EFFECTIVITY OF "KEMANGI GUNUNG" (*Elsholtzia pubescens Benth*) HYDROSOL SOLUTION ON THE MORTALITY AND MORPHOLOGICAL DAMAGE OF *Aedes Aegypti* LARVAE

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ABSTRACT
Dengue Hemorrhagic Fever (DHF) remains a major public health concern in Indonesia, with increasing cases in recent years. Using synthetic insecticides in DHF vector control has caused negative impacts, prompting the search for safer alternatives. This study aimed to evaluate the effectiveness of "Kemangi Gunung" leaves (Elsholtzia pubescens Benth) on mortality and morphological damage of Aedes aegypti larvae. The research employed a True Experimental Design with treatment groups exposed to "Kemangi Gunung" hydrosol (concentrations of 3.125%, 6.25%, 12.5%, 25%, and 50%) and control groups. Results showed that dried mountain basil leaf hydrosol was effective at 25% concentration with a 48-hour exposure time, killing 50% of test larvae, while fresh leaf hydrosol was effective at 50% concentration with 51% larval mortality. One Way ANOVA statistical test showed significant differences between concentrations (p<0.05). Morphological observations revealed damage to the abdominal region of larvae exposed to hydrosol. This research demonstrates the potential of "Kemangi Gunung" leaves as a natural larvicide for DHF vector control.
***Keywords**: Aedes Aegypti; Elsholtzia pubescens Benth; Hydrosol Solution; larvicide; Morphology*

ABSTRAK
Demam Berdarah Dengue (DBD) masih menjadi masalah kesehatan utama di Indonesia dengan peningkatan kasus dalam beberapa tahun terakhir. Penggunaan insektisida sintetik dalam pengendalian vektor DBD telah menimbulkan dampak negatif, mendorong pencarian alternatif yang lebih aman. Penelitian ini bertujuan mengevaluasi efektivitas daun kemangi gunung (Elsholtzia pubescens Benth) terhadap kematian dan kerusakan morfologi larva Aedes aegypti. Penelitian menggunakan True Experimental Design dengan kelompok perlakuan yang dipaparkan hidrosol daun kemangi gunung (konsentrasi 3,125%, 6,25%, 12,5%, 25%, dan 50%) dan kelompok kontrol. Hasil penelitian menunjukkan hidrosol daun kemangi gunung kering efektif pada konsentrasi 25% dengan waktu pemaparan 48 jam, mampu membunuh 50% larva uji, sedangkan hidrosol daun segar efektif pada konsentrasi 50% dengan kematian larva 51%. Uji statistik One Way ANOVA menunjukkan perbedaan signifikan antar konsentrasi (p<0,05). Pengamatan morfologi menunjukkan kerusakan pada bagian perut larva yang dipaparkan hidrosol. Penelitian ini membuktikan potensi daun kemangi gunung sebagai larvasida alami untuk pengendalian vektor DBD.
***Keywords**: Aedes Aegypti; Hidrosol; Kemangi Gunung; Larvasida; Morfologi*

INTRODUCTION

Dengue Hemorrhagic Fever (DHF) remains one of Indonesia's primary public health concerns. According to data from the Indonesian, the number of DHF cases in Indonesia has increased in recent years, with an incidence rate reaching 71.78 per 100,000 population(Kemenkes RI, 2022). This disease is transmitted through the bite of Aedes aegypti mosquitoes infected with the dengue virus (Ferreira-de-Lima & Lima-Camara, 2018). Vector control for DHF has primarily relied on the use of synthetic insecticides. However, the continuous use of chemical insecticides causes various negative effects such as vector resistance, environmental pollution, and harmful effects on human health (Ritchie et al., 2021). This condition has triggered efforts to search for safer and more environmentally friendly vector control methods, one of which is through the utilization of botanical insecticides (Handayani, 2021). Using natural plant materials as botanical insecticides has become a research focus in recent years. One promising plant in this role is Elsholtzia pubescens Benth, which belongs to the Family Lamiaceae and is known to have various benefits in traditional medicine.

Elsholtzia pubescens Benth is one of the plants with potential as a botanical insecticide. This plant has various special characteristics, including its distinctive fragrance, 0.5 - 1.2 m leaves, oval shape with sharp ends, and many dotted glands (Chen et al., 2022). This plant contains bioactive compounds such as flavonoids, tannins, saponins, and essential oils known to have larvicidal activity. Previous research has shown that extracts from several Elsholtzia species have inhibitory activity against various insect pests (Liang et al., 2020).

The secondary metabolite compounds in *Elsholtzia pubescens Benth* leaves are suspected to affect the growth and development process of Aedes aegypti larvae. Flavonoids work as respiratory inhibitors and can damage the larval nervous system. Saponins can reduce the surface tension of the larval digestive tractus mucosa, making the digestive tractus wall corrosive. Meanwhile, tannins can interfere with the digestive process by binding proteins needed for larval growth (Kumara, 2021).

Several studies have examined the potential of various Elsholtzia species as botanical insecticides, but specific studies on the effectiveness of Elsholtzia pubescens Benth leaves against mortality and morphological damage of Ae. aegypti larvae are still limited. Therefore, this research is important to evaluate the potential of *Elsholtzia pubescens Benth* leaves as a safe and environmentally friendly alternative for DHF vector control.

METHOD

This research employs a True Experimental Design with research objects divided into two groups. The first group serves as the treatment group exposed to *Elsholtzia pubescens Benth* with varying doses, and the second group serves as the negative control group not exposed to *Elsholtzia pubescens Benth*.

The population used originates from mosquito larvae in the Bogor area, which will be bred into adult mosquitoes. The sample used is *Aedes sp* from the larval stage, using *Elsholtzia pubescens Benth* with five dilution variations: 3.125%, 6.25%, 12.5%, 25%, and 50%. The *Elsholtzia pubescens Benth* used originates from Mount Penanggungan, Kedungundi village, Mojokerto regency.

A total of 100 grams of *Elsholtzia pubescens Benth* leaf samples are finely chopped and placed in a steam distillation apparatus set with the lower flask containing 700 mL of distilled water. Steam distillation is conducted for 5 hours at 105°C. The obtained essential oil is separated from the hydrosol and dried with sodium sulfate.

The data to be used in this research is primary data obtained by directly observing the mortality rate and morphological damage of *Aedes sp.* after exposure to *Elsholtzia pubescens Benth* with various dose variations. The data will be tested using statistical analysis, specifically the *One Way ANOVA* test. This test is conducted to observe differences in samples using the same population with different treatments.

The treatment for the test group involves transferring 20 cultured larvae into *beaker glasses* containing distilled water for negative control and temephos for positive control, then observing larval mortality for 2x24 hours, placing the dead larvae on glass slides, and examining the morphology under a microscope.

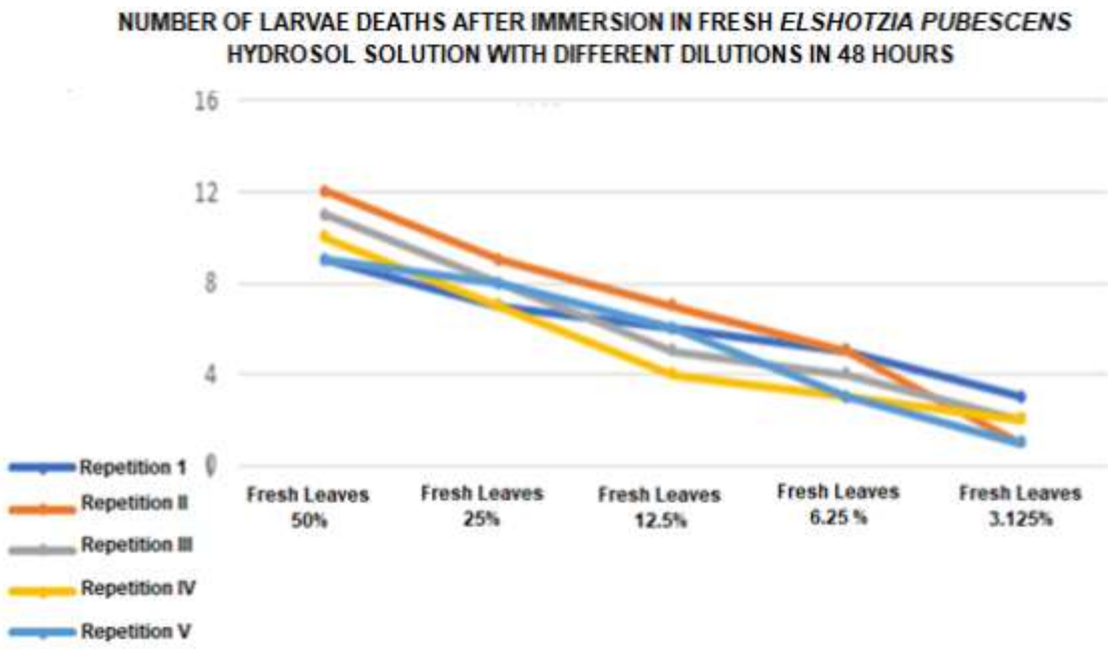
RESULTS AND DISCUSSION

1. Deaths of Larvae Sample

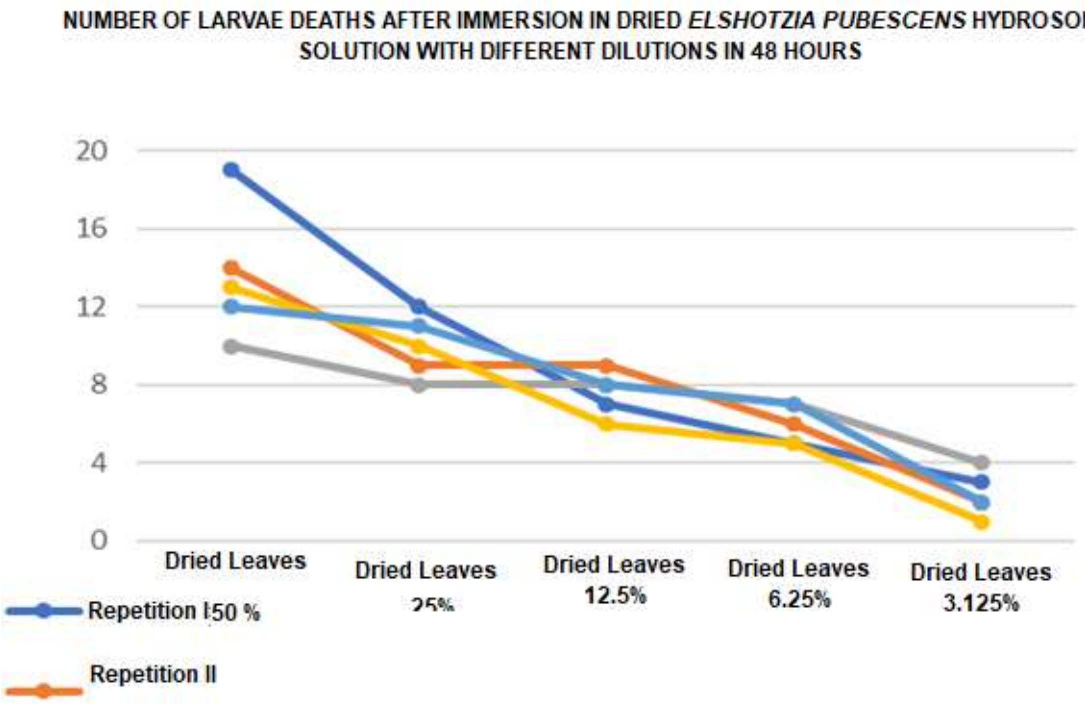
Table 1. Mean and Percentage of the deaths of 20 Aedes sp. Larvae with Five Different Dilution Solution of *Elsholtzia pubescens Benth* Hydrosol within 2 x 24 hour Observation

Observation Time		Aedes sp. Larvae Deaths			
		24 Hours		48 Hours	
Type of Leaves	Dilution	Mean	%	Mean	%
Positive Control	-	20	100%	20	100%
Negative Control	-	0	0%	0	0%
Dried Leaves	3.125%	1	5%	2	12%
	6.25%	1	6%	6	30%
	12.5%	5	25%	8	38%
	25%	6	31%	10	50%
	50%	10	50%	14	68%
Fresh Leaves	3.125%	1	4%	2	9%
	6.25%	1	6%	4	20%
	12.5%	3	15%	6	28%
	25%	4	22%	8	39%
	50%	8	41%	10	51%

Based on the data above, it shows that *Elsholtzia pubescens Benth* dried leaves at 25% dilution achieved 50% effectiveness at 48 hours, and fresh *Elsholtzia pubescens Benth* leaves at 50% dilution achieved 51% effectiveness at 48 hours. Therefore, it can be concluded that *Elsholtzia pubescens Benth* at 50% dilution is effective in killing *Aedes sp.* larvae with a percentage of 68% for dried leaves while fresh leaves achieved 51%.



Picture 1 Graph of Number of Larvae Deaths After Immersion in Fresh *Elshotzia pubescens* Hydrosol Solution with Different Dilutions in 48 Hours



Picture 2. Graph of Number of Larvae Deaths After Immersion in Dried *Elshotzia pubescens* Hydrosol Solution with Different Dilutions in 48 Hours

The graph above shows that the decrease in test larval mortality occurs at 3.125% dilution for both dried and fresh *Elsholtzia pubescens Benth* hydrosol solution. Based on these data, it can be concluded that higher dilution rates result in more effective hydrosol solution in killing *Aedes sp.* larvae. In the larval stage, using various dilutions of fresh and dried *Elsholtzia pubescens Benth* hydrosol at 3.125%, 6.25%, 12.5%, 25%, and 50% with 5 repetitions for 2 x 24 hours, different numbers of dead Aedes sp. larvae were obtained from each dilution and repetition.

"Kemangi Gunung" (*Elsholtzia pubescens Benth)* is an herbal plant used as a medicinal plant due to its active compound content, including eugenol, ursolic acid, carvacrol, linalool, methyl chavicol, and sitosterol, as well as saponins, flavonoids, triterpenoids, and tannins. The compounds found in basil leaves can be utilized by processing them into essential oils. Essential oil is a liquid obtained using aromatic plant parts through extraction processes. This material serves as a source of components in various fields such as pharmaceuticals, cosmetics, and food (Sari et al., 2020). Hydrosol liquid is the residual liquid from the extraction process. Essential oils are mostly composed of terpenoids (often referred to as terpenes, the most important natural components). Essential oils can be extracted through several methods: hydrodistillation, solvent-free microwave extraction, steam distillation, microwave hydrodiffusion and gravity, and enzyme-assisted hydrodistillation. These various methods will affect the properties and quality of essential oils (Jugreet et al., 2020)

The control solutions used to test larvae are clean water from wells as negative control and temephos solution as positive control. Clean water from the ground contains substances including iron (Fe) and Manganese (Mn) levels (Usman et al., 2021). Larvae need water to live and move. "Abate" solution is an organic phosphate compound containing phosphorotiate groups; "Abate" has anticholinesterase properties that can inhibit the action of cholinesterase enzymes in vertebrates and invertebrates, disrupting neural activity due to acetylcholine accumulation at nerve endings which can result in death (Eldefrawi & Eldefrawi, 2020).

2. Statistical Analysis of Larvae Sample
    a. Normality Test

Table 2. Table of Normality Test of Larvae Sample

	Type of Leaves and Concentration	*Statistic*	*df*	*Sig*
Larvae Deaths	Dried 3.125%	.961	5	.814
	Dried 6.25%	.987	5	.119
	Dried 12.5%	.961	5	.814

Dried 25%	.987	5	.967
Dried 50%	.925	5	.560
Fresh 3.125%	.881	5	.314
Fresh 6.25%	.821	5	.119
Fresh 12.5%	.961	5	.814
Fresh 25%	.881	5	.314
Fresh 50%	.902	5	.421

The normality test table using the Shapiro Wilk test obtained significant results > alpha (sig > 0.050), based on these results it can be said that the residual data has normal distribution.

b. Homegenity and One Way Anova Test

Table 3. Homogenity and One Way Anova Test

	Levene Statistic				
Homogenity Test	Number of Larvae Sample Deaths				
	Levene Statistic	df2	df2	Sig.	
	1.628	9	40	0.140	
	Anova				
		Number of Larvae Sample Deaths			
		Sum of squares	df	Mean Square	F	Sig.
Difference test	Between Groups	619,300	9	68,811	30,180	0,000
	Within Groups	91,200	40	2,280		
	Total	710,500	49			

The homogeneity test value using the Levene test above has a significant value of 0.140, which means > alpha (0.140 > 0.050), indicating that the variance of the treatment group data is the same. The ANOVA test value using one-way ANOVA test shows that the resulting probability value is 0.000, which means < 0.050, therefore the average number of test larval deaths at dilutions of 3.125%, 6.25%, 12.5%, 25%, and 50% from both dried and fresh leaves are not the same or there are differences.

Data in table 2 and 3 shows that higher concentrations of hydrosol solution used are more effective in killing larvae. The negative control using clean water resulted in 0% dead larvae. This indicates that the water used contains no substances that can kill the larvae. However, solutions mixed with *Elsholtzia pubescens Benth* hydrosol from both fresh and dried leaves caused larval mortality. Research conducted showed that the use of temephos as a larvicide was able to kill all Aedes sp. larval samples at hour 4 or minute 240 (Hanafiah, 2019). It can be concluded that temephos use is effective against mosquito larval mortality.

3. Morphology of Larvae Sample

Larva with 50% Dried *Elshotzia pubescens* Hydrosol

Larva with 50% Fresh *Elshotzia pubescens* Hydrosol

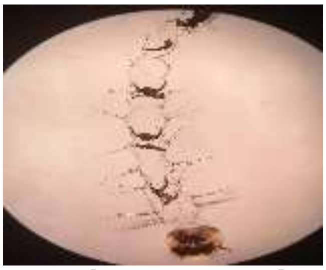
Larva with 25% Dried *Elshotzia pubescens* Hydrosol

Larva with 25% Fresh *Elshotzia pubescens* Hydrosol

Larva with 12.5% Dried *Elshotzia pubescens* Hydrosol

Larva with 12.5% Fresh *Elshotzia pubescens* Hydrosol

Larva with 6.25% Dried *Elshotzia pubescens* Hydrosol



Larva with 6.25% Fresh *Elshotzia pubescens* Hydrosol

Larva with 3.125% Dried *Elshotzia pubescens* Hydrosol

Larva with 3.125% Fresh *Elshotzia pubescnes* Hydrosol

Negative Control Larva

Positive Control Larva

The figure above shows the larval changes after being immersed in *Elsholtzia pubescens Benth* hydrosol solution from both fresh and dried leaves for 48 hours with various concentrations; these images show morphological damage has occurred in the larval abdomen.

Before treatment, Aedes sp. larvae were seen actively moving and swimming in water. In the positive control study using temephos solution, dead larvae sank to the bottom of the container, indicating no weight reduction in larvae. This is due to the specific gravity of the larvae being greater than the specific gravity of water. In larvae immersed in *Elsholtzia pubescens Benth* hydrosol, dead larvae float on the water surface. Macroscopically, the abdomen appears thinner and smaller. When observed under a microscope, larvae that died due to “Abate” showed no significant changes in body wall or parts. However, for larvae immersed in hydrosol solution, damage was visible in the abdomen and the abdominal wall appeared thinner.

During the research, after several minutes of larvae being immersed in hydrosol solution, living larvae viewed under the microscope showed slower movement compared to living larvae in the negative control. Larval death began with increasingly slower larval movement, which that larvae move at a speed of ≥ 1 mm s^{-1} (Lutz et al., 2020).

CONCLUSION (12pt)

There are differences in *Elsholtzia pubescens Benth* leaf doses in killing and damaging the morphology of *Aedes sp.* larval stages. At the larval stage, dried *Elsholtzia pubescens Benth* leaf hydrosol solution is effective at 25% dilution with 48 hours immersion time, capable of killing 50% of test larvae. Meanwhile, fresh *Elsholtzia pubescens Benth* leaf hydrosol is effective at 50% dilution with 48 hours immersion time, capable of killing 51% of larvae.

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