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Efficacy of culantro (*Eryngium foetidum* L.) extract and essential oil as biolarvicides against *Culex quinquefasciatus* larvae

Sri Ayu Winarti, Lela Lailatul Khumaisah^{ID*}, Devi Indah Anwar

ABSTRACT

Background: Filariasis, a priority tropical disease in Indonesia, is transmitted by the *Culex quinquefasciatus* mosquito. The use of conventional synthetic larvicides can lead to environmental and health issues, including poisoning and resistance in target insect populations.

Objective: This study explores the utilization of biolarvicides derived from culantro (*Eryngium foetidum*) to mitigate these adverse effects, focusing on analyzing the components and evaluating the larvicidal efficacy of both the extract and essential oil of *E. foetidum*.

Method: This study employed a molecular docking approach to examine *in silico* biolarvicidal activity against the odorant binding protein (OBP) receptor and conducted *in vitro* experiments on *Cx. quinquefasciatus* larvae using varying concentrations of *E. foetidum* extract (100, 250, and 500 ppm) and essential oil (10, 50, and 100 ppm).

Results: The *in silico* study identified hynokiflavone and longifenaldehyde as the compounds with the most potent activity, demonstrating binding affinities of -10.2 and -9.5 kcal/mol, respectively. The *in vitro* assays revealed that the *E. foetidum* extract achieved 75% larval mortality at an LC₅₀ of 78.59 ppm, while the essential oil resulted in 88% mortality with an LC₅₀ of 10.13 ppm.

Conclusion: The extract and essential oil of *E. foetidum* exhibit significant biolarvicidal activity against *Culex quinquefasciatus*, offering promising plant-based alternatives to traditional larvicides, with implications for safer and more sustainable vector control strategies.

Keywords: *Eryngium foetidum*, biolarvicidal activity, *Culex quinquefasciatus*, natural insecticides, vector control

Introduction

Indonesia's tropical climate contributes to its status as a hotspot for infectious diseases transmitted by bacteria, viruses, and parasites [1]. Among these, filariasis—primarily spread by the *Cx quinquefasciatus* mosquito—poses a significant health concern [2]. The Indonesian Ministry of Health reported 8,365 filariasis cases in 2022 [2]. In combating this disease, the government has initiated mass drug administration strategies, which include synthetic larvicides for

mosquito control. However, these measures face challenges such as potential toxicity, environmental pollution, and the development of insect resistance [3], prompting interest in safer and eco-friendly alternatives, such as plant-based biolarvicides.

Research has identified several plants with biolarvicidal properties against *Cx. quinquefasciatus* larvae, including betel leaves [4], vetiver [5], and grapefruit leaves [6]. Notably, culantro (*Eryngium foetidum* L.) emerges as a promising candidate due to its wide-ranging bioactivities, such as antibacterial, antioxidant, antifungal, antilarval, and antidiabetic properties [7]. The larvicidal efficacy of *E. foetidum* has been demonstrated in studies showing its essential oil's potency against *Aedes albopictus* larvae, with an LC₅₀ of 33.3 ppm [8].

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This study aims to further explore *E. foetidum*'s potential by analyzing its components and evaluating the efficacy of its extract and essential oil as bio-larvicides against *Cx. quinquefasciatus* larvae. It employed both *in vitro* methods and *in silico* molecular docking to investigate the interactions with odorant binding protein (OBP) receptors, which are critical for insect olfaction. This dual approach seeks to identify the most effective larvicidal compounds and understand their mechanisms, contributing to the development of safer and more sustainable mosquito control strategies.

Method

Materials

The larvicidal activity was assessed on *Cx. quinquefasciatus* instar III larvae, obtained from Lokaltibang Kesehatan Pangandaran, West Java, Indonesia.

Plant material

The aerial parts of the *E. foetidum* plant were collected from Pasawahan, Takokak District, Cianjur Regency, West Java, Indonesia. The plant material was authenticated at the Biology Laboratory of Universitas Muhammadiyah Sukabumi.

Extraction

The *E. foetidum* was cleaned, air-dried, and then ground into a powder. Using the maceration method, one kilogram of this powdered sample was extracted with methanol as the solvent over a period of 72 hours. The mixture was then filtered through a Buchner funnel, and the filtrate was concentrated under reduced pressure at 50°C using a rotary vacuum evaporator.

Distillation

For this process, 14.8 kilograms of the *E. foetidum* samples underwent water and steam distillation. The plant material was placed in a distillation kettle, which was filled with water and separated from the plant material by a perforated filter. This setup allowed the steam to permeate the plant material and then travel through a connecting pipe to a condenser. In the condenser, the steam reverted to liquid, allowing the collection of the essential oil as it dripped from the end of the pipe into a designated container [10].

Phytochemical screening of sample extract

Phytochemical screening was conducted to detect the presence of various compounds, including flavonoids, alkaloids, saponins, tannins, terpenoids, triterpenoids, steroids, phenolics, and glycosides [11,12].

Flavonoids: A mixture of 1 mL of the extract, 1 gram of magnesium powder, and 1 mL of concentrated HCl was prepared in a test tube. The appearance of a red, yellow, or orange indicated a positive test for flavonoids.

Alkaloids: 1 mL of the extract was combined with a few drops of 1% HCl in a test tube. After the solution dissolved, 1 mL of Mayer's reagent was added. The formation of a white precipitate or a cloudy solution indicated the presence of alkaloids.

Saponins: 10 mL of distilled water was added to 1 mL of the extract and shaken for 10 seconds. Persistent foam formation after 10 minutes suggested the presence of saponins.

Tannins: A mixture of 1 mL of the extract and 5 mL of distilled water was treated with a few drops of 5% FeCl₃ solution. A dark green or bluish-green color change indicated the presence of tannins.

Terpenoids: A test tube containing 2 mL of CHCl₃, 2 mL of the extract, and 3 mL of concentrated H₂SO₄ was mixed and heated for 3 minutes. A brownish color change in the solution indicated terpenoids.

Triterpenoids and steroids: For these analyses, 1 mL of the extract was mixed with acetic acid and 1 mL of concentrated H₂SO₄ in a test tube. Steroids were indicated by a blue or purple solution color, while triterpenoids were suggested by a red coloration in the lower layer of the solution.

Phenolics: 1 mL of the extract was reacted with 1% FeCl₃ in a test tube. The presence of phenolic compounds was characterized by the formation of green, red, purple, blue, or solid black coloration.

Glycosides: A 0.1 mL extract sample was evaporated on a hot plate, dissolved in 5 mL of glacial acetic acid, and treated with ten drops of concentrated sulfuric acid. Positive results were indicated by a blue or green color change (Liebermann Burchard reaction).

Identification of the chemical composition of *E. foetidum* extract

The chemical composition of the *E. foetidum* extract was analyzed using QTOF Liquid Chromatography-Mass Spectrometry (LC-MS/MS) equipped with an

ESI (+) source. The chromatographic separation was performed on an ARC-18 Raptor column, which has a particle size of 2.7 mm, an internal diameter of 2.1 mm, a length of 100 mm, and a pore size of 90Å.

Identification of the chemical composition of *E. foetidum* essential oil

The constituents of the *E. foetidum* essential oil were examined using a Shimadzu QP 2010 GC/MS instrument. The analysis was conducted under the following conditions: a DB-5 column (30 m in length and 0.25 mm in diameter), with helium as the carrier gas flowing at 226.0 mL/min. The linear velocity, pressure, and separation ratio settings were 38.6 cm/sec, 70.0 kPa, and 200, respectively. The injection temperature was maintained at 280°C. The column oven temperature was programmed to start at 70°C and increase by 8°C per minute until reaching 270°C. Components were identified by comparing their spectra with those in the WILEY7 library.

In silico study of extract components and essential oil samples

In silico analysis of compounds in the extracts and essential oil samples was conducted using a molecular docking approach, comprising the following steps. An odorant binding protein (OBP) receptor (PDB ID: 3OGN) was obtained from the Protein Data Bank. The receptor was isolated from unnecessary molecules and its ligands using AutodockTools software. Hydrogen atoms were added to the receptor's polar areas, and Gasteiger charges were computed. A grid box was then created around the native ligand. The molecular structure of the compounds was initially drafted in two-dimensional (2D) form using ChemDraw Ultra software and subsequently converted to three-dimensional (3D) form with Chem3D.

Molecular docking was performed using Autodock Vina, operated through the Command Prompt (CMD). The validation of the docking method involved calculating the Root Mean Square Deviation (RMSD) value using PyMOL software. The docking method was considered valid if the RMSD value was ≤ 2.00 Å. The results of the docking were visualized using Discovery Studio software to examine the interactions between the test ligands and the receptor. Key parameters included the receptor amino acid residues and the types of bonds/interactions formed.

In vitro biolarvicide activity test

Biolarvicidal activity testing was conducted on *Cx. quinquefasciatus* instar III larvae using both the extract and essential oil samples. Abate was used as the positive control, and distilled water (aquadest) served as the negative control. The extract was tested at three different concentrations: 100, 250, and 500 ppm. The essential oil was tested at concentrations of 10, 50, and 100 ppm. Each concentration in the experimental groups was replicated three times. Twenty test larvae were placed in a beaker containing 200 mL of the sample solution [13].

Larval mortality was monitored over a 24-hour period, with observations made at 6, 12, 18, and 24 hours. Larvae were considered dead if they showed no movement upon mechanical stimulation with a pipette. Larval mortality was calculated through the formula:

$$\% \text{ mortality} = \frac{\text{number of dead larvae}}{\text{total number of larvae}} \times 100\%$$

Data analysis

Test result data were analyzed probit using SPSS.

Results

Maceration extraction

The extraction of *E. foetidum* was conducted using the cold maceration method, which is favored for its simplicity and effectiveness in preserving thermolabile compounds. Methanol was selected as the solvent due to its universal solvent properties, enabling the extraction of a broader range of compounds [14].

During the evaporation process, temperatures were maintained at or below 50°C, under methanol's boiling point (64.7°C). This approach, utilizing a vacuum system, ensures evaporation below the solvent's boiling point, thereby preventing damage to the extracted compounds. The yield extract was a blackish-green liquid, weighing 88 grams and yielding 8.8%. Such a yield is within the typical range (4 – 40%) for methanol extractions in various studies [15,16], suggesting that the *E. foetidum* methanol extract's yield is consistent with other methanol-based extractions.

Essential oil extraction

The extraction of essential oil from *E. foetidum* was carried out using water and steam distillation. The extracted oil was yellow-brown, had a pungent odor, and yielded 0.07%. This yield is within the

reported range for *E. foetidum* essential oils, which varies from 0.1 – 0.95% [17]. Factors influencing this yield include the preparation methods, type of raw materials, equipment used, and precision in the extraction process [18].

Phytochemical screening of extracts

Phytochemical screening was carried out to determine the class of secondary metabolites in *E. foetidum* plants qualitatively (Table 1).

Table 1. Phytochemical compounds of *E. foetidum* extract

No.	Compound classes	Results
1	Flavonoids	+
2	Alkaloids	-
3	Tannins	+
4	Saponins	+
5	Terpenoids	+
6	Triterpenoids	+
7	Steroids	-
8	Glycosides	+
9	Phenolic	+

Flavonoid identification showed positive results, evidenced by yellowish color changes after reacting the sample with HCl and magnesium powder (Table 1). This indicates the reduction of the benzopyrone core by the reagent. Phytochemical tests using FeCl_3 revealed the presence of condensed tannin compounds, which are flavonoid polymers containing numerous phenolic hydroxy groups commonly found in plants [20].

Furthermore, positive results are shown in saponins characterized by the formation of stable foam in which indicates the presence of glycosides hydrolyzed into glucose and aglycone [21]. Triterpenoid and steroid tests show positive results on triterpenoids as evidenced by the formation of red at the bottom of the solution. Similarly, tests for terpenoids, involving the addition of

chloroform and concentrated sulfuric acid, resulted in a brownish discoloration, indicating their presence. The tests for glycosides and phenolics were also positive, with the solution turning green.

Analysis and identification of extract and essential oil components

The identification of *E. foetidum* extract was carried out with the LC-MS/MS instrument consisting of two different biosynthetic pathways as in Table 2.

Table 2 shows natural aromatic compounds that include phenylpropanoids, phenolics, and flavonoids. Phenylpropanoids are classified into cinnamic acid, coumarin, allyl phenol, and propenylphenol groups [22]. Based on their structure, phenylpropanoid compounds in Table 2 show coumarin groups, while flavonoid compounds show groups of compounds with the basic structure of flavones and flavonols [22].

In addition, there are compounds in *E. foetidum* extract from the acetate-malonate pathway which is a group of polyketides and terpenoids that have a certain basic structure. Polyketide compounds have an aromatic basic framework composed of several carbon atom units and form a linear carbon chain, the results of the analysis of polyketide compounds in Table 2 are macrolide groups (macrocytic lactones). Terpenoid compounds in *E. foetidum* extract consist of monoterpenoids, sesquiterpenoids, and triterpenoids [22]. The analysis of *E. foetidum* essential oil with GC/MS obtained 69 compounds (Table 3).

In silico biolarvicide activity study

Parameters used as references in *in silico* studies include binding affinity, RMSD, and interactions between ligands and amino acid residues. The interaction that occurs affects the conformation and causes a decrease in activation energy indicated by a small affinity [23]. The smaller the binding affinity, the more stable the interaction between ligands and receptors [23]. The docking results of *E. foetidum* extract compounds and essential oils are shown in Table 4.

Table 2. Components of *E. foetidum* extract from LC-MS/MS analysis

No.	Compounds	Subclasses	Classes
Compounds with the shikimate biosynthesis pathway and a combination of the shikimate-acetate biosynthesis pathway			
1	6-aldehydo-isooaphio-pogonone B	Coumarin	Phenylpropanoids
2	6-hydroxykaemferol-3-O-glucoside	Flavonols	Flavonoids
3	7-hydroxy-1-methoxy-2-methoxyxanthone	Xanthone	Phenolic
4	7-methyltectorigenin	Flavones	Flavonoids
5	Apigenin-6-C-galac-tosyl-8-C-arabinoside	Flavones	Flavonoids
6	Buddlenoid A	Flavonols	Flavonoids
7	Cyanidin 3,5-diglucoside_1	Flavilium salt	Flavonoids
8	Eupatin	Flavonols	Flavonoids
9	Hynokiflavone	Flavones	Flavonoids
10	Isoquercitrin	Flavonols	Flavonoids
11	Kaemferol	Flavonols	Flavonoids
12	Kaemferol 3-O-β-D-glucopyranoside	Flavones	Flavonoids
13	Kaemferol 3-O-β-D-glucuronide	Flavonols	Flavonoids
14	Quercetagetin-6,7,3',4'-tetramethyl ether	Flavonols	Flavonoids
15	Quercetin-3-gentiobioside	Flavonols	Flavonoids
16	Quercetin-3-O-α-D-glucuronide	Flavonols	Flavonoids
17	Undulatoside A	Coumarin	Phenylpropanoids
18	Wogonoside	Flavones	Flavonoids
19	1β,3α,9β-trihydroxyeudesma-5,11(13)-dien-12-oic-acid	Sesquiterpenoids	Terpenoids
20	3-O-β-D-glucopyranosyl-14, 19-dideoxyandrographolide	Sesquiterpenoids	Terpenoids
21	4, 8, 12-trimethyl-tridecanoic acid	-	Fatty acid
22	6-O-acetyl shanzhiside methyl ester_1	Monoterpenoid	Terpenoids
23	Apocyanoside II	Monoterpenoid	Terpenoids
24	Brefeldin A	Macrolides	Polyketides
25	Dendronobilin F	Sesquiterpenoid	Terpenoids
26	Isopropyl-p-benzalcohol	Monoterpenoid	Terpenoids
27	Lablaboside B	Triterpen saponins	Terpenoids
28	Lactiflorin	Monoterpenoids	Terpenoids
29	Lactinolide	Monoterpenoids	Terpenoids

Table 3. Compounds in *E. foetidum* essential oil

Peak	Compounds	Retention time (Minutes)	% Area
1	Methanol	1.301	0.14
2	α -pinene	4.725	0.16
3	β -phellandrene	5.304	0.30
4	β -pinene	5.419	0.55
5	1,3,5-trimethylbenzene (mesitylene)	5.629	0.14
6	δ -3-cerene	5.903	0.07
7	p-cymene	6.107	0.26
8	(-)-limonene	6.190	0.25
9	1,8-cineole	6.271	3.26
10	γ -terpinene	6.665	0.26
11	Tridecane	7.212	0.54
12	Nonanal	7.319	0.51
13	Cantharene	7.395	0.10
14	β -citronellal	8.175	0.34
15	Safranal	8.425	0.13
16	4-terpineol	8.773	0.51
17	Linalyl propanoat	8.976	0.10
18	Decanal	9.049	4.69
19	2-Octylfuran	10.537	0.17
20	Safrole	10.634	0.21
21	Undecanal	10.729	0.43
22	Duraldehyde	11.156	0.34
23	Eugenol	11.687	0.54
24	Duraldehyde (mesitaldehyde)	11.873	5.93
25	Trans-2-dodecenal	12.186	2.29
26	Isolongifolene-oxide	12.267	0.20
27	Myristaldehyde	12.370	12.92
28	α -longipinene	12.637	0.12
29	Trans-Caryophyllene	12.912	0.38
30	Trans-2-dodecenal	13.028	0.79
31	Z- β -farnesene	13.109	0.23
32	(E)-isoeugenol	13.165	0.21
33	Trans-2-dodecenal	13.322	28.68
34	Trans-2-nonenol	13.487	3.24

Table 3. (Continued)

Peak	Compounds	Retention time (Minutes)	% Area
35	α -curcumen	13.645	0.78
36	Butyric acid	13.758	0.29
37	Cis-caryophyllene	14.017	0.20
38	(-)- beta-elemene	14.143	0.30
39	Myristicin	14.254	4.18
40	Citronellol-epoxide	14.451	0.22
41	Elemicin	14.563	0.19
42	δ -nerolidol	14.739	0.79
43	Tridecanol	14.883	4.60
44	Longifenaldehyde	15.031	2.48
45	Trans-2-tridecenal	15.184	0.22
46	Myristaldehyde	15.350	1.67
47	(-)-Caryophyllene-oxide	15.411	0.32
48	3a(1H)-azulenol	15.583	0.32
49	Alloaromadendrene	15.885	0.24
50	Alloaromadendrene	16.023	1.21
51	Palmitaldehyde, diallyl acetal	16.120	0.18
52	Trans-2-Dodecenal	16.201	3.88
53	Artemisia ketone	16.398	0.13
54	Patchouli alcohol	16.629	0.12
55	Artemisia ketone	16.921	0.13
56	(+) Spathulenol	18.054	0.23
57	Neophytadiene	18.300	1.47
58	Phytol	18.835	0.21
59	p-cymene-8-ol	18.943	0.88
60	Citronellyl butyrate (citronellyl butanoate)	23.297	0.09
61	Patchouli alcohol	24.536	0.28
62	2-Butyl-octanol	26.472	0.26
63	Palmitaldehyde	26.596	0.68
64	Longiborneol	27.217	1.09
65	Tridecanedial	27.383	0.45
66	1-phenyl-2-methylbutane	27.495	0.57
67	Tetrahydroedulan A	27.593	0.51
68	Myristyl chloride	27.694	0.59
69	5,4'-dihydroxy,7-di-O-glucoside-flavone	28.083	1.30

Table 4. Affinity of compound docking results in *E. foetidum* extract

No.	Types of ligands	Affinity (kcal/mol)
Compounds in the extract		
1	Native ligand	-7.9
2	Temephos (positive control)	-8.5
3	6-aldehydo-isoophiopogonone B	-9.1
4	6-hydroxykaempferol-3-O-glucoside	-8.1
5	7-Hydroxy-1-methoxy-2-methoxyxanthone	-8.3
6	7-Methyltectorigenin	-8.0
7	Apigenin-6-C-galac-tosyl-8-C-arabinoside	-4.7
8	Cyanidin 3,5-diglucoside_1	-6.0
9	Hynokiflavone	-10.2
10	Kaempferol	-9.2
11	Kaempferol 3-O- β -D-glucopyranoside	-7.4
12	Undulatoside A	-8.2
13	1 β ,3 α ,9 β -Trihydroxyeudesma-5,11(13)-dien-12-oic acid	-9.1
14	3-O- β -D-Glucopyranosyl-14,19-dideoxyandrographolide	-8.2
15	4,8,12-Trimethyl-tridecanoic acid	-6.9
16	Apocynosidell	-8.4
17	Brefeldin A	-9.0
18	Dendronobilin F	-7.6
19	Isopropyl-p-benzalcohol	-6.6
20	Lablaboside B	-3.7
21	Lactinolide	-6.9
Compounds in essential oils		
22	Trans-2-dodecenal	-5.9
23	Decanal	-5.3
24	1,8-cineol	-6.8
25	Longifenaldehyde	-9.5
26	Myristicin	-7.0
27	Myristaldehyde	-6.0
28	Tridecanol	-6.1
29	Duraldehyde	-6.9
30	Trans-2-nonenol	-5.5

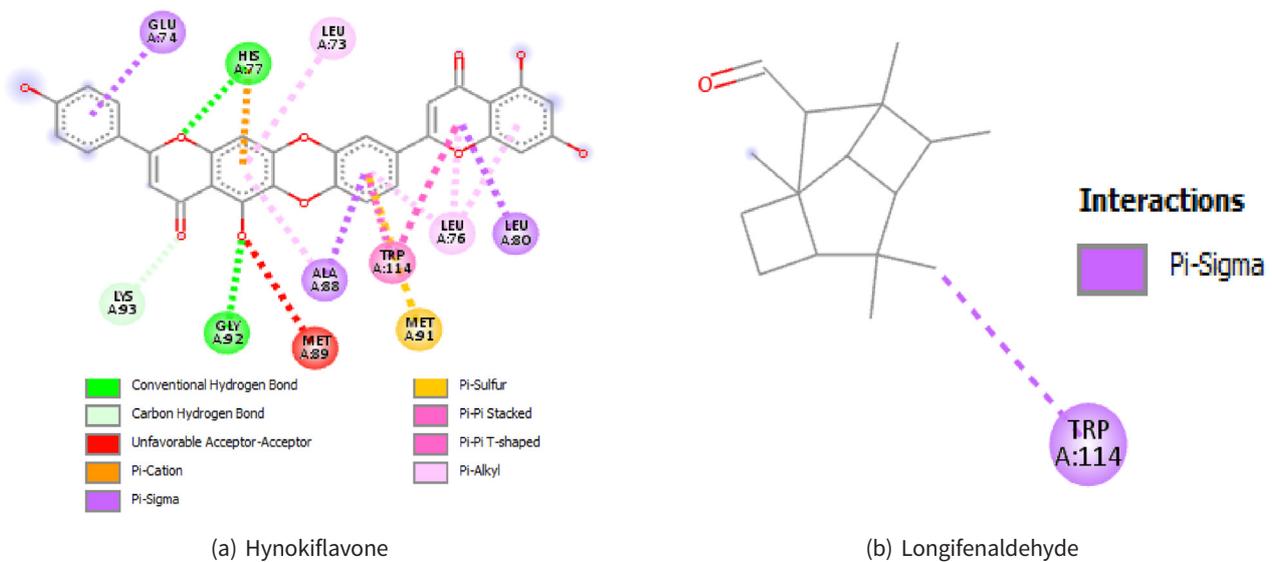


Figure 1. Visualization of test ligands with the best activity in *E. foetidum* extracts and essential oils.

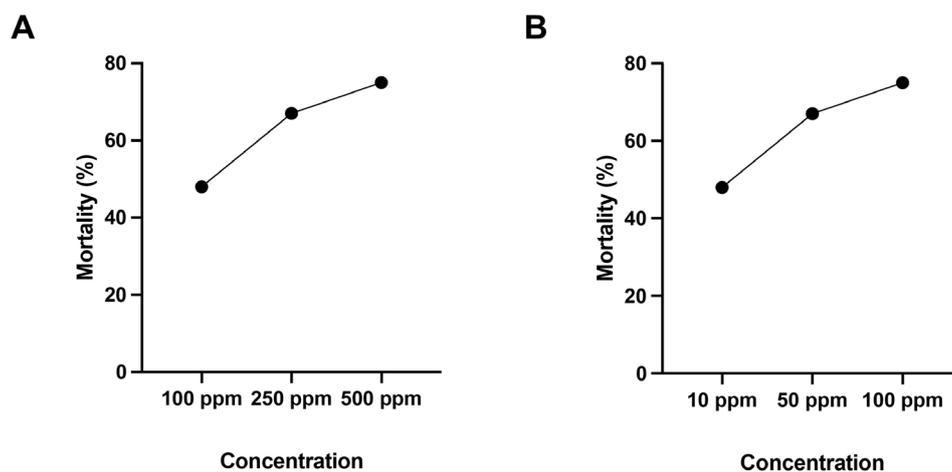


Figure 2. Percentage of larval death (a) in the extract; (b) on essential oils

Nine teen compounds from the results of LC-MS/MS analysis were selected based on the similarity of structure and the presence or absence of sugar bound to the compound. Likewise, *E. foetidum* essential oil selected 9 dominant compounds with the largest percentage of area. Of the 28 test compounds, 2 compounds with the best activity were hynokiflavone and longifenaldehyde, which has a lower bond energy than positive control and natural ligands. Visualization of both compounds is presented in Figure 1.

Biolarvicide activity test

Biolarvicide activity test was carried out at Lokalibang Kesehatan Pangandaran on *Cx. quinquefasciatus* instar III larvae. Room temperature conditions at the time of the study were 25°C, water temperature 25 – 28°C and pH 7. Based on this, larvae live in good condition because basically larvae are able to survive at air temperatures ranging from 8 – 37°C [24]. The death of the larvae is presented in Figure 2. Based on larval mortality, it was shown that biolarvicide treatment in the form of essential oils and

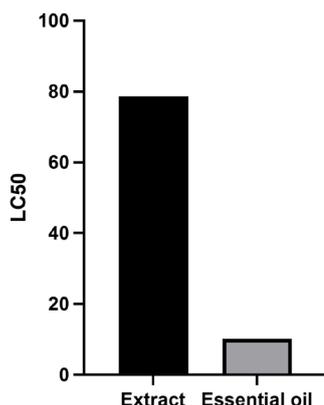


Figure 3. LC₅₀ in *E. foetidum* extract and essential oil

extracts resulted in mortality percent of 88 and 75%. From the death of larvae after 24 hours, LC₅₀ values can be calculated by probit analysis is shown in Figure 3.

Discussion

Filariasis is a significant problem in countries with tropical climates such as Indonesia. Efforts to break the chain of spread can be done by controlling vectors through eradication of larvae [5]. In this study, *E. foetidum* extract and essential oil produced good activity against *Cx. quinquefasciatus*. According to the study [24], phytochemicals have a role in the death of larvae. Phytochemical screening results show that *E. foetidum* extract contains flavonoids, tannins, saponins, terpenoids, triterpenoids, glycosides, and phenolics.

The *in silico* study identified the most effective biolarvicidal compounds as belonging to the flavonoid and sesquiterpenoid groups, specifically hynokiflavones in extracts and longifenaldehyde in essential oils, acting as competitive inhibitors. Both compounds demonstrated lower affinity values, -10.2 and -9.5 kcal/mol respectively, compared to positive controls and native ligands. Lower affinity values are indicative of higher compound activity, attributed to the nature of interaction between the compound and the receptor.

Compounds from the flavonoid group (hynokiflavones) can increase enzyme inhibition in which the OH group at C₅ forms hydrogen bonds with amino acid residues. Figure 4 shows the relationship of flavonoids to flavone skeletons as biolarvicides.

Moreover, the presence of terpenoids contributes to the activity of *E. foetidum* extract and essential oil as insecticides, due to their capacity to disrupt larval metamorphosis and inhibit insect development [5].

This study reveals a correlation between *in silico* and *in vitro* findings, showing that *E. foetidum* extract and essential oil exhibit good activity against target larvae. The LC₅₀ values for *E. foetidum* extract and essential oil are below 5000 ppm, aligning with the criteria for bioinsecticides in Integrated Pest Management (IPM) strategies, which consider bioinsecticides effective if extract concentrations are less than 5000 ppm and pure compounds are below 5 ppm [25]. The *in silico* results corroborate the broad-spectrum activity of *E. foetidum* extract and essential oil as biolarvicides, potentially minimizing target insect resistance to natural insecticides.

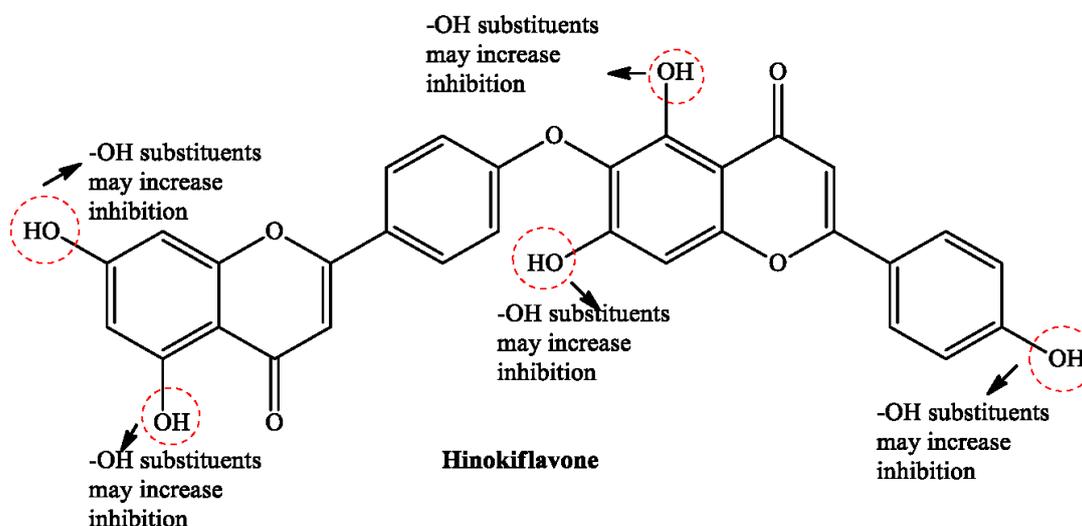


Figure 4. The relationship of flavonoids to the flavone skeleton as biolarvicide.

Conclusion

In conclusion, *E. foetidum* extract contains 18 components derived from the shikimate biosynthetic pathway and its combination with shikimate-acetate, as well as 11 components from the acetate-malonate pathway. In contrast, the essential oil of *E. foetidum* comprises 69 compounds, including nine primary components. Furthermore, both *in silico* and *in vitro* tests on compounds from *E. foetidum* extracts and essential oils demonstrate effective larvicidal activity against *Culex quinquefasciatus*.

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Author Contributions

SAW, LLK, and DIA contributed to the design of the study. Data processing and collection is done by SAW, while writing is done by SAW and LLK. Guidance and direction are carried out by LLK and DIA, and all authors contribute to the interpretation of the data and the final approval of the manuscript.

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