larvae increased with the concentration of *U. lobata* leaf extract (Table 6). All midgut structures appeared normal in the control group (score 0). At 0.3%, mild (score 1) alterations were observed across epithelial cells, nuclei, microvilli, basal membranes, peritrophic membranes, and cytoplasmic vacuoles. Damage intensified at 0.5%, with moderate to severe (scores 2–3) degeneration, particularly in the nuclei and basal membranes. The highest concentration (0.7%) caused severe (score 3) destruction in nearly all midgut components.

Table 6. Effect of *U. lobata* leaf extract at different concentrations on larval midgut damage

Type of damage	95% Confidence limits			
	Control	0.3%	0.5%	0.7%
(EC) epithelial cell	0	1	2	3
(N) nucleus	0	1	3	3
(M) microvilli	0	1	2	3
(BM) basal membrane	0	1	3	3
(PM) peritrophy membrane	0	1	3	3
(V) vacuolization	0	1	2	3

Level of damage: 0 = normal, 1 = mild, 2 = moderate, 3 = severe

4. Discussion

This experiment demonstrated a clear concentration-dependent effect of the extract on larval mortality. As the extract concentration increased, a corresponding increase in larval mortality was observed, indicating that the extract contains bioactive compounds toxic to larvae. This finding aligns with previous studies showing a dose-dependent relationship between exposure to plant extracts and Ae. mortality rates. *aegypti* larvae (Aditama *et al.* 2019, Subahar *et al.* 2020).

The LC_{50} value of *U. lobata* leaf extract in this study is 0.5%, which means that the concentration of *U. lobata* leaf extract required to cause death in 50% of *Ae. aegypti* larvae is 0.5%. Thus, *U. lobata* leaf extract at 0.5% concentration showed potential as a plant-based larvicide, effectively reducing the test larval population by 50%.

The results of phytochemical analysis using LC-MS showed that the ethanol extract of *U. lobata* leaves contained secondary metabolites such as flavonoids, alkaloids, saponins, tannins, and triterpenoids. The mortality of third-instar *Ae. Aedes aegypti* larvae following treatment were caused by the secondary metabolites present in *U. lobata* leaf extract. Various plants have been identified as containing bioactive compounds with insecticidal activity. Toxic substances are more readily absorbed into the insect's body due to the hydrophobic and lipophilic nature of the cuticle that lines its body. Bioactive compounds act as larvicides through the nervous, respiratory, and digestive systems. Nonpolar bioactive compounds easily penetrate the cuticle and enter the insect's body (Souza *et al.* 2019).

Research by Gea *et al.* (2021) identified flavonoids, alkaloids, saponins, tannins, and steroid compounds in the acetone extract of *U. lobata* leaves, which were found to be toxic to *Artemia salina* larvae. Additionally, Njoku *et al.* (2021) reported that phytochemical analysis of *U. lobata* stems revealed the presence of flavonoids, tannins, saponins, pitate alkaloids, and cyanogenic glycosides. The differences in the compounds identified in *U. lobata* plants are attributed to the production of secondary metabolites, which are influenced by the plants' biochemical and physiological conditions, including environmental factors such as light, soil moisture content, fertility, salinity, carbon dioxide levels, and temperature. Similar secondary metabolites have also been found in plants that act as larvicides for *Ae. aegypti*, such as *Gliricidia sepium* flower extract, *Annona squamosa* seed extract (Goyal *et al.* 2019), and *Sphaeranthus amaranthoides* leaf extract (Thanigaivel *et al.* 2019).

The most identified bioactive compounds in the U. lobata leaf extract in this study were flavonoids. According to Sayono *et al.* (2022), the highest larvicidal potential of *n*-hexane extract from *Derris elliptica* roots is influenced by the flavonoid content. Inaba *et al.* (2022) reported that flavonoids act on the nobo (Noppera-bo) protein in *Ae. aegypti* at the atomic, enzymatic, and organismal levels. Nobo protein is an enzyme used in the biosynthesis of the insect steroid hormone ecdysone in *Ae. aegypti* (yellow fever mosquito). Certain flavonoids have been found to inhibit acetylcholinesterase (AChE), an enzyme vital for neurotransmission in insects, leading to neurotoxicity and mortality in mosquito larvae (Perumalsamy *et al.* 2015).

Histopathological examination of the midgut in this study revealed similar damage in all groups treated with *U. lobata* leaf extract. Damage to the microvilli that border the midgut epithelial cells' surface. According to de-Lemos *et al.* (2018), the larval midgut is normally composed of a single layer of cuboidal cells with microvilli on their surfaces. Bioactive compounds enter the digestive system through the mouth. The midgut is the central digestive tract of insects, responsible for acquiring and storing most of the nutrients that support metamorphosis and the adult insect's life cycle (Godoy *et al.* 2023).

Damage to the peritrophic membrane in the treated group increases the likelihood of larval mortality, as this structure functions as a critical protective barrier separating the midgut epithelium from toxic substances present in ingested material (Suryani *et al.* 2020). The peritrophic membrane plays essential roles in food digestion, microbial defense, and the protection of midgut cells from mechanical injury. It represents the first line of defense against the penetration of

toxic compounds into the midgut and the insect body (Denecke *et al.* 2018). When this barrier is disrupted, harmful substances can directly contact the epithelial cells, leading to structural damage, impaired digestion, and reduced nutrient absorption. Such disturbances have been demonstrated to cause larval mortality in *Ae aegypti* (Procópio *et al.* 2015).

Phytochemical analysis of *U. lobata* leaf extract in this study identified seven quercetin compounds (Table 6). Quercetin is a natural flavonol widely found in foods and medicinal plants (Aghababai and Hadidi 2023). Pessoa *et al.* (2018) reported that quercetin nanosuspension is a potential larvicide for controlling *Ae. aegypti*, causing larval mortality and inhibiting larval survival from hatching. Additionally, it shows no ecotoxicity to non-target organisms.

Another compound found in *U. lobata* leaf extract is tannin. Tannin reduces larval appetite, growth, and survival (Moniharapon *et al.* 2023). Growth inhibition occurs because tannin causes damage and thinning of the microvilli, thereby impairing nutrient absorption. Tannins also synergize with other compounds to induce cytoplasmic vacuolization and apoptosis in cells. The appearance of large vacuoles in the cytoplasm was also observed in the midgut epithelial cells of treated larvae. Such cytoplasmic vacuolization results from osmotic imbalance.

Insects' bodies are covered by a cuticle, which serves as an external skeleton with various mechanical properties (Ohkubo *et al.* 2023). Saponins can damage the cuticular membrane and the outer body membrane. The penetration of saponins through the body wall causes trauma to the larval skin (Aditama *et al.* 2019). Saponins' ability to penetrate the cuticle is due to their molecular structure, consisting of hydrophilic sugar groups linked to a hydrophobic aglycone. The combination of hydrophilic (polar) and hydrophobic (apolar) elements in a single molecule imparts soap-like properties (Roopashere and Naik 2019). In-depth mechanistic studies have shown that saponins in *Camellia oleifera* seed extract damage the epidermal wax layer in *Ectropis obliqua* larvae. This damage leads to significant water loss and can penetrate the larval gut. Other damage caused by saponins includes shortening of intestinal villi and disruption of intestinal wall cavities, leading to larval death (Cui *et al.* 2019).

According to Subahar *et al.* (2020), damage to the larval body membrane allows other metabolite compounds in the extract to enter the body more easily. Further, Sharma *et al.* (2022) reported that saponins in *Achyranthes aspera* leaf extract cause severe disruption of the internal midgut structure in *Ae. aegypti* larvae, such as disintegrated cells, damaged peritrophic membranes and microvilli, disrupted epithelial layers, and ruptured and displaced basal membranes. Other studies have reported that saponins play a significant role in the mortality of various mosquito species, including *Ae. aegypti* (Aditama *et al.* 2019).

Another compound contributing to midgut structural damage in this study is triterpenoid, a terpenoid derivative. Triterpenoids are commonly associated with mosquito larvicidal activity (Raja *et al.* 2024). Silverio *et al.* (2020) reported that phenolic and terpenoid compounds exhibit larvicidal activity against *Ae. Aegypti*. Terpenoid derivatives can affect the respiratory system and damage acetylcholinesterase enzymes (Sarma *et al.* 2019). Inhibition of acetylcholinesterase enzymes increases muscle impulses, causing continuous muscle contraction, leading to muscle spasms, and ultimately resulting in the death of the insect (Sharifi *et al.* 2018)

In conclusion, the *U. lobata* leaf extract has potential as a natural larvicide against *Ae. aegypti* larvae, with a clear concentration-dependent increase in mortality. Bioactive compounds, including flavonoids, tannins, saponins, and terpenoids, contribute to the observed insecticidal activity through multiple mechanisms, such as enzyme inhibition and damage to midgut epithelial cells. The experiments were conducted under laboratory conditions, which may not fully represent natural environmental scenarios. Field trials were not performed, limiting the ecological relevance of the findings. Future research should therefore include ecotoxicity assessments on non-target organisms, and field evaluations to determine the efficacy, persistence, and environmental safety of *U. lobata* extract under real-world conditions.

Declaration of Competing Interest

The authors affirm that there are no conflicts of interest regarding the publication of this article.

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