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Effectiveness of Seed Extract Hutun (*Barringtonia asiatica* KURZ), on LARVA Aedes aegypti Vector Disease Dengue Fever

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Abstract: The effectiveness of vegetable insecticide extracts of hutun (*Baringtonia asiatica* Kurz) seeds on larvae of dengue hemorrhagic fever (DHF) vector Aedes aegypti mosquito has been conducted. The test results indicate that there are differences in the real (significant) mortality level of mosquito larvae at various levels of concentration.
The test result of Probit Analysis shows the LC_{50} mortality concentration value of *Ae. aegypti* larvae with the provision of concentration of 35.572 ppm is a concentration quantity value of the methanol extract of the hutun seeds which is the most effective way to kill the larvae of *Ae. aegypti* as much as 50% during 24 hours of treatment. According to the toxicity criteria, based on *Australian Petroleum Energy Association,* the concentration of 35.572 ppm of seeds hutun methanol extract or (LC_{50} = 35.572 ppm) at 24 hours of observation is included in the criteria for Toxic Toxicity.
Keywords: Extraction, vegetable insecticides, Hutun seeds, Aedes aegypti.

Introduction

Indonesia in general has a risk of contracting dengue hemorrhagic fever (DHF) because the cause vector, i.e. Aedes aegypti mosquito, is spread in residential areas as well as in public places, except the area which lies at an altitude of over 1000 meters above sea level[1]. North Sulawesi on January 27, 2015 was classified as Extraordinary Events the five years cycle of Dengue Hemorrhagic Fever (DHF) epidemic that afflicted eight regencies/cities in North Sulawesi, had killed eight people who tested positive for the virus transmitted by Aides aegypti mosquito.

Until now, the vaccine of the virus that causes dengue hemorrhagic fever (DHF) has not been found. One way to prevent the spread of dengue hemorrhagic fever (DHF) is done with the prevention of dengue virus transmission, i.e. by controlling and eradicating the vector to cut the disease transmission[2].

Fogging is one of the mechanical control methods. The target of the fogging is to kill adult mosquitoes. Unfortunately, fogging is considered less effective because it tends to repel mosquitoes from the nest, not kill mosquitoes. The chemical method that is used is to larvicides spreading like abate in mosquito breeding sites. The method is proved to be effective in controlling populations of Aedes aegypti compared with the fogging method. Therefore, larvicides are specific to the target, i.e. the pre-mature phase (egg, larva, pupa). The

larvicides work by inhibiting the growth of mosquito larvae. The form can be a contact poison or stomach poison.

The use of chemical larvicides is indeed success in controlling the larva of Aedes aegypti, but the use of chemical larvicides continuously cause resistance and various environmental issues in addition to the use of DDT also can cause health problems and environmental problems[3,4]. The use of abate in Indonesia has been done since 1976 or has been used for more than 30 years, so the continuous use of insecticides may increase the risk of pesticide residue contamination in water, especially drinking water[5].

Syahputra *et al.*[6] reports from various regencies in Indonesia, there are more than 40 species of plants that can potentially be used as a botanical insecticide. One of the plants that have been isolated by researchers, which contain plant-based insecticide active compounds, are seeds of soursop (Annona muricata) with LC_{50} = 117. 27 ppm.[7]

Plants that have the potential to be developed as a phyto-insecticide is *Barringtonia asiatica* Kurz (Lecythidaceae) with the common name '*Pohon Racun Laut*'(Sea Poison Tree) or in Indonesia known as Bitung[8] . *B. Asiatica* is known to have active compounds which are known to cause the death of the insect pests. Methanol extract of *B. asiatica* seeds are toxic for Crocidolomia pavonana with LC_{50} values of 0.66% at 7 days after treatment. The application of B. asiatica seed extract also has affected the oviposition with the effective concentration of 0.96% that causes female C. pavonana do not lay eggs on plants. Response of larvae shows that the extract of *B. Asiatica,* besides having a poisonous character, it also has antifeedant activity[9]. One of the active compounds in the *B. Asiatica* seeds is a saponin[10].

In some places, B. asiatica is used as a medicine and poison of fish. Active compounds in the B. Asiatica seeds that poison fish is saponin compounds group[8]. One of the most toxic compounds to fish from B. asiatica seed extract is ranuncide VIII[10]. Research on seed extract of *B. Asiatica* has been carried out but their toxicity against Dengue Hemorrhagic Fever vector Aedes aegypti mosquito larva is unknown. Therefore, the use of alternative insecticides that are relatively safe for the environment and have minimal side effect or no adverse effects on non-target organisms, is necessary. An alternative method to control the Aedes aegypti mosquito larvae by phyto-insecticides from the extract of *B. Asiatica* seeds.

More intensive research is expected to be able to extract the Hutun *(B.asiatica)* seed to kill Aedes aegypti larvae that could help overcome the problem of Dengue Hemorrhagic Fever in Indonesia, especially Manado city.

2. Materials and Methods

2.1. Research methods

Tool

The tools used in this study are: knife, blender, analytical balance, beaker glass, funnel, erlenmeyer, micro pipette, rotary vacuum evaporator, desiccators, pipette, test tubes, glass, and wristwatch.

Material

The chemicals used in this study is ethanol, methanol, chloroform, butane, concentrated sulfuric acid, ethyl acetate, acetic acid, dimethylsulfoxide, Hutun *(Baringtonia asiatica)* seeds*, A. aegypty* mosquito larvae.

Work Procedures

Materials used in this study is old Hutun *(Baringtonia asiatica)* seed that have been collected from Malalayang coast in Manado. The preparations of materials are crop determination, materials collecting, cleaning, drying by blowing (not under direct sun) and milling to powder using blender.

Biolarvacide Toxicity Test of Hutun Seed Methanol Extract

Hutun *(Baringtonia asiatica)* seed powder is extracted by maceration using technical methanol until all components are extracted. The obtained methanol extract is evaporated with a vacuum rotary evaporator until thick. Thick Hutun seed extract is then tested its toxicity to the larvae *of Aedes aegypti* as its bioindicator.

The media of *A. aegypti* mosquito larvae is made by filling the container with water. The eggs of *A. aegypti* larvae are stored in a damp place until the eggs of the mosquito larvae hatch and ready for use in testing. Ten beakers are prepared for testing, where for each sample takes nine beakers and a beaker as a control.

Concentrated extract is weighed as much as 0.02 g and diluted with 2 mL of ethanol. The solution is put in pipette as much as 5; 50; 500 µL. Each is put in a small bottle and the solvent is evaporated for 24 hours.

Insert into the bottle 2 mL of water, 50 mL of dimethylsulfoxide, 10 mosquito larvae of *A. aegypti.* Then the Hutun seed extract solution is added with water until the volume is 5 mL in a concentration of 10; 100; 500: 1000 ppm. For control, into a small bottle put 2 mL of water, 50 mL of dimethylsulfoxide, 10 mosquito larvae *A. aegypti* then add water until the volume is 5 mL. The observations are made after 24 hours of the death of the mosquito larvae. Data analysis is performed to find the death concentration (LC_{50}).

2.2 Research Model

This research is an experiment research with *experimental design* and the type of design is *Completely Randomize Design* or equivalent to the Analysis of Variance (ANOVA).

2.3. Probit analysis

To determine the lowest concentration that shows that the application of the active compounds from the extracts and fractions of hutun seeds gives mortality impact (% mortality) and the most effective concentration of LC_{50} to kill larvae of *Ae. Aegepti* and the highest concentration in the death impact (% mortality), the test of mortality rate pattern recognition is conducted at various levels of concentration in the range of 10 ppm to 1000 ppm, by *probit analysis.* This analysis is conducted to determine the extent of the pattern of the mosquito larvae mortality

3. Result and Discussion

3.1. Sample Extraction

The method used in extracting the waste of hutun seed is maceration using technical methanol. The technical methanol is selected as solvent because methanol is an organic solvent that can dissolve almost all secondary metabolites. The samples are soaked with technical methanol for 24 hours. The common extracting method for organic compounds of natural ingredients is maceration. The maceration extraction method is the process of soaking the sample by using an organic solvent which is used at room temperature. The selection of solvents for maceration process will provide high effectiveness by observing the solubility of compounds of natural materials such solvents[11].

The hutun seeds are dried (by wind) and blended in the form of powder. As much as 1.0 kg is extracted by maceration for 1 X 24 hours by using ±10 L of technical methanol until all the components are extracted. The obtained methanol extract is evaporated using Vacuum *rotary evaporator* until thick. The maceration result of 1.0 Kg of hutun dry seeds powder is 130 g of thick dark brown methanol extract. The biological activity of condensed methanol extract obtained is then tested on the *Ae. Aegypti* mosquito larvae.

3.2. Biolarvacide Toxicity Test of Hutun Seed Methanol Extract

The lethal/acite toxicity test of the methanol extract of hutun seed waste as biolarvacide of *Ae. aegypti* is conducted at the Laboratory of Chemistry and Biology, Faculty of Mathematics and Natural Science,

UNIMA in Tondano, for 2 months. During the research, the room temperature ranges from 21-27^{0}C and the water temperature is 21 to 25^{0}C and the pH of the water ranges in 7.0 to 7.1. Based on the conditions of the environmental factors, it is possible to the test larvae to be able to live and grow well, because the larvae of *Ae. Aegypti* is able to live at temperatures of 8-37^{0}C or at room temperature condition that is warm and humid[12]. *Ae. aegypti* can live in water with a pH between 5.8 to 8.6 (Hidayat *et al,*)[13], so it can be said that environmental factors had no effect during the study. This is seen in the observations in the control treatment (without giving biolarvacide / methanol extract of hutun seeds) which shows the average of mortality percentage of 0%.

1. Comparison Test of the Mortality Rate of *Ae. Aegypti* Mosquito Larvae on the Application of Methanol Extracts

Data presents the number of deaths and the mortality rate of *Ae. aegypti* larvae at five concentration levels of 1000 ppm, 500 ppm, 100 ppm, 10 ppm, and 0 ppm (control). The data used are the data rate of death (mortality) in the form of percentage score from 0% to 100%. Figures 0% state that among 10 mosquito larvae, there is no one died, while the 100% states that among 10 mosquito larvae, they all have died.

The first step before the analysis is to conduct research variable description (statistical descriptive), which includes the presentation of the average value and variation (standard deviation) of each concentration of the methanol extract of hutun seeds.

Table 3.1. The Average and Variation Value Description of the Application of Each Methanol Extracts Concentration

Concentration	The average	Variation
0 ppm (Control)	0.00	0.00
10 ppm	23.33	5.77
100 ppm	93.33	5.77
500 ppm	100.00	0.00
1000 ppm	100.00	0.00

Source: Primary Data Processed

Graphically according to the data contained on Table 3.1 above, it can be presented as follows:

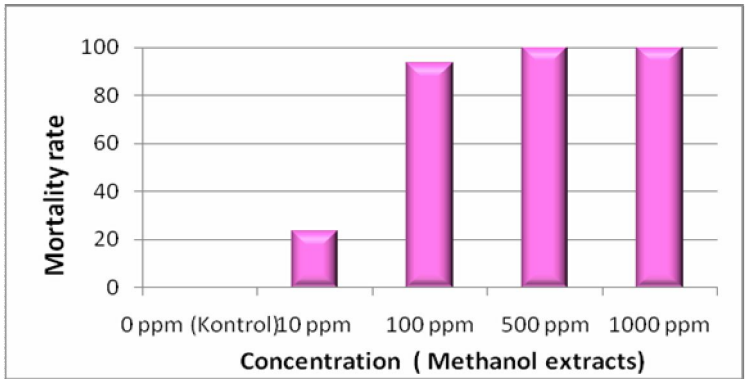

Figure 3.1. The Average and Variation Value Description of the Application of Each Methanol Extracts Concentration

In the figure 3.1, the height of bar charts states the average of each concentration, while the vertical line on the average value of each concentration states the data deployment or variation (standard deviation). From the above table and the image, it appears that there are differences in the mortality rate of *Ae. Aegypti* larvae at different levels of concentration of 0 ppm (control), 10 ppm to 1000 ppm. To determine whether there are

significant differences in the mortality rate of *Ae. aegypti* larvae at the five concentrations, the One Way ANOVA test or equivalent to *Completely Randomize Design* is conducted.

Further, the One-way ANOVA is performed. The treatment or the methanol extract concentration of the hutun seeds is stated as significantly different if the value of $F_{count} > F_{table}$ or the Sig F (P-value) < 0.05 (5% of error rate).

Table 3.2. The Results of One-Way ANOVA of The Application Concentration Data of Methanol Extracts

Mortality rate					
	Sum of Squares	**df**	**Mean Square**	**F**	**Sig.**
Between Groups	125,583	3	41,861	251,167	,000
Within Groups	1,333	8	,167		
Total	126,917	11			

The test results in Table 3.2 show that the F_{count} value is 251.167, and Sig F is 0.000. From the Statistics-F table, the F_{tables} is 3.2. Because $F_{count} > F_{table}$ and Sig F > 0.05, it indicates that there are significant differences in the mortality level of mosquito larvae at various levels of concentration. The research hypothesis which states there is a difference in the mortality rate of *Ae. aegypti* larvae in various concentrations in the application of methanol extract of the seeds hutun is accepted.

To determine the concentration which gives the highest mortality rate, the post hoc test is conducted, i.e. *of* the Tukey test. If the concentrations are given the same notation (same subset), it indicates that there are similarities between concentrations, on the contrary if the concentrations are notated different (different subset), it indicates that there is a difference between concentrations. The following test results are presented in full:

Table 3.3. Further Test Using Tukey Test: The Concentration Data of The Methanol Extracts Application

Concentration	**The average**	**Notation**
0 ppm (Control)	0.00	a
10 ppm	23.33	a
100 ppm	93.33	b
500 ppm	100.00	c
1000 ppm	100.00	c

Description: The same notation indicates that the difference is not significant, while the different notations indicate significant differences.

In Table 3.3 above, it shows that by the application of a concentration of 0 ppm (control, or no methanol extract), it will cause the lowest mortality level of *Ae. aegypti* larvae that is equal to 0.00% or there will be no dead *Ae. aegypti* larvae. With the increase in the concentration of the methanol extract to 10 ppm, it will cause the same (same notation) mortality level of *Ae. aegypti* larvae when compared to the concentration of 0 ppm, i.e. at 23.33%. That is, the provision of the methanol extract concentration of 10 ppm, do not give a better mortality rate of *Ae. aegypti* larvae than without the methanol extract (0 ppm). On the other hand, with an increase in the concentration of the methanol extract to 100 ppm, it will cause the better (different notation) mortality rate when compared to the concentration of 0 ppm and 10 ppm, which amounted to 93.33%. Meanwhile, with the increase in concentration to 500 ppm in methanol extract, it will provide better (different notation) mortality level of *Ae. aegypti* larvae when compared to the concentration of 100 ppm, which is the death rate of 100.00%. By applying a higher concentration, i.e. 1000 ppm in methanol extract, it does not cause higher and better (same notation) mortality level of *Ae. aegypti* larvae when compared to the concentration of 500 ppm, which reached 100.00% mortality rate. Thus, it is concluded that by applying a concentration of 1000

ppm, it will not cause the higher mortality level of *Ae. aegypti* larvae when compared with the concentration of 500 ppm.

2. The concentration of most effective hutun seed methanol extract to kill *Aedes aegypti* Linn mosquito larvae

In the previous analysis, it is concluded that the difference in the mortality rate of *Ae. aegypti* larvae in various types of concentrations ranging from 0 ppm to 1000 ppm. However, to determine the concentration of the methanol extract of seeds hutun which is the most effective to kills the *Ae. aegypti* larvae, it is required the more in-depth analysis tool, i.e. Probit Analysis (*Finney Method)* by using the software Minitab 14.

According to Frank C. Lu[14], to determine the LC_{50} in an acute toxicity test, it requires three dose ranges in the study so that the range of doses which will achieve the LC_{50} can be estimated with precision. The data used in the probit analysis test are data on the number of deaths and the mortality rate of *Ae. Aegypti* larvae at four levels of concentration of the methanol extract of 1000 ppm, 500 ppm, 100 ppm, and 10 ppm. The data used is the number of deaths (mortality) which is a number from 0 to 10. Number 0 states among 10 mosquitoes larvae, there is no one died, while the 10 states that among 10 mosquito larvae, all have died.

The data used are obtained from 10 *Ae. aegypti* larvae at each repetition (there are 3 repetitions) in order to obtain 30 larvae of *Ae. aegypti* as a whole. The following table 3.4 presents the parameter of probit analysis model estimation:

Table 3.4. The estimation parameter of probit analysis model of hutun seed methanol extract of Ae. aegypti larvae

Parameter Estimates				
	Standard	95,0 %	Normal CI	
Parameter	Estimate	Error	Lower	Upper
Location	35,572	7,725	20,432	50,713
Scale	35,131	6,973	23,809	51,838

Graphically, the curve of probit analysis is presented as follows:

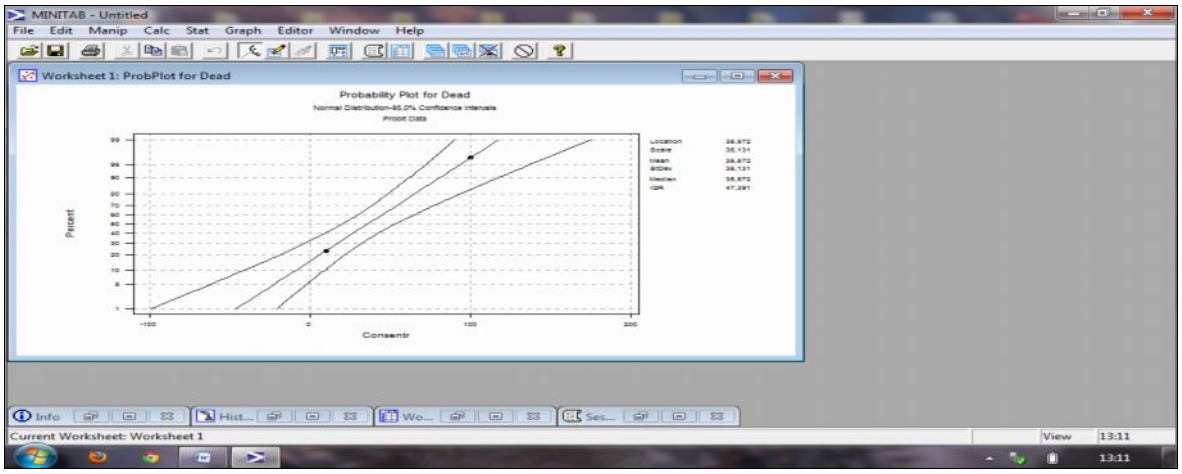

Figure 3.2. LC_{50} value of the methanol extract of hutun seed samples on *Ae.aegypti* larvae after 24 hours of treatment.

Table 3.5 presents the value of LC_{50} or *Mean Lethal Concentration* of methanol extract of seeds hutun based on the results of Probit Analysis.

Table 3.5. LC_{50} values or *Mean Lethal consentration* based on the results of Probit Analysis of methanol extract.

Characteristics of Distribution				
	Estimate	Standard Error	95.0% Normal CI Lower	Upper
Mean Lethal Consentration	35,572	7,725	20,432	50,713

The test results demonstrate the LC_{50} concentration value of *Ae. aegypti* larvae by the application of the concentration of 35.572 ppm. Thus, the concentration of 35.572 ppm is the most effective concentration quantity of the methanol extract of the hutun seeds to kill the *Ae. aegypti* larvae as much as 50% during 24 hours of treatment. According to the criteria of toxicity based on *Australian Petroleum Energy Association* (1994), the concentration of 35.572 of the hutun seeds methanol extract or (LC_{50} = 35.572 ppm) at 24 hours of observation is included in the criteria for Toxic Toxicity.

4. Conclusion

1. There is a significant difference in the mortality rate of *Ae. aegypti* larvae in various types of concentrations ranging from 0 ppm to 1000 ppm.
2. The test results of biolarvacide activity on *Ae. aegypti* larvae shows the methanol extract of the hutun seeds is active as an agent of larvicides and effectively kill larvae of *A e. aegypti* with death concentration value LC_{50} = 35.572 ppm.
3. According to the criteria of toxicity based on *Australian Petroleum Energy Association* (1994), the concentration of 35.572 of the hutun seeds methanol extract (LC_{50} = 35.572 ppm) at 24 hours of observation is included in the criteria for Toxic Toxicity.

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