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Test Toxic Tuba Root Extract as a Natural Insecticide on Larvae of *Aedes aegypti* Mosquito Vector of Dengue Fever

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Abstract: Test toxic tuba root extract as a natural insecticide on larvae of *Aedes aegypti* mosquito vector of dengue fever has been completed. The test results indicate that there are different levels of mosquito larvae mortality significantly (significant) at various levels of concentration. The test results show the concentration value Probit Analysis LC₅₀ mortality of larvae of *Ae. aegypti* with the provision of 44.7526 ppm concentration is the magnitude of the concentration of the ethanol extract of the roots of tuba most effective way to kill the larvae of *Ae. aegypti* as much as 50% during 24 hours of treatment. According to the criteria of toxicity by the Australian Petroleum Energy Association on 44.7526 ppm concentration of the ethanol extract of the roots of tuba or (LC₅₀ = 44.7526 ppm) at 24 hours of observation included in the criteria for Toxic Toxicity.

Keywords: .Extraction, tuba root, a natural insecticide, *Aedes aegypti*.

Introduction

Until now the murderers not found a vaccine virus that causes dengue fever. One way to prevent the spread of dengue hemorrhagic fever (DHF) is done with the prevention of transmission of dengue virus, by controlling and eradicating the disease transmitting vectors to decide¹. Fumigation (fogging) is one method of controlling mechanically. The target of the fumigation is to kill adult mosquitoes. Unfortunately, fogging is considered less effective because it tends to repel mosquitoes from the nest, not kill mosquitoes.

North Sulawesi on January 27, 2015 is classified as Extraordinary Events (KLB), cycle of five annual outbreaks of Dengue Hemorrhagic Fever (DHF) that afflicts eight regencies / municipalities in North Sulawesi, has killed eight residents were positively infected by a virus transmitted by mosquitoes *Aedes aegypti*. Until now the murderers not found a vaccine virus that causes dengue fever. One way to prevent the spread of dengue hemorrhagic fever (DHF) is done with the prevention of transmission of dengue virus, by controlling and eradicating the disease transmitting vectors to decide¹.

The use of chemical larvicides indeed managed to control the larvae of *Aedes aegypti*, but the use of chemical larvicide continuously it will cause resistance and environmental issues, in addition to the use of DDT can cause health problems and environmental problems^{2,3}. The use of abate any companies already since 1976 or been used for more than 30 years, so that the repeated use of insecticide can increase of the risk of residual pesticide contamination in the water, especially drinking water⁴. According to Perez B, D. and Rubio, S in

Subhash Chandra *et al.*⁵.Pesticides have potentially adverse effects on vegetables, fruits, animal resources and human health.Uncontrolled use of pesticides will lead to various health problems and environmental pollution (Yusnani, inJamaludin,*et al.*)⁶.

Alternative businesses more effective in controlling Populations and the spread of mosquitoes as vectors of disease that is easily available and environmentally *Aedes aegypti* friendly, indispensable. Vegetable insecticide leaves no residue in the air, water and soil and have a higher level of security when compared with poison-toxic inorganic.

Indonesian state has various plants that produce active compounds as insecticidal, repellent andAntifeedant that are readily biodegradable and leaves no residue⁷.Syahputra, et.al.⁸ reported from various districts in Indonesia, there are more than 40 species of plants that could potentially be used as a botanical insecticide. Plants that have been isolated by the researchers contains active compounds are plant-based insecticide seed soursop (*Annonam uricata*) with 27 ppm LC₅₀ = 117.ppm⁹, and seed extract hutun (*Baringtonia asiatica* Kurz) with LC₅₀ = 35.72 ppm¹⁰.

One of the plants that can act as a biopesticide in mosquito control is the root of the plant tuba (*Derris elliptica*). Tuba plant contains a substance called rotenone. The content of rotenone on the plant tuba very useful, these compounds are widely used in agriculture as a biopesticide that is safe for use by farmers and can also be used as larvicides moth (*Plutella xylostella* Linn.)¹¹.

Tuba root extract contains a substance called rotenon / tubotoxin, but it also contained other substances such as deguelin, elliptone, sumatrol and toxicarol. However, the substance most commonly found in the roots of poison and has been used extensively in human life is rotenon¹¹. Tuba root can be used to eradicate the larva of *Aedes* sp.¹² and the content of flavonoids in tuba root extract can kill larvae of *Aedes aegypti*¹³. In addition extract tuba root has been used to kill larvae in the fly¹⁴.

Based on the above reasons, need to be developed biological control techniques population using bioactive compounds from plant roots tuba as natural insecticide which is expected to be more effective, efficient, and environmentally friendly. More intensive research is expected to be at the root tuba fractionation and extraction in isolated and identified the active compounds are the most toxic to kill mosquito larvae *Ae. Aegypti* vector of dengue hemorrhagic fever.

Research Methods

Model Research

This research is a research experiment with the design of experiments (experimental design) with the type of design is completely randomized design (Completely Randomize Design) or equivalent with Analysis of Variance (ANOVA)

Probit Analysis.

To determine the lowest concentration of the active compound showed that administration of extracts and fractions from plant roots begin to affect mortality tuba (% mortality) and the most effective concentration to kill larvae LC₅₀*Ae. Aegypti* and the highest concentration in the mortality impact (% mortality), testing the mortality pattern recognition at various levels of concentration in the range of 10 ppm to 1000 ppm, with probit analysis. This analysis was conducted to determine the extent of the pattern (shape) of the mosquito larvae mortality.

Result and Discussion

Sample Extraction

The method used in extracting the roots of the plant tuba is maceration using technical ethanol. Selection of technical ethanol as a solvent for ethanol is an organic solvent that can dissolve almost all of secondary metabolites. Samples were soaked with technical ethanol solvent for 24 hours. Methods for

extracting organic compounds commonly used natural ingredients are macerated. Maceration extraction methods is the soaking process the sample using organic solvents used at room temperature. Selection of solvents for maceration process will provide high effectiveness by observing the solubility of compounds of natural materials such solvents¹⁵.

Plant roots tubal wind dried and blender in the form of 250 g of powder was extracted by maceration for 1 X 24 hours using technical \pm 8 L of ethanol until all components extracted exhausted. The ethanol extract obtained Vacuum evaporated with a rotary evaporator until thick. Results maceration of 250 g of dry powder 22g tuba root extract obtained dark brown viscous ethanol. The ethanol extract thick were then tested their biological activities against larvae of *Ae. aegypti*, dengue fever vector.

Toxicity Test Natural Insecticide Ethanol Extract Plant Roots Tuba

Research lethal toxicity tests / acute ethanol extract of the roots of plants tuba as natural insecticide *Ae. Aegypti* conducted at the Laboratory of Chemistry Manado State University (UNIMA) in Tondano, for two months. During the study room temperatures ranging from 20 – 28 °C and for the water temperature is 20 to 25 °C and the pH of the water ranges in the 7.0 to 7.1. Based on the conditions of the environmental factors, the possible test larvae can live and grow well, because the larvae of *Ae. Aegypti* can live at temperatures 8 – 37 °C or the condition of the room that is warm and moist¹⁶. The larvae of *Ae. aegypti* can live in water with a pH between 5.8 to 8.6¹⁷, so it can be said that environmental factors do not influence during the study. This is seen in the observations in the control treatment (without giving natural insecticide / ethanol extract of the roots of plants tuba) which shows the percentage of average mortality of 0%.

1. Comparison Test Mosquito Larvae Mortality *Ae. Aegypti* on Giving Ethanol Extract.

Data presents the number of deaths and the mortality rate of larvae of *Ae. aegypti* at five concentration levels of 1000 ppm, 500 ppm, 100 ppm, 50 ppm, 10 ppm, and 0 ppm (control). The data used is the data rate of death (mortality) in the form of a percentage score from 0% to 100%. Score 0% stated that of the 10 mosquito larvae, no one died, while the figure of 10 100% said the overall mosquito larvae died.

The first step before the analysis is to conduct a description of the study variables (descriptive statistics), which includes the presentation of the average value and variation (standard deviation) of each concentration of the ethanol extract of the roots of plants tuba.

Table 3.1. Description Average Value and Variation Each Concentration Giving Ethanol Extracts

Concentration	The average	Variation
0 ppm (Control)	0.00	0.00
10 ppm	0.00	0.00
50 ppm	80.00	10.00
100 ppm	100.00	0.00
500 ppm	100.00	0.00
1000 ppm	100.00	0.00

Source: Primary Data Processed, 2016

Graphically the data contained in table 3.1 above can be presented as follows:

In Figure 3.1, high bar charts stated on average each concentration, whereas the line that runs vertically in the average value of each concentration expressed deployment or data variation (standard deviation). From the table and the picture above appears there are differences in the mortality rate of larvae of *Ae. Aegypti* in different levels of concentration of 0 ppm (control), 10 ppm to 1000 ppm. To determine whether there are differences in the mortality rate of larvae of *Ae. Aegypti* real (significant) at the concentrations tested five One Way ANOVA or the equivalent of completely randomized design (Completely Randomize Design).

Further testing One-way ANOVA. Treat (treatment) or the concentration of the ethanol extract of the root tubawas significant (significant difference) if the value of F count> F table or the Sig F (P-value) of <0.05 (5% error rate).

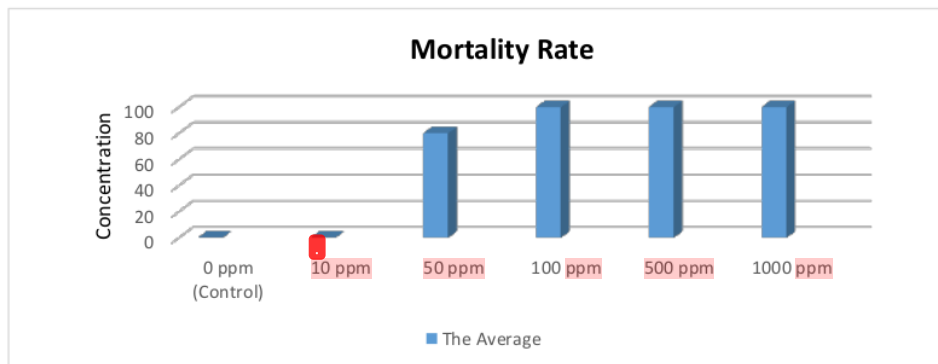


Figure 3.1. Description Average Value and Variation Each Concentration Giving Ethanol Extracts

Table 3.2. Results One-Way ANOVA Data Extract Ethanol Concentration Giving Root Tuba

Mortality rate					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	225.600	4	56.400	282.000	,000
Within Groups	2,000	10	,200		
Total	227.600	14			

The test results in Table 3.2 shows that the value of F amounted to 225 600, and Sig F amounted to 282,000. Statistics from the table-F with F table gained 3.1. Because of the value of F count > F table and Sig F > 0.05 indicates that there are differences in the level of real mosquito larvae mortality (significant) at various levels of concentration. To determine the concentration which gives the highest mortality rates used further test (post hoc test) of the Tukey test (or least significant difference test / honestly significance difference). If concentrations are given the same notation (a subset of the same), indicating there are similarities between concentration, otherwise if the concentration were given a different notation (a different subset), indicating there is a difference between concentration. The following test results presented in full:

Table 3.3. Using the Advanced Test Tukey Test (BNT) Provision Concentration Ethanol Extract Data.

Concentration	The average	Notation
0 ppm (Control)	0.00	a
10 ppm	0.00	a
50 ppm	80.00	b
100 ppm	100.00	c
500 ppm	100.00	c
1000 ppm	100.00	c

Description: The same notation shows the difference was not significant, while a different notation indicates a significant difference.

In Table 3.3 above shows that by administering a concentration of 0 ppm (control, or without ethanol extract), will give a mortality rate of larvae of *Ae. aegypti* low in the amount of 0.00% or there will be no larvae of *Ae. aegypti* dead. With the increase in the concentration of ethanol extract to 10 ppm, will give a mortality rate of larvae of *Ae. aegypti* same (same notation) when compared to the concentration of 0 ppm, ie by 0.00%. That is, the concentration of the ethanol extract giving 10 ppm, did not give a death rate of larvae of *Ae. aegypti* are better than without the ethanol extract (0 ppm). On the other hand, with the increase of ethanol extract concentration to 50 ppm, will provide mosquito larvae mortality rates are better (different notation) when

compared to the concentration of 0 ppm and 10 ppm, which amounted to 80.00%. While the increase in the concentration to 100 ppm in the ethanol extract, will give a mortality rate of larvae of *Ae. aegypti* better (different notation) when compared to a concentration of 50 ppm, with the mortality rate 100.00%. By administering a higher concentration, namely 500 and 1000 ppm of ethanol extract, does not provide the level of mortality of larvae of *Ae. aegypti* higher and better (same notation) when compared with a concentration of 100 ppm, which reached 100.00% mortality rate. So it concluded that the administration of 500 and 1000 ppm concentration would not give the death rate of larvae of *Ae. aegypti* is higher than the concentration of 100 ppm.

2. Test the most effective concentration to kill larvae of *Aedes aegypti* Linn.

To find out at what concentration of ethanol extract of the roots of plants tuba most effectively kill mosquito larvae *Ae. aegypti* required more in-depth analysis tools that Probit analysis (Finney Method) by using the software Minitab 17. By Frank C. Lu¹⁸, to determine LC₅₀ in an acute toxicity test, required three doses in the range of research so that the range of doses will achieve the LC₅₀ can be estimated precisely. Data used in the testing of probit analysis are data on the number of deaths and the mortality rate of larvae of *Ae. Aegypti* at four levels of ethanol extract concentration of 1000 ppm, 500 ppm, 100 ppm, 50 ppm and 10 ppm. The data used is data on the number of death (mortality) which is a number from 0 to 10. Number 0 states of the 10 mosquito larvae, no one died, while the 10 states of the 10 overall mosquito larvae died.

The data used as a whole is obtained from 10 larvae of *Ae. aegypti* at each repetition (there were 3 replications) in order to obtain 30 larvae of *Ae. aegypti* as a whole. 3.4 The following table is presented parameter estimation models probit analysis:

Table 3.4. Parameter estimation of probit analysis model roots of tuba ethanol extract of larval *Ae.aegypti*

Parameter Estimates				
Parameter	Estimate	Standard Error	95,0 % Lower	Normal CI Upper
Location	44.7526	236.509	-418.797	508.303
StDev	6.2348	281.012	0.0000	*

In the graph, the curve of probit analysis are presented as follows:

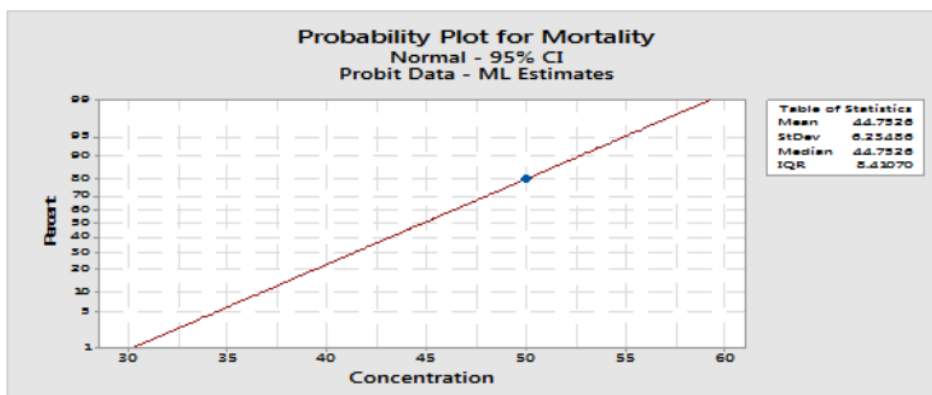


Figure 3.2. LC₅₀ value of the ethanol extract of the roots of plants sample tube at *Ae.aegypti* larvae after 24 hours of treatment.

Table 3.5. LC₅₀ value or Mean Lethal concentration based on the Probit Analysis Ethanol extracts.

Characteristics of Distribution	Estimate	Standard Error	95.0% Normal CI Lower	Upper
	Mean Lethal Concentration	44.7526	236.509	-418.797

Table 3.5 presents the LC₅₀ value or Mean Lethal concentration of the ethanol extract of the roots of plants tuba based on Probit Analysis. The test results showed the concentration LC₅₀ mortality of larvae of *Ae. aegypti* is the provision of 44.7526 ppm concentration. Thus, the rate of 44.7526 ppm concentration is a massive concentration of ethanol extract of the roots of plants tuba most effective way to kill the larvae of *Ae. aegypti* as much as 50% during 24 hours of treatment. According to the criteria of toxicity by the Australian Petroleum Energy Association²¹ (1994) 35.572 concentrations of ethanol extract of the roots of plants tuba or (LC₅₀ = 44.7526 ppm) at 24 hours of observation included in the criteria for Toxic Toxicity.

Conclusion

1. There is a significant difference in the mortality rate of larvae of *Ae. aegypti* in various types of concentrations ranging from 0 ppm to 1000 ppm.
2. Test results natural insecticide activity on the larvae of shows the ethanol extract of the roots of plants tuba active as larvicidal agent and effectively kill mosquito larvae *Ae. aegypti* with the concentration mortality LC₅₀ = 44.7526 ppm.
3. According to the criteria of toxicity by the Australian Petroleum Energy Association (1994) 44.7526 concentration of ethanol extract of the roots of tuba or (LC₅₀ = 44.7526 ppm) at 24 hours of observation included in the criteria for Toxic Toxicity.

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