

ARTICLE

EXPLORATION OF THE POTENTIAL OF ASTERACEAE FAMILY PLANTS ETHANOL EXTRACT AS BIOLARVICIDES AGAINST THIRD-INSTAR *Aedes aegypti* LARVAE

[Eksplorasi Potensi Ekstrak Etanol Tanaman Famili Asteraceae Sebagai Biolarvasida Terhadap Larva *Aedes aegypti* Instar III]

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ABSTRACT

The improper use of synthetic larvicides results in detrimental environmental impacts. To mitigate these effects, biolarvicides serve as a viable alternative. Plants within the Asteraceae family contain bioactive compounds with potential biolarvicidal properties. This research aimed to explore the potential of Asteraceae species as biolarvicides. This research utilizes *Artemisia vulgaris*, *Cosmos caudatus*, *Eclipta prostrata*, and *Tagetes erecta*. Extraction was conducted by the maceration method using 96% ethanol. The resulting extracts were analyzed for secondary metabolite content. Data were analyzed using SAS 9.0 software Two-way ANOVA, followed by Tukey's post-hoc test. Larvicidal bioassays were performed on third-instar *Aedes aegypti* larvae at concentrations of 1,000-10,000 ppm. The parameters measured included mortality percentage, LC₅₀, and LC₉₀. Morphological changes in dead larvae were observed and compared against a control group. The results indicated that secondary metabolite in the leaf ethanolic extracts were higher than those in the stems and roots. The leaf ethanolic extract of *C. caudatus* exhibited the highest content of flavonoids (928.3±368.7 mg/g), phenolics (310.2±84.9 mg/g), tannins (187.1± 55.5 mg/g), and saponins (314±95.4 mg/g extract). Conversely, the highest alkaloid content was recorded in the leaf extract of *T. erecta* (27.8±7.1 mg/g). The highest mortality rate was observed in larvae exposed to *A. vulgaris* leaf extract, reaching 100% mortality across all concentrations within 24 hours. Consequently, LC₅₀ and LC₉₀ values for *A. vulgaris* could not be determined. For *C. caudatus*, the LC₅₀ values were 7204.7 ppm (24 h) and 6839.7 ppm (48 h), while LC₉₀ values were 9077.3 ppm (24 h) 8226.4 ppm (48 h), respectively. *E. prostrata* yielded an LC₅₀ of 10255.6 ppm and an LC₉₀ of 18048.9 ppm at 24 hours; however, these values were not detectable at 48 hours. *T. erecta* showed an LC₅₀ of 6569.6 ppm (24 h) and 6397.1 ppm (48 h), with LC₅₀ values of 9682.1 ppm and 9343.3 ppm, respectively. Morphological observations of dead larvae exposed the extracts alterations, including pigmentation, elongation, and shrinkage. While the leaf ethanolic extract of *C. caudatus* contained the highest concentration of secondary metabolites. The leaf ethanolic extract of *A. vulgaris* demonstrated the greatest potential as a biolarvicide.

Keywords: *Aedes aegypti*, asteraceae, bioactive compounds, biolarvacide, lethal concentration, mortality, secondary metabolite

ABSTRAK

Larvasida sintetik yang digunakan tidak tepat memiliki dampak negatif. Untuk mengatasi hal tersebut larvasida nabati dapat menjadi alternatif pengganti. Tanaman Asteraceae memiliki senyawa bioaktif yang berpotensi sebagai biolarvasida. Tujuan penelitian ini untuk mengeksplorasi potensi tanaman Asteraceae sebagai biolarvasida. Penelitian menggunakan empat spesies tanaman Asteraceae, yaitu *Artemisia vulgaris*, *Cosmos caudatus*, *Eclipta prostrata* dan *Tagetes erecta*. Ekstraksi dilakukan menggunakan metode maserasi dengan pelarut etanol 96%. Ekstrak yang diperoleh kemudian diukur kandungan metabolit sekunder. Data yang diperoleh kemudian dianalisis menggunakan SAS 9.0 dengan analisis Two-way ANOVA dengan uji lanjutan Tukey. Selanjutnya dilakukan uji larvasida dengan menggunakan larva *Aedes aegypti* instar III menggunakan konsentrasi 1.000-10.000 ppm. Hasil yang diperoleh dilanjutkan dengan menghitung persen mortalitas, LC_{50} dan LC_{90} . Larva yang mati diamati morfologinya dan dibandingkan dengan kontrol. Hasil menunjukkan metabolit sekunder ekstrak etanol daun lebih tinggi dibandingkan dengan bagian batang dan akar. Metabolit sekunder ekstrak etanol daun *C. caudatus* memiliki kandungan tertinggi pada flavonoid ($928,3 \pm 368,7$ mg/gr ekstrak), fenolik ($310,2 \pm 84,9$ mg/gr ekstrak), tanin ($187,1 \pm 55,5$ mg/gr ekstrak) dan saponin ($314 \pm 95,4$ mg/gr ekstrak). Sedangkan alkaloid tertinggi pada ekstrak etanol daun *T. erecta* ($27,8 \pm 7,1$ mg/gr ekstrak). Mortalitas tertinggi ditunjukkan pada paparan ekstrak etanol daun *A. vulgaris*, pada jam ke 24 mortalitas larva mencapai 100% pada tiap konsentrasi. Nilai LC_{50} dan LC_{90} ekstrak etanol daun *A. vulgaris* tidak menunjukkan hasil dikarenakan keseluruhan larva mati. LC_{50} *C. caudatus* jam ke 24 sebesar 7204,7 ppm dan jam ke 48 sebesar 6139,7 ppm, LC_{90} jam ke 24 sebesar 9077,3 ppm dan jam ke 48 sebesar 8226,4 ppm. Pada jam ke 24 LC_{50} ekstrak *E. prostrata* sebesar 10255,6 ppm dan LC_{90} sebesar 18048,9 ppm. Pada jam ke 48 LC_{50} dan LC_{90} tidak dapat muncul. LC_{50} ekstrak etanol *T. erecta* jam ke 24 sebesar 6569,6 ppm dan jam ke 48 sebesar 6397,1 ppm, LC_{90} jam ke 24 sebesar 9682,1 ppm dan jam ke 48 sebesar 9343,3 ppm. Morfologi larva yang mati terpapar ekstrak menunjukkan adanya perubahan, seperti pigmentasi, elongasi, dan penyusutan ukuran tubuh. Berdasarkan penelitian yang telah dilakukan menunjukkan bahwa ekstrak etanol daun *C. caudatus* memiliki kandungan metabolit sekunder tertinggi. Sedangkan ekstrak etanol daun *A. vulgaris* memiliki potensi tertinggi sebagai biolarvasida.

Kata kunci: *Aedes aegypti*, asteraceae, biolarvasida, konsentrasi letal, metabolit sekunder, mortalitas, senyawa bioaktif

INTRODUCTION

Dengue Hemorrhagic Fever (DHF) is a vector borne disease caused by the dengue virus. *Aedes aegypti* serves as the primary vector for DHF transmission, while *Aedes albopictus* acts as a secondary vector (Sumekar & Nurmaulina, 2016). Temephos is a larvicide commonly utilized to reduce cases of vector-borne diseases. However, improper application may result in negative impacts on both target and non-target organisms. These include the development of resistance in *Ae. aegypti* larvae (Handayani *et al.*, 2016) and an increase in detoxification enzymes within the larvae (Adhikari & Khanikor, 2021). Furthermore, research has indicated an intensification of hepatic detoxification processes and gill damage in fish (Abe *et al.*, 2019), as well as potential genotoxicity (Cobanoglu & Cayir, 2020).

Botanical larvicides offer a potential solution to mitigate the adverse effects of synthetic larvicides. Due to their plant-based origins, botanical larvicides possess residues that are more biodegradable and degrade more rapidly in the environment (Mahfud *et al.*, 2021). Secondary metabolites in plants function through various larvicidal mechanisms and can exhibit toxic properties. Alkaloids act as stomach poisons and can inhibit the activity of the acetylcholinesterase enzyme (Basundari *et al.*, 2018). Flavonoids function as anti-feedants (Kaihena & Ukratalo, 2021), disrupting the metabolic processes and respiratory systems of the larvae (Ahyanti & Yushananta, 2022). Saponins act as stomach poisons by reducing larval digestive activity and can exert corrosive effects on the walls of the digestive tract (Utami *et al.*, 2016). Tannins function as anti-feedants and inhibit the larval digestive system (Laksono *et al.*, 2022).

Various plant species have been investigated to determine their potential as larvicides. Plants from the Asteraceae family have been extensively studied and are known to contain bioactive compounds with potent larvicidal properties. These compounds include alkaloids, flavonoids, saponins, phenolics, and tannins. Such compounds are found in *Tagetes erecta* (Artini,

2021), *Cosmos caudatus* (Al Kausar *et al.*, 2023), *Artemisia vulgaris* (Budiana *et al.*, 2017), and *Eclipta prostrata* (Herapathdeniya *et al.*, 2020). Several studies have been reported regarding the testing of Asteraceae plants as biolarvicides. These include the use of essential oil from *A. vulgaris* leaves (Balasubramani *et al.*, 2018), methanol extract from *E. prostrata* leaves (Mahalakshmi *et al.*, 2018), ethanol extract from *C. caudatus* leaves (Wasilah & Budi Setiawan, 2019) against *Ae. aegypti* larvae and extract *T. erecta* leaves against *Culex* sp. larvae (Ahad *et al.*, 2024)

Several studies have been conducted on various Asteraceae plant species separately. However, a research gap remains in these studies regarding the comparison of biolarvicidal potential among these species under uniform testing conditions. Furthermore, most previous research has focused more on larval mortality rates and has not measured the variations in active compound content in each plant. Therefore, this study possesses novelty by providing a perspective through direct comparative testing to determine which species have higher potential, as well as identifying various secondary metabolite contents that have potential as biolarvicides. This study aims to explore the potential of various Asteraceae plants as biolarvicides.

MATERIALS AND METHODS

Materials and Apparatus

The apparatus utilized in this study included a spectrophotometer (Shimadzu UVmini-1240), a microscope (Portable LCD Digital Microscope KS601203), analytical balances (Ohaus Pioneer dan Shimadzu TXB622L), a dehydrator (Maksindo MKS-FDH10), a grinder (Maksindo MKS-ML100), micropipettes (DLAB), wooden sticks, plastic cups dan various laboratory glassware (Duran, Pyrex).

The materials used in this research consisted of plant samples (*A. vulgaris*, *C. caudatus*, *E. prostrata* dan *T. erecta*), third-instar *Ae. aegypti* larvae, distilled water, ethanol 96% (Medika), DMSO (Merck), chloroform (Merck), NaNO₂ (Merck), AlCl₃ (Merck), NaOH (Merck), Folin-Ciocalteu (Merck), Na₂CO₃ (Merck), acetic anhydride (Merck), H₂SO₄ (Mallinckrodt), Bromocresol Green (Merck), Na₂HPO₄.12H₂O (Merck), citric acid (Merck), quercetin (Sigma-aldrich), tannic acid (Merck), gallic acid (Merck), saponin quillaja bark (Sigma-aldrich), atropine (Sigma-aldrich) dan temephos (BASF).

Plant Collection and Identification

Sample collection was conducted in the Semarang Regency and Salatiga City areas. The collected specimens consisted of whole plants (leaves, stems, and roots). Initial identification was performed through morphological determination. Furthermore, formal identification and authentication were carried out by the UPF Pelayanan Kesehatan Tradisional in Tawangmangu.

Extraction

The collected plant samples were cleaned and separated into their respective parts (roots, stems, and leaves). The fresh weight of each part was recorded before being dried using a dehydrator. The leaves were dried at 45°C, while the stems and roots were dried at 50°C until a constant weight was achieved. The dried samples were subsequently pulverized into a fine powder using a grinder. The powdered samples underwent extraction via the maceration method using 96% ethanol as the solvent. A sample-to-solvent ratio of 1:10 (w/v) was employed. The maceration process was conducted for 3 x 24 hours. The resulting filtrate was then evaporated to remove the solvent, yielding a concentrated crude extract (Kasmiyati & Kristiani, 2022).

Quantification of Bioactive Compounds

Phenolic

The total phenolic content was determined spectrophotometrically using the Folin-Ciocalteu reagent. A standard calibration curve was constructed by measuring the absorbance of gallic acid at various concentrations (0, 10, 20, 30, 40, and 50 ppm). For sample preparation, 0.0010 g of the concentrated extract was dissolved in 96% ethanol to a final volume of 10 mL. A

0.1 mL aliquot of the extract or standard was mixed with 0.75 mL of 10% Folin-Ciocalteu reagent and incubated for 5 minutes. Subsequently, 0.75 mL of 7% Na₂CO₃ was added, and the mixture was shaken and further incubated for 90 minutes. The absorbance was measured at a wavelength of 550 nm using a spectrophotometer. The total phenolic was calculated based on the gallic acid linear regression equation and expressed using the formula $GAE = c(V/m)$ (Azlim Almey *et al.*, 2010).

Tannin

The total tannin content was determined spectrophotometrically using the Folin–Ciocalteu reagent. A standard calibration curve was constructed by measuring the absorbance of tannic acid at various concentrations (0, 10, 20, 30, 40, and 50 ppm). For sample preparation, 0.0010 g of the concentrated extract was dissolved in 96% ethanol to a final volume of 10 mL. A 0.1 mL aliquot of the extract or standard was mixed with 0.75 mL of 10% Folin-Ciocalteu reagent and incubated for 5 minutes. Subsequently, 0.75 mL of 7% Na₂CO₃ was added, and the mixture was shaken and further incubated for 90 minutes. The absorbance was measured at a wavelength of 550 nm using a spectrophotometer. The total phenolic was calculated based on the gallic acid linear regression equation and expressed using the formula $TAE = c(V/m)$ (Gurning *et al.*, 2021).

Flavonoid

The total flavonoid content was determined using the aluminum chloride (AlCl₃) colorimetric method. A quercetin standard curve was established using a concentration series of 20, 40, 60, 80, and 100 ppm. To prepare the sample, 0.0010 g of the concentrated extract was dissolved in 96% ethanol to a final volume of 10 mL. For the assay, a 0.2 mL aliquot of the sample or standard was mixed with 0.8 mL of distilled water and 0.06 mL of 5% NaNO₂, followed by a 5-minute incubation period. Subsequently, 0.06 mL of 10% AlCl₃ was added and incubated for another 5 minutes. The mixture was then treated with 0.4 mL of 1M NaOH and 0.48 mL of distilled water. The absorbance was measured at a wavelength of 510 nm using a spectrophotometer. The flavonoid concentration was calculated using the quercetin linear regression equation, expressed as $QE = c(V/m)$ (John *et al.*, 2014).

Saponin

The total saponin content was determined using the Liebermann-Burchard reagent, with measurements performed via spectrophotometry. A series of standard concentrations were prepared at 0, 100, 200, 300, 400, and 500 ppm. To quantify the saponin content in the extracts, 3.5 mL of the Liebermann-Burchard reagent was added to 1 mL of the diluted extract. The solution was then stirred and allowed to stand for 30 minutes at room temperature. Subsequently, the absorbance of the samples was measured at a wavelength of 578 nm using a spectrophotometer (Mora-Ocación *et al.*, 2022; Gianna, 2013)

Alkaloid

The total alkaloid content was quantified using spectrophotometric analysis. Atropine standard solutions were prepared at concentrations of 0.4, 0.6, 0.8, 1.0, and 1.2 ppm. A 1 mL aliquot of the solution was transferred into a separating funnel and washed three times with 10 mL of chloroform. The pH of the sample solution was subsequently adjusted to neutral by adding 0.1 N NaOH. Following pH adjustment, 5 mL of Bromocresol Green (BCG) solution and 5 mL of phosphate buffer were added, and the mixture was shaken thoroughly. The resulting mixture was extracted with successive volumes of 1, 2, 3, and 4 mL of chloroform through vigorous shaking. The extracts were then collected in a 10 mL volumetric flask and diluted to the volume mark with chloroform. The absorbance was measured at a wavelength of 470 nm using a spectrophotometer (Fadhil *et al.*, 2007).

Larvicidal Bioassay

The larvicidal bioassay was performed at treatment concentrations of 1,000-10,000 ppm in triplicate. Third-instar *Ae. aegypti* larvae were sourced from the Balai Besar Laboratorium Kesehatan Lingkungan Salatiga. Fifteen third-instar *Ae. aegypti* larvae were utilized for each concentration. The larvae were maintained in plastic cups containing 50 mL of the test solution. This volume was selected as a modification of the (Paul & Mathew, 2023) and (World Health Organization, 2005), the volume was modify to optimize the use of the limited extract while ensuring sufficient exposure and larval movement. Temephos (10.000 ppm) served as the positive control, while distilled water was employed as the negative control and DMSO for control. The larvae were incubated for 48 hours at room temperature (25–28°C). Observations were conducted, and the cumulative mortality was recorded at 24 and 48 hours to determine the mortality percentage. The Lethal Concentration (LC), LC₅₀ and LC₉₀ values were determined through Probit analysis using SPSS software version 23 (World Health Organization, 2005).

Morphological Observation of Larvae

Morphological observations of the third-instar *Ae. aegypti* larvae were focused on the digestive tract (midgut) and the overall body structure. The assessment was performed using microscopy by comparing the dead larvae exposed to the extracts with the untreated larvae (control) (Ravi *et al.*, 2018; Lim *et al.*, 2023).

Statistical Analysis

The secondary metabolite data were subjected to a Two-way Analysis of Variance (ANOVA) followed by Tukey's post-hoc test using SAS software version 9.0. For the larvicidal bioassay, the data were processed using IBM SPSS Statistics version 23. Probit analysis was employed to determine the LC₅₀ and LC₉₀ value.

RESULTS

Sample Plants Identification

The plant identification was conducted by the UPF YANKESTRAD (Traditional Health Services) in Tawangmangu. The results confirmed that the botanical specimens were consistent with the species intended for this study. Figure 1 show as the plant species utilized in the research, which is *A. vulgaris*, *C. caudatus*, *E. prostrata*, and *T. erecta*.

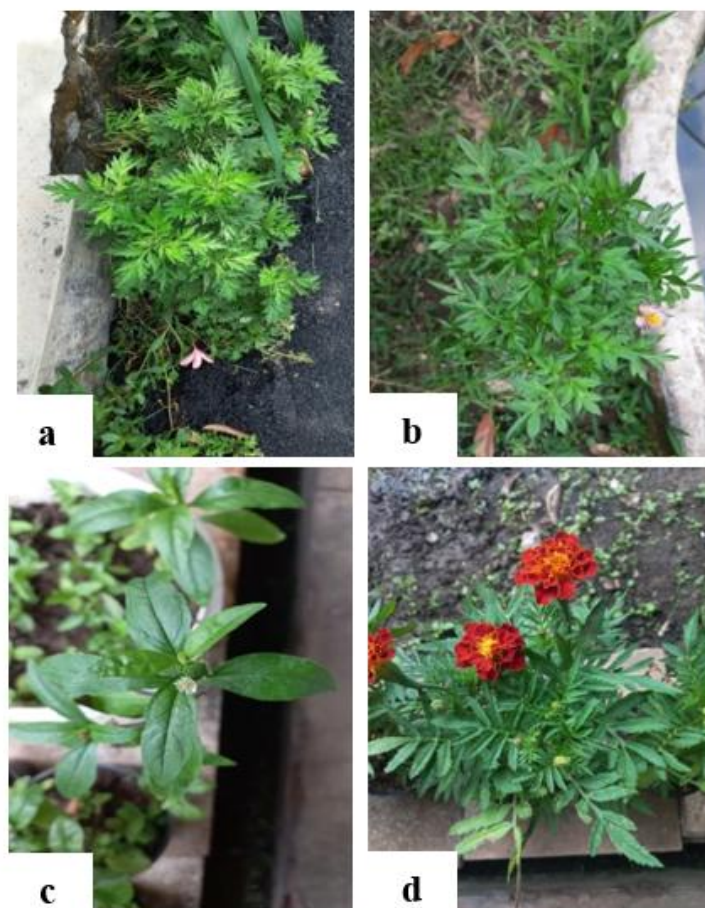


Figure 1. The identification results of the plants to be used in the research. (a) *A. vulgaris*; (b) *C. caudatus*; (c) *E. prostrata*; (d) *T. erecta*. (Hasil identifikasi tanaman yang akan digunakan untuk penelitian. (a) *A. vulgaris*; (b) *C. caudatus*; (c) *E. prostrata*; (d) *T. erecta*).

Secondary Metabolite Content

Quantification of secondary metabolites across the four plant species and their respective organs revealed that the highest concentrations were localized in the leaves. The analysis result showed a significance value p-value (<0.0001), which means there is an effect of plant species, plant organ, and the interaction of plant with plant organ on bioactive compounds. Table 1 summarizes the secondary metabolite profiles of the ethanolic extracts for each species and plant part. The ethanolic leaf extract of *C. caudatus* exhibited the highest levels of flavonoids (928.3 ± 368.7 mg/g extract), phenolics (310.2 ± 84.9 mg/g extract), tannins (187.1 ± 55.5 mg/g extract), and saponins (314 ± 95.4 mg/g extract). Meanwhile, the highest alkaloid content was observed in the leaf extract of *T. erecta* (27.8 ± 7.1 mg/g extract).

Table 1. Secondary metabolite measurement results (*Hasil pengukuran senyawa metabolit sekunder*).

Part of Plants (Organ tanaman)	Species (Spesies)	Secondary Metabolite Content (mg/g extract) (Kandungan Metabolit Sekunder (mg/gr ekstrak))				
		Flavonoid (Flavonoid)	Phenolik (Fenolik)	Tannin (Tanin)	Alkaloid (Alkaloid)	Saponin (Saponin)
Leaf (Daun)	<i>E. prostrata</i>	275.2±109.9 ^b	214.5±31.7 ^b	124.5±20.7 ^b	4.7±0.7 ^{cd}	101.6±14.2 ^e
	<i>C. caudatus</i>	928.3±368.7 ^a	310.2±84.9 ^a	187.1±55.5 ^a	12±5.3 ^b	314±95.4 ^a
	<i>T. erecta</i>	283.5±63.9 ^b	108.5±34.4 ^c	55.1±22.5 ^c	27.8±7.1 ^a	197.8±26.4 ^{bcd}
	<i>A. vulgaris</i>	367.3±41.3 ^b	120.1±21 ^c	62.7±13.7 ^c	16.4±2.9 ^b	198.2±13.4 ^{bcd}
Stem (Batang)	<i>E. prostrata</i>	351±23.7 ^b	205.2±47.8 ^b	118.4±31.2 ^b	5.3±2.7 ^c	161±24.5 ^{de}
	<i>C. caudatus</i>	57.7±22.5 ^c	103.2±14.7 ^c	51.7±9.6 ^c	1±0.3 ^{de}	175.5±31.9 ^{cd}
	<i>T. erecta</i>	26.5±13.6 ^c	104±10.4 ^c	52.3±6.8 ^c	3.5±1.3 ^{cde}	169.3±44.5 ^d
	<i>A. vulgaris</i>	318.1±92.4 ^b	90.5±14.2 ^c	43.4±9.3 ^c	2.4±0.2 ^{cde}	233.8±23.3 ^{bc}
Root (Akar)	<i>E. prostrata</i>	407.3±240.9 ^b	198±72.7 ^b	113.7±47.5 ^b	1.2±0.8 ^{de}	216.9±4.6 ^{bcd}
	<i>C. caudatus</i>	84.4±65 ^c	119.4±13.6 ^c	62.3±8.9 ^c	0.5±0.6 ^e	242.7±17.3 ^b
	<i>T. erecta</i>	28.1±15.6 ^c	108±9.8 ^c	54.8±6.4 ^c	0.6±0.4 ^e	245±93.2 ^b
	<i>A. vulgaris</i>	44.4±16.1 ^c	83.1±13.7 ^c	38.6±9 ^c	0.3±0.1 ^e	206.8±26.6 ^{bcd}

Note: Means followed by the same letter do not differ significantly according to Tukeys's HSD post-hoc test at p-value (<0.0001). (Keterangan: huruf yang sama notasinya dibelakang angka menunjukkan tidak beda nyata pada uji lanjutan Tukey HSD pada p-value (<0,0001)).

Larval Mortality

As presented in Table 2, the ethanolic leaf extract of *A. vulgaris* exhibited the highest mortality rate. Larval mortality reached 100% across all tested concentrations at both 24 and 48 hours of exposure. For the *C. caudatus* ethanolic leaf extract, the maximum mortality recorded was 93% at both exposure intervals. In the case of *E. prostrata* leaf extract, the peak mortality was 50% after 24 hours, increasing to 100% at 48 hours. Similarly, the *T. erecta* leaf extract reached a maximum mortality of 93% and 97% at 24 and 48 hours, respectively.

Table 2. Larval mortality percentage (*Persentase mortalitas larva*).

Sample (Sampel)	Concentration (ppm) (Konsentrasi (ppm))	Time Exposure (h) (Waktu Pemaparan (jam))	Mortality (%) (Mortalitas (%))	
<i>A. vulgaris</i>	1,000	24	100	
		48	100	
	2,500	24	100	
		48	100	
	5,000	24	100	
		48	100	
	7,500	24	100	
		48	100	
	10,000	24	100	
		48	100	
	<i>C. caudatus</i>	1,000	24	0
			48	0
2,500		24	0	
		48	0	
5,000		24	0	
		48	13	
7,500		24	67	
		48	93	
10,000		24	93	
		48	93	

Sample (Sampel)	Concentration (ppm) (Konsentrasi (ppm))	Time Exposure (h) (Waktu Pemaparan (jam))	Mortality (%) (Mortalitas (%))	
<i>E. prostrata</i>	1,000	24	0	
		48	0	
	2,500	24	0	
		48	0	
	5,000	24	7	
		48	23	
	7,500	24	20	
		48	33	
	10,000	24	50	
		48	100	
	<i>T. erecta</i>	1,000	24	0
			48	0
2,500		24	0	
		48	0	
5,000		24	20	
		48	23	
7,500		24	63	
		48	63	
10,000		24	93	
		48	97	
Temephos		24	100	
		48	100	
DMSO	24	0		
	48	0		
Aquadest	24	0		
	48	0		

As shown in Table 3, the LC₅₀ and LC₉₀ values for the *A. vulgaris* ethanolic leaf extract at 24 and 48 hours of exposure could not be determined due to 100% larval mortality across all concentrations. For the *C. caudatus* extract, the LC₅₀ values were 7204.7 ppm at 24 hours and 6139.7 ppm at 48 hours, while the LC₉₀ values were 9077.3 ppm and 8226.4 ppm, respectively. The *E. prostrata* extract exhibited an LC₅₀ of 1025.5 ppm and an LC₉₀ of 18048.9 ppm at the 24-hour interval; however, these values could not be calculated at 48 hours. For the *T. erecta* extract, the LC₅₀ was 6569.6 ppm at 24 hours and 6497.1 ppm at 48 hours, with corresponding LC₉₀ values of 9682.1 ppm and 9343.3 ppm.

Table 3. LC₅₀ and LC₉₀ (LC₅₀ dan LC₉₀).

Sample (Sample)	Time Exposure (Waktu pemaparan) (h) (jam)	LC ₅₀ (ppm)	LC ₉₀ (ppm)
<i>A. vulgaris</i>	24	-	-
	48	-	-
<i>C. caudatus</i>	24	7204.7	9077.3
	48	6139.7	8226.4
<i>E. prostrata</i>	24	10255.6	18048.9
	48	-	-
<i>T. erecta</i>	24	6569.6	9682.1
	48	6497.1	9343.3
Temefos	24	-	-
	48	-	-
DMSO	24	-	-
	48	-	-
Aquadest	24	-	-
	48	-	-

Larval Morphology

Morphological observations revealed distinct differences between the control larvae and those exposed to the plant ethanolic leaf extracts. Figure 2 presents the occurrence pigmentation on siphon (picture 1-4 c) and midgut (picture 1-4 b), elongation on neck (picture 1-4 a), and body shrinkage (picture 5) in the treated larvae. These morphological alterations were particularly prominent in the cervical region, the digestive tract, and the siphon and.

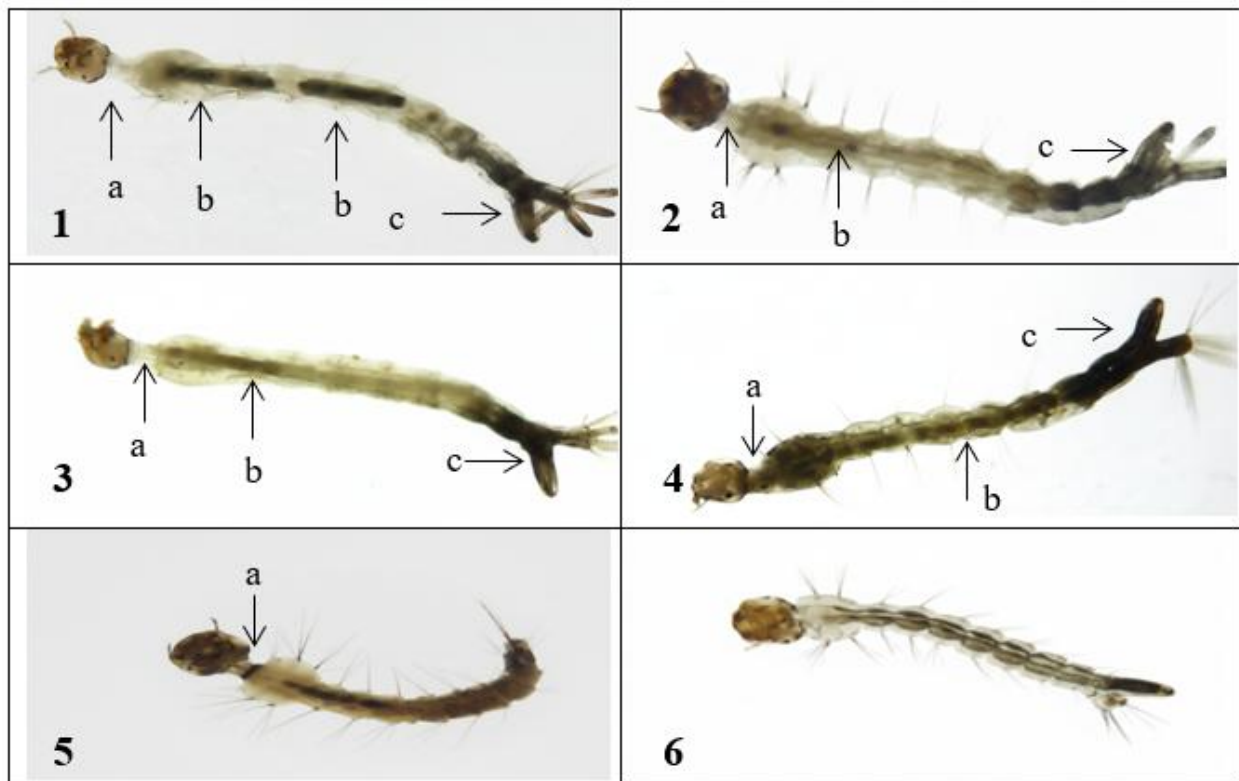


Figure 2. Morphology of control and plants extract exposed *Aedes aegypti* larvae (magnification 40x). (1-5) extract exposed larvae; (6) control larvae (a) neck; (b) midgut; (c) siphon and. (Morfologi Larva *Ae. aegypti* kontrol dan terpapar ekstrak tanaman (perbesaran 40x). (1-5) larva terpapar ekstrak; (6) larva kontrol; (a) leher; (b) saluran pencernaan; (c) sifon).

DISCUSSION

Secondary metabolite concentrations exhibit variation across different plant organs, which can be influenced by an array of factors. In this research, the leaves of each species contained the highest phenolic content compared stems and roots. The highest phenolic content in this research was observed in *C. caudatus* leaves, at 310.2 ± 84.9 mg/g. Phenolic compounds serve as a defense mechanism against environmental stressors, pathogens, and predators (Kim, 2016). Ecological conditions, such as climate, altitude, and soil characteristics, also exert a substantial influence on phenolic synthesis (Zargoosh *et al.*, 2019). Furthermore, the distribution of secondary metabolites within a plant is characteristically non-uniform. In several species, phenolic compounds primarily accumulate in the reproductive organs, with the lowest concentrations typically found in the stems. Fluctuations in phenolic content are also associated with the plant's ontogenetic period and phenological phases, such as flowering or fruiting (Feduraev *et al.*, 2019).

Tannin content across the studied species was also found to be highest in the leaves compared stems and roots. *C. caudatus* leaves contain the highest phenolic content, which was 187.1 ± 55.5 mg/g. Tannins play a crucial role in plant defense against pathogens and herbivores due to their antidiigestive and antimicrobial properties. Furthermore, these compounds contribute to plant growth and development. The regulation of tannin biosynthesis is mediated by various phytohormones, including salicylic acid, jasmonic acid, ethylene, and abscisic acid (Iqbal & Poór, 2025).

Leaves also exhibited substantially higher flavonoid concentrations compared to stems and roots plants. In this research, *C. caudatus* leaves had highest flavonoid content, at 928.3 ± 368.7 mg/g. This accumulation occurs as a response to environmental conditions or abiotic stress (Chu *et al.*, 2024). Beyond stress response, flavonoids play pivotal roles in regulating plant development, pigmentation, and biosynthesis, as well as mediating defense and signaling pathways between plants and microorganisms (Mathesius, 2018). The synthesis of flavonoids is modulated by plant growth regulators, including auxin, cytokinin, gibberellin, and abscisic acid (Hu *et al.*, 2021). Previous research by (Savina *et al.*, 2023) similarly demonstrated that flavonoid levels in the leaves of *Filipendula ulmaria* were higher than those in the stems and roots. Furthermore, a downward trend in flavonoid content was observed from the upper to the lower leaves. This decline in the lower leaves is likely attributable to the translocation of nutrients and energy toward actively developing tissues (Hu *et al.*, 2021).

Measurement results across all plant species indicated that the highest saponin concentrations were localized in the leaves. Saponin content highest had a *C. caudatus* leaves, which was 314 ± 95.4 mg/g. Saponins play a significant role in enhancing plant immunity against various pests, pathogens, and herbivores. The saponin content can also be influenced by various biotic stimuli associated with pest infestations, pathogenic infections, and mutualistic symbioses with rhizobial bacteria and mycorrhizal fungi (Hussain *et al.*, 2019). Furthermore, saponins exhibit antifeedant properties. Certain saponin compounds have been shown to modulate hormonal pathways, leading to developmental abnormalities and reproductive inhibition in target pests. Additionally, saponins can affect essential enzymes in pests, thereby disrupting metabolic processes for survival and development (Hameed *et al.*, 2025).

This research indicate that the highest alkaloid concentrations were in the leaves. *T. erecta* leaves had highest alkaloid content, which was 27.8 ± 7.1 mg/g. These results contrast with a study by (Zhaleh *et al.*, 2025), which reported that in *Berberis integerrima*, the highest alkaloid content was found in the roots, followed by the stems, and was lowest in the leaves and fruits. The variation in alkaloid levels among plants can be attributed to both intrinsic plant characteristics and environmental factors. For instance, nitrogen-fixing plants and those growing in nitrogen-rich environments tend to accumulate higher alkaloid concentrations in their leaves. Alkaloids play a vital role in plant defense mechanisms. Abiotic stress conditions, such as drought, salinity, and high temperatures, can significantly influence alkaloid accumulation. Additionally, certain alkaloid

compounds found in the leaves, roots, and other plant tissues exhibit allelopathic properties (Bhambhani *et al.*, 2022).

Probit analysis for the *A. vulgaris* extract could not determine the LC₅₀ values, as 100% larval mortality was observed across all tested concentrations. Similarly, the LC₉₀ values for the *E. prostrata* extract could not be calculated for the same reason. In a previous study by (Ninditya *et al.*, 2020), the ethanolic leaf extract of *A. vulgaris* against *Ae. aegypti* larvae yielded LC₅₀ values of 65.8 ppm and 18.6 ppm after 1 and 24 hours of exposure. Furthermore, (Mya *et al.*, 2016) reported a LC₅₀ of 792.40 ppm for the same extract against *Ae. aegypti* larvae. The *C. caudatus* extract exhibited LC₅₀ values of 7204.7 ppm at the 24 hour observation and 6139.7 ppm at 48 hours. These findings vary from previously reported data. A study by (Wasilah & Setiawan, 2019) demonstrated that the ethanolic leaf extract of *C. caudatus* against third-instar *Ae. aegypti* larvae yielded LC₅₀ of 0.19% at 24 hours. In contrast, (Aminu *et al.*, 2020) reported that the ethanolic fraction of *C. caudatus* extract resulted no larval mortality.

Exposure to the *E. prostrata* extract resulted in an LC₅₀ value of 10255.6 ppm ppm. This finding deviates from previous studies, (Dass & Mariappan, 2016) reported an LC₅₀ of 106.057 ppm when third-instar *Culex quinquefasciatus* larvae were exposed to the methanolic leaf extract of *E. prostrata* for 24 hours. Similarly, (Govindarajan & Karuppnanan, 2011) demonstrated an LC₅₀ of 127.64 ppm for the methanolic leaf extract of *Eclipta alba* against third-instar *Ae. aegypti* larvae. A 48 hour exposure to the ethanolic extract of *E. prostrata* leaves yielded no determinable LC₅₀ and LC₉₀ values via probit analysis. This suggests that larval mortality was either insufficient or excessive, thereby failing to meet the required range for statistical estimation (Pillai *et al.*, 2021). Furthermore, the 24-hour exposure to the *E. prostrata* extract exhibited lower efficacy compared to ethanolic extracts from other plant species. Regarding the *T. erecta* extract, LC₅₀ values were recorded at 6569.6 ppm at 24 hours and 6397.1 ppm at 48 hours. These results contrast with those of (Nikkon *et al.*, 2011), who observed an LC₅₀ of 918.62 µg/ml against third-instar *Cx. quinquefasciatus* larvae over a 24 hour period. Thus, the efficacy observed in the current study was lower than that reported in previous literature. The results obtained from the test using ethanolic leaves extracts of *C. caudatus*, *E. prostrata*, and *T. erecta* showed lower efficacy compared to existing literature. Meanwhile, in this study, larval mortality reached 100% in test using the ethanolic extract of *A. vulgaris* leaves. Several factors may influence the LC₅₀ values recorded in prior research, including variations in secondary metabolite profiles and the distinct environmental conditions where the plants were cultivated.

Observations of *Ae. aegypti* larvae that succumbed to the plant extracts revealed significant morphological alterations, such as pigmentation, elongation, and body shrinkage. These findings are consistent with studies by (Alyahya, 2023) and (Sharawi, 2024), which reported similar abnormalities, including cervical elongation and bodily pigmentation. The observed body shrinkage is likely due to the degradation of the larval exoskeleton (chitin), which serves as a protective barrier and regulates osmoregulation across the body wall. Furthermore, the transition of larval color to a pale hue or the presence of intense pigmentation indicates widespread tissue damage or necrosis. Larvae exposed to the extracts also exhibited siphon damage, characterized by pigmentation. The siphon is a vital respiratory organ its degradation leads to the loss of the hydrophobic surface, resulting in oxygen deprivation. Consequently, damage to the siphon and anal papillae significantly compromises larval viability. Additionally, the midgut, which plays a crucial role in secreting digestive enzymes, showed signs of epithelial cell protrusion and swelling, which ultimately disrupts the digestive processes (Subahar *et al.*, 2020).

Morphological changes in larvae can be induced by the presence of secondary metabolites. Exposure to plant-derived alkaloids has been shown to cause structural damage to the anterior and posterior epithelial cells of the *Aedes aegypti* midgut. Furthermore, the posterior midgut region exhibits a high density of dark cells within the nucleus, leading to systemic larval body pigmentation (Perumalsamy *et al.*, 2013). Investigations into the activity of saponins from *Camellia oleifera* against *Ectropis obliqua* demonstrated significant midgut degradation. Observed histopathological alterations include the disruption of the brush border, nuclei with non-condensed

chromatin, and prominently visible nucleoli. Perforations were also detected along the midgut wall. Additionally, saponins adversely affect the epidermal layer, resulting in a toughened texture and severe wrinkling. The chitin layer exhibits thinning and ablation, while the intestinal tract undergoes ulceration. These physiological disruptions culminate in systemic dehydration and bodily shrinkage of *E. obliqua* (Cui *et al.*, 2019). In a study by Tan *et al.* (2022), the administration of tannins (tannic acid) to *Hyphantria cunea* larvae resulted in elevated levels of hydrogen peroxide (H₂O₂) and malondialdehyde (MDA) within the larval midgut. The accumulation of these compounds indicates an increase in oxidative stress and subsequent physiological damage to the midgut tissues of *H. cunea*.

Pigmentation occurring in the larval siphon is potentially attributed to the degradation of the anal papillae. Dysfunctional anal papillae may lead to the disruption of osmotic balance and ionic regulation, subsequently compromising the integrity of the hydrophobic walls. This structural failure facilitates the infiltration of water into the trachea, thereby debilitating the larval respiratory system (Leandro *et al.*, 2023). The mechanism of phenolic toxicity in larvae is potentially mediated by the elevation of compounds that facilitate the degradation of the larval epithelial membrane and the subsequent upregulation of antioxidant responses (Baganha *et al.*, 2015). Furthermore, flavonoid compounds act by inhibiting the larval respiratory system and obstructing electron transport, leading to a significant reduction in ATP production and oxygen consumption (Babu *et al.*, 2025).

CONCLUSION

Based on the results of this research, the ethanolic leaf extracts of the tested plants exhibited higher secondary metabolite content compared to the roots and stems. The leaf extract of *C. caudatus* contained the highest levels of flavonoids (928.3±368.7 mg/g), phenolics (310.2±84.9 mg/g), tannins (187.1±55.5 mg/g), and saponins (314±95.4 mg/g), while *T. erecta* leaf extract showed the highest alkaloid (27.8±7.1 mg/g). The four Asteraceae plant extracts demonstrated potential as biolarvicides. The ethanolic leaf extract of *A. vulgaris* achieved a mortality rate up to 100%. The obtained LC₅₀ at 24 hours observation period values were 7204.7 ppm (*C. caudatus*), 6569.6 ppm (*T. erecta*) dan 10255.6 ppm (*E. prostrata*). while the LC₉₀ values were 9077.3 ppm (*C. caudatus*), 9682.1 ppm (*T. erecta*), dan 18048.9 ppm (*E. prostrata*). The dead larva also exhibited morphological changes, such as neck elongation, pigmentation in the siphon and digestive track as well as body shrinkage. These results may serve as a reference for the development of eco-friendly larvicides. Further research is recommended to isolate and identify the bioactive compounds in *A. vulgaris* that exhibit significant potential as a biolarvicide. Identifying the specific compounds responsible for this larvicidal activity will provide a crucial reference for the future development of biolarvicides.

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AUTHOR CONTRIBUTIONS

SAD: collecting research data, drafting the article, processing research data; SK: Creating research concept, processing research data; EBK: Creating research concepts, checking and verifying research result.

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