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Toxic Test of Lavender Leaf *(Lavandula angustifolia)* Ethanol Extract as Biolarvicide for Aedes aegypti Mosquitoes Vectors of Dengue Hemorrhagic Fever

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Abstract**—** *Toxic test of ethanolic extract of lavender (Lavandula angustifolia) leaf on mortality of Aedes aegypti mosquito larvae as vector of dengue hemorrhagic fever has been completed. The aim of the study was to determine the effective concentration of lavender (Lavandula angustifolia) leaf ethanol extract against the mortality of Aedes aegypti and LC_{50} mosquitoes for 24 hours. This study used a completely randomized design with 5 extract treatments, namely: 10ppm; 50ppm; 100ppm; 500ppm and 1000ppm and 1 control group with 3 replications. The results of the study were analyzed using one-way analysis of variance. and continued with the BNT test at a significance level of 0.05. The results showed that the ethanolic extract of lavender (Lavandula angustifolia) leaves was toxic to the larvae of the Aeders aegypti mosquito, which was indicated by the increasing number of larval mortality. Based on the test results, the concentration of 500 ppm lavender leaf ethanol extract was able to kill 100% of mosquitoes, and the effective concentration to kill 50% of test mosquito larvae was 87.0285 ppm. The ethanol extract of lavender (Lavandula angustifolia) leaves has the potential to be developed as a biolarvicide for the Aedes aegypti mosquito vector of dengue hemorrhagic fever.*

Keywords**— Lavandula angustifolia leaves, biolarvicides, Aedes aegypti.**

I. INTRODUCTION

North Sulawesi in January 2015 was categorized as an Extraordinary Event (KLB), the five-year cycle of the Dengue Hemorrhagic Fever (DHF) outbreak that hit eight districts/cities in North Sulawesi, killing eight people who were positive for the virus transmitted by the Aides aegypti mosquito.

Until now, no vaccine has been found to kill the virus that causes dengue fever. One way to prevent the spread of dengue hemorrhagic fever (DHF) is to prevent dengue virus transmission, namely by controlling and eradicating vectors to cut off disease transmission. (WHO, 2005).

Fogging is one of the mechanical control methods. Unfortunately, smoking is considered less effective because it tends to repel mosquitoes from their nests, not kill them. The chemical method used is the spread of larvicides such as abate in mosquito breeding places. Indeed, the use of chemical larvicides has succeeded in controlling *Aedes aegypti* larvae, but the continuous use of chemical larvicides will actually cause resistance and various environmental problems. cause environmental pollution, poisoning, death of non-target organisms, and produce residues.

Due to the negative impact caused by chemical insecticides, it has encouraged experts to look for alternatives to vector eradication, namely by using natural insecticides that are safer, easier, cheaper, and do not have a toxic impact on humans.

Plants that have been isolated by researchers containing active compounds of vegetable insecticides in *Aedes aegypti* mosquito larvae are soursop seeds *(Annona muricata)* with LC_{50} = 117. 27 ppm (Komansilan *et al.* 2012), Hutun seeds *(Barringtonia asiatica Kurz)* with Lethal Concentration LC_{50} = 35.72 ppm (Komansilan and Suriani. 2016) and tuba root *(Derris elliptica)* with Lethal Concentration LC_{50} = 44.7526 ppm (Komansilan *et al.* 2017).

Lavender *(Lavandula angustifolia)* is one of the plants that can be used as a natural insecticide, because it is effective in controlling insects (mosquitoes). This is because lavender plants have kairomone as a chemical that causes an odor that mosquitoes don't like. Lavender plants also have active ingredients in the form of flavonoids; Rosmarinic acid, Chlorogenic acid, Caffeic acid 2-(3,4 dihydroxyphenyl) ethenyl ester (found in flowers), Flavonoids; Hypolaetin, Scutellarein, Salvigenin, Malvidin, Xanthomicrol, Delphinidine (found in leaves), and Terpenoi; Linalil acetate, Linalol, 1,8-

Cineole, Camphor, Ursolic acid, Oleanolic acid which acts as a repellent (insect repellent) by working as a contact poison and respiratory poison (Kherissat, 2009).

Based on research from Lekitoo (2009), it is known that the flowers and leaves of the lavender plant *(Lavandula angustifolia)* have no statistically different effect as a repellent against the *Aedes aegypti* mosquito.

Regarding the toxicity of lavender *(Lavandula angostifolia)* leaf extract, the results of research from Nindatu, *et al.* (2011} showed that lavender *(Lavandula angostifolia)* leaf extract was good and effective for controlling Culex sp mosquitoes, with an LC_{50} value of 0.259%

This study aims to determine the toxicity of the ethanolic extract of lavender *(Lavandula angustifolia)* leaves as a biolarvicide to the *Aedes aegypti* mosquito vector of dengue hemorrhagic fever.

II. RESEARCH METHODS

Location of Research Time

This research was conducted at the Integrated Science Laboratory, Faculty of Mathematics and Natural Sciences, Manado State University. This research was conducted from May to September 2021

Tools and Materials

The tools used are: vial, mosquito cage, blender, buchner, rotary evaporator, desiccator, digital scale, measuring cup and micro pipette. Research materials: Lavender leaves, ethanol, aquades, Aedes aegypti mosquito larvae, fish feed, filter paper .

Research design

The design used was a completely randomized design (CRD), with 6 treatments and 3 replications, namely: K1 = Control, K2 = .10ppm, K3 = .50ppm, K4 = 100ppm, K5 = 500ppm and K6 = 1000ppm

Observation

The parameter observed was the mortality percentage of *Aedes aegypti* mosquito larvae, which was calculated using the formula proposed by Kundra (1981):

M = a/b x 100%

Where: M = percentage of mosquito mortality *Ae. aegypti*

a = number of mosquitoes *Ae. Aegypti* Dead

b = number of mosquitoes *Ae. aegypti* who used.

Work procedures

1. Reproduction of *Aedes aegypti* mosquito larvae

a) *Aedes aegypti* mosquito larvae media is made by filling a plastic container with water and the inner wall is lined with filter paper. Filter paper serves as a place for female mosquitoes to attach their eggs.

b) Eggs attached to filter paper are then dried at room temperature and stored in a closed container. For hatching eggs, filter paper is dipped in a plastic tray filled with water and after 24 hours the eggs will hatch and grow into first instar larvae.

c) First instar larvae will develop into second, third (4 days) and IV instar (2 days) instar larvae. Once every 2 days, the larvae were fed 1-2 grams of fish pellets. III/IV instar larvae used in the test.

2. Extract Making

The manufacture of lavender leaf extract is as follows:

a) Lavender leaves are separated from the stems, washed, and air-dried to dry indoors and away from sunlight.

b) The dried lavender leaves are mashed using a blender.

c) The mashed leaves were extracted by maceration using technical ethanol until all the components had been extracted.

d) The ethanol extract obtained was evaporated with a vacuum rotary evaporator until all solvent evaporated.

3. Toxicity Test.

a) Provide a solution of lavender leaf extract *(Lavandula angustifolia)* in a vial with concentrations of: 0ppm, 10ppm, 50ppm, 100ppm, 500ppm and 1000ppm.

b) In each vial, 10 larvae of *Aedes aegypti* mosquitoes were inserted with 3 replications

c) The calculation of mortality was carried out after 24 hours of treatment.

d) In the control tube using plain water.

Data analysis

The toxicity of lavender leaf extract to Aedes aegypti mosquito larvae was determined based on the LC_{50} value, namely the concentration where the test larvae died by 50%. Determination of LC_{50} was carried out using probit analysis. To distinguish the toxicity between treatments of several concentrations of lavender leaf extract against *Aedes aegypti* mosquito larvae, it was analyzed using one-way ANOVA analysis at a 95% confidence level (α = 0.05), followed by the BNT test.

III. RESULTS AND DISCUSSION

Based on mortality data of Aedes mosquito larvae that were tested for 24 hours with lavender leaf extract (Lavandula angustifolia), the average mortality obtained is presented in table 1.

Table 1. Average Mortality of Aedes aegypti Mosquito Larvae Testing With Lavender Leaf Extract (Lavandula angustifolia) For 24 Hours.

Test Concentration (ppm)	Average Mortality (%)
Control	0
10	3.33
50	23.33
100	60
500	100
1000	100

According to table 1, it can be seen that the average mosquito mortality at the lowest concentration, namely the 10ppm treatment was only able to kill 3.33% of the number of mosquito larvae tested and the 50ppm concentration treatment was able to kill 23.33% of the number of mosquito larvae tested. Furthermore, in the treatment with a concentration of 100 ppm the mortality of mortality was good, reaching 60% of the number of mosquitoes tested. While at concentrations of 500ppm and 1000ppm mortality mortality of Aedes aegypti mosquito larvae has reached 100%. %. The mortality data of *Aedes aegypti* mosquito larvae can be seen in the histogram in Figure 1.

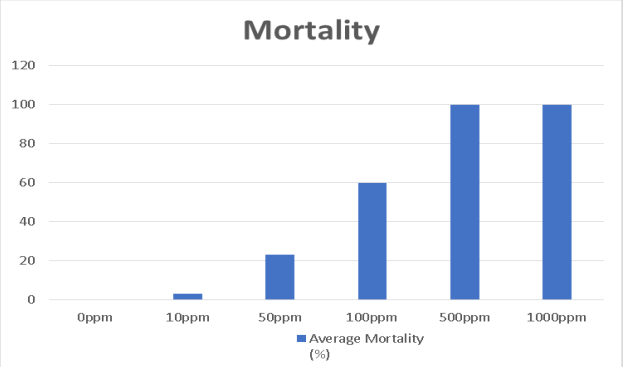

Fig.1. Histogram of Average Mortality of Larvae Ae. aegypti For 24 Hours Treatment with Lavender Leaf Extract (Lavandula angustifolia).

Furthermore, based on one-way analysis of variance of the toxicity of the ethanolic extract of lavender leaves on the mortality of *Ae. aegypti* data can be seen in table 2.

Table 2. Results of one-way analysis of variance on the toxicity of lavender leaf extract (Lavender angustifolia) on mortality of Ae. aegypti after 24 hours of treatment.

ANOVA

Death Rate

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	313.778	5	62.756	225.920	.000
Within Groups	3.333	12	.278		
Total	317.111	17			

From the results of one-way analysis of variance (ANOVA) in table 2, it shows that the calculated F value > table F (P < = 0.05). This means that the treatment given has a significant effect on the mortality of *Ae. aegypti*. Furthermore, to determine the toxicity of lavender *(Lavender angustifolia)* leaf extract which was significantly different to the mortality of *Ae. aegypti*, data analysis was continued with the Least Significant Difference (BNT) test using the SPSS for windows 15.0 program.

From the results of the BNT test shown in the table above, it shows that the control is not significantly different from a concentration of 10ppm, but significantly different from 50, 100,500 and 1000ppm. The concentration of 10ppm is not significantly different from 0ppm, but significantly different from 50, 100, 500 and 1000ppm. As for the concentrations of 50ppm 100ppm, 500ppm and 1000ppm, all of them looked significantly different both to the control and between treatments.

Based on the results of the calculation of the average mortality data and the BNT test, it can be seen that at all concentrations used, the higher the concentration of lavender leaf extract, the higher the mortality percentage of *Ae. Aegypti* as in Figure 2.

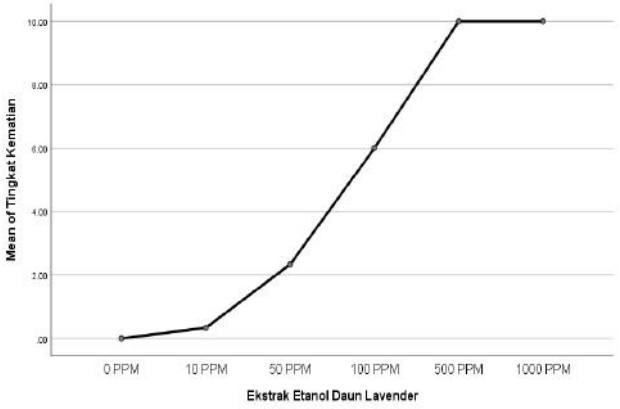

Fig.2. Graph of the relationship between the concentration of lavender leaf extract (Lavandula angustifolia) and mortality of Ae. Aegypti

Based on the results of the BNT test, probit analysis was carried out to determine the LC_{50} value of the effective concentration of lavender leaf extract *(Lavandula angustifolia).* kill 50% of *Ae. aegypti* which was tested for 24 hours, the data were analyzed using Probit Analysis (Finney Method) using Minitab 17 software.

The data used as a whole were obtained from 10 larvae of Ae. aegypti in each replication (there were 3 replications) so that 30 larvae of *Ae. aegypti* as a whole. Table 3. below presents the estimation parameters of the probit analysis model:

Table 3. Parameters of the estimated probit analysis model of clove leaf extract on Ae. aegypti larva larvae

Parameter Estimates				
		Standard	95,0 % Normal Cl	
Parameter	Estimate	Error	Lower	Upper
Location	87.0285	8.35754	70.6480	103.409
St Dev	45.3707	9.79414	29.7186	69.2666

Graphically, the probit analysis curve is presented as follows:

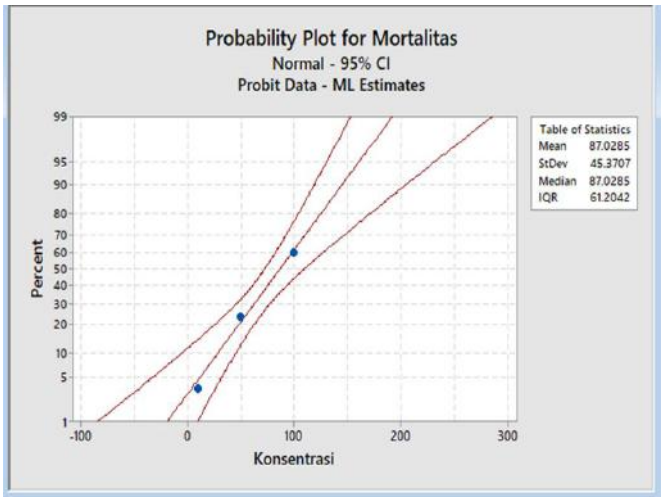

Fig.3. LC_{50} value of ethanol extract of lavender leaf samples on Ae. aegypti larvae after 24 hours of treatment.

Table 2 and Figure 3 present the LC_{50} or Mean Lethal Concentration values of the ethanol extract of lavender leaves based on the results of Probit Analysis. The test results show the value of the LC_{50} mortality concentration of *Ae. aegypti* is by giving a concentration of 245,802 ppm. Thus, the concentration figure of 245,802 ppm is the concentration of the lavender leaf ethanol extract which is the most effective for killing *Ae. aegypti* as much as 50% for 24 hours of treatment. According to the toxicity criteria based on the Australian Petroleum Energy Association (1994) the concentration of 245,802 from the ethanolic extract of lavender leaves or (LC_{50} = 245,802 ppm) at 24 hours of observation was included in the criteria for Toxic Poisoning.

IV. CONCLUSION

Conclusion

1. There is a significant difference between the mortality rate of *Ae. aegypti* at various concentrations of lavender leaf ethanol extract ranging from 0 ppm to 1000 ppm.

2. The results of the test of biolarvicide activity on the larvae of *Ae. aegypti* showed that lavender leaf ethanol extract was active as a larvicidal agent and effectively killed *Ae. aegypti* with a mortality concentration value of LC_{50} = 44.7526 ppm. included in the criteria for Toxic Poison.

Suggestion

1. It is necessary to separate and further identify the ethanolic extract of lavender leaves using chromatography and GC-MS spectrometer techniques.

2. The results of the separation, purification and identification of the compounds contained in the ethanol extract of lavender leaves were tested for their biolarvicidal activity on the larvae of *Ae. aegypti* to obtain the most toxic isolates as an ingredient in the formula for anti mosquito dengue fever.

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