Biolarvicide of Herba Ethanol Extract of *Phyllanthus niruri* L on *Aedes aegypti* Mosquito Larva Vector Of Dengue Hemorrhagic Fever Disease (DHF)

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**Abstract.** Until now, Dengue Hemorrhagic Fever (DHF) has not found a cure or vaccine, so one of the prevention is to break the chain of transmission of this disease. *Phyllanthus niruri* L is a wild plant that can live in moist and rocky places. This plant has the ability to cure hepatitis, anti-mosquito, anti-inflammatory, fever, launching urine, expectorant, launching menstruation and increasing appetite. This research aims to determine the biolarvicide and LC50 and LC90 values of the *Phyllanthus niruri* L herbal extract against *Aedes aegypti* mosquito larvae. This study uses a completely randomized design (CRD) with 5 treatments and 3 replications. Three hundred instar mosquito larvae III were put into each container containing the treatment determined P0 = without administration of extract, P1 = given abate 1% (Control +), P2 = given Ethanol Extract Herb *Phyllanthus niruri* 25% concentration, P3 = Given Ethanol Extract Herba *Phyllanthus niruri* concentration of 50% and P4 = Given Ethanol Extract Herba *Phyllanthus niruri* concentration of 75%. Each container is filled with 20 larvae. Observation of the larvicidal activity of the ethanol extract of the herb *Phyllanthus niruri* L was carried out at 0, 6, 12, 18 and 24 hours. Data from observations of *Aedes aegypti* mosquito larvae mortality were then analyzed using ANOVA. To determine the value of LC50 and LC90 extract of *Phyllanthus niruri* L, it was analyzed using probit analysis. The results showed that the calculated F value was 281.558 with a F probability of 0.000 (p <0.05), which means that the ethanol extract of the herb *Phyllanthus niruri* L affected the mortality of *Aedes aegypti* larvae. LC50 value of 2.644% with an upper limit of 1.040 and a lower limit of 3.674. While the LC90 value of 5.772% with an upper limit of 4.424 and a lower limit of 12.042.

1. Introduction

One infectious disease that is still a public health problem in Indonesia is Dengue Hemorrhagic Fever (DHF). Dengue hemorrhagic fever appears as an Extraordinary Event (KLB), causing panic in the community because of the risk of causing death and its spread very quickly [1]. World Health Organization said Dengue Fever was first reported in Southeast Asia in 1954 in the Philippines precisely in Manila, then subsequently spread to various regions [2].

Data from the Ministry of Health in 2018 recorded the number of cases of Dengue Hemorrhagic Fever there were around 11,000 cases. The number of cases is still relatively small compared to 2017 there were approximately 22,000 cases of dengue hemorrhagic fever [3]. Dengue hemorrhagic fever is caused by one of four dengue virus serotypes (DENV-1, -2, -3, and -4), which are members of the genus Flavivirus and family Flaviviridae [4].
Dengue virus can enter the human body by the Aedes aegypti and Aedes albopictus mosquitoes [5]. Aedes aegypti mosquitoes can be found in almost all provinces in Indonesia because they are very easy to adapt to the surrounding environment. Dengue Hemorrhagic Fever Disease until now has not found a drug or vaccine so that one of the prevention is to break the chain of transmission of this disease by eradicating the vector.

There is no vaccine for the prevention of dengue fever and there are no specific medicines for its cure, thus efforts to control DHF depend on eradicating the Aedes aegypti mosquito. So far the dengue eradication program has not been maximized, because it still depends on spraying insecticide (fogging) to kill adult mosquitoes. Implementation is also limited because fogging requires special operations and high costs. Prevention and eradication measures will be more sustainable if done by eradicating larval sources. In this case an integrated approach to mosquito control is needed by using all appropriate methods (environmental, biological and chemical) that are inexpensive, safe and environmentally friendly [6].

Phyllanthus niruri L grows wild in damp and rocky places, such as along waterways, bushes, and soil between grasses. This plant can grow in areas up to an altitude of 1,000 m above sea level. This herb tastes rather bitter, sweet, cool, astringent. Efficacious for hepatitis, anti-inflammatory, fever (anti-pyretic), launching urine (diuretic), expectorant, launching menstruation, explaining vision, increasing appetite [7].

Empirical experience of the community in Danar Sare Village, Kei Kecil sub-district, South East, Southeast Maluku Regency, recognizes Phyllanthus niruri L by the name Enmur. In its use as a traditional medicine, people often use it as an antimalarial drug, energy booster, fever and dengue fever. The use of herbal enmur or Phyllanthus niruri L as a drug is generally used by boiling and drinking water. Phyllanthus niruri L. is also one of the plants that contains active ingredients such as saponin, flavonoids, tannins, and alkaloids and can kill vector mosquito larvae that cause DHF[8].

Lethal Concentration (LC 50) and LC 90 tests are tests conducted to determine the effective concentration of Phyllanthus niruri L extract in killing 50% and 90% of Aedes aegypti larvae population. This research aims to determine the biolarvicide and LC 50 and LC 90 values of Phyllanthus niruri L herbal extracts against Aedes aegypti mosquito larvae.

2. Experimental

2.1 Research type
This research is a quasi-experimental study (quasi experiment) with a posttest design with a control group (post test only control group design) [9].

2.2 Research design
This research was conducted using a completely randomized design (CRD) with 5 treatments and 3 replications. The details are as follows:

- P0 : Without extract
- P1 : Abate 1% (Control +)
- P2 : Given Ethanol Extract of Herb Phyllanthus niruri 25% concentration
- P3 : Given Ethanol Extract of Herba Phyllanthus niruri 50% concentration
- P4 : Given Ethanol Extract of Herba Phyllanthus niruri 75% concentration

2.3 Tools and materials
The tools used in this study are pipettes, plastic trays, 15 plastic containers (as containers), glass beaker, analytical balance, spatulas, chemical cups, measuring tubes, cloth (as a protector so that mosquitoes that become adults do not fly out), blenders, Loops, glass stir bar, label paper and knives. The materials used in this study are: Phyllanthus niruri Herba, ethanol, clean water, Abate 1%, Aedes aegypti larvae, Fish food for larval food.

2.4 Preparation of mosquito larvae
Aedes aegypti eggs were obtained from the Animal Source Disease Control Laboratory (P2B2) Banjarnegara Health Research and Development Agency (Balitbangkes). 10 g of feed was put in a plastic container containing ± 1000cc of clean water for 6 hours. Aedes aegypti mosquito eggs are
inserted and hatched in the container. Then left for 2-3 days. Hatched larvae are fed fish food every
day. The larvae are kept until stage III, approximately for 2 days, then used for research

2.5 Test material preparation
a. Sampling of Phyllanthus niruri
Phyllanthus niruri samples were taken from Telaga Kodok Hamlet in Leihitu District, Central Maluku
Regency. Samples are taken from stems, leaves, flowers, fruit, roots or all parts of the plant.

b. Simplesia setup
Samples were separated from impurities both foreign bodies and parts of plants that have been
damaged and then washed using clean running water. Washing is done to remove soil and other
foreign matter that is in the simplicia. The Phyllanthus niruri L herb is then dried in a place protected
from direct sunlight. The sample is dried and then mashed using a blender to get powder from the
herb Phyllanthus niruri L.

2.6 The method of making Phyllanthus niruri L. extract
Dry powder weighed as much as 170 g, put into 4 L flask. Then put 95% ethanol as much as 1.8 liters
to flood the whole powder. Furthermore, the powder is soaked for 6 hours while stirring occasionally
then refluxed for 3 hours. The filtered reflux results are transferred to another tube, the pulp is
refluxed in the same way. The results of reflux were concentrated using a rotary evaporator until a
thick extract extract of 15.8 g was obtained.

2.7 Testing of larvicide activity
Three hundred instar mosquito larvae III were put into each container containing the treatment
determined P0 = without administration of extract, P1 = given abate 1% (Control +), P2 = given
Ethanol Extract Herb Phyllanthus niruri 25% concentration, P3 = Given Ethanol Extract Herba Phyllanthus niruri concentration of 50% and P4 = Given Ethanol Extract Herba Phyllanthus niruri concentration of 75%. Each container is filled with 20 larvae. Each treatment consisted of 3
replications. Observation of the larvicidal activity of the ethanol extract of the herb Phyllanthus niruri L was carried out for 24 hours with observations of 0, 6, 8, 12, 18 and 24 hours. Calculation of time
begins after the larvae are put in a container.

2.8 Data collection
The data collected is by counting the number of larvae that die in each container. Dead larvae are
counted during the observation, recorded in tabular form. Dead larvae are larvae that sink to the
bottom of the container, do not move, leaving other larvae that can move clearly and do not respond
to stimuli.

2.9 Data analysis
Observation data will be analyzed by Analysis Of Variance (ANOVA) using the SPSS program. If
there are significant differences, further tests will be carried out using the Least Significant Difference
(LSD) test at a 0.05% confidence level. To determine the value of LC 50 and LC 90 extract of
Phyllanthus niruri L, it was analyzed using probit analysis.

3. Results and Discussion

3.1 Mortality of Aedes aegypti mosquito larvae
The results of observations of the average mortality of Aedes aegypti larvae for 24 hours can be seen
in Table 1. ased on the results in Table 1 it can be seen that in negative control (without treatment)
there was no death of Aedes aegypti larvae. On positive control (abate 1%) the average mortality of
Aedes aegypti larvae was 16.00±8.28. At the concentration of the ethanol extract of the herb
Phyllanthus niruri L concentration 25%, 50% and 75%, the average mortality of Aedes aegypti
larvae was 8.87±6.57, 14.33±7.98 and 15.53±8.09.
Table 1. Average mortality of *Aedes aegypti* larvae per each treatment

| Observational Time (Hours) | Mortality of *Aedes aegypti* mosquito larvae per each treatment |
|---------------------------|---------------------------------------------------------------|
|                           | K (-)             | (+)                  | 25% concentration | 50% concentration | 75% concentration |
| 0                         | 0,00              | 0,00                 | 0,00              | 0,00              | 0,00              |
| 6                         | 0,00              | 20,00                | 4,67              | 14,33             | 18,33             |
| 12                        | 0,00              | 20,00                | 8,67              | 18,00             | 19,33             |
| 18                        | 0,00              | 20,00                | 15,67             | 19,33             | 20,00             |
| 24                        | 0,00              | 20,00                | 15,33             | 20,00             | 20,00             |
| Total ± SD                | 0,00±0,00         | 16,00±8,28           | 8,87±6,57         | 14,33±7,98        | 15,53±8,09        |

Note: Superscripts with the same letter are not significantly different (P< 0.05).

Based on the analysis of Variance (ANOVA) two lines using the SPSS 16.0 program (Appendix 2) shows that the calculated F value is 281,558 with a F probability of 0,000 (p<0.05), which means that the ethanol extract of the herb *Phyllanthus niruri* L affect the mortality of *Aedes aegypti* larvae. The Least Significant Difference Results (LSD) showed that in all treatment groups that were given were significantly different between each concentration, but between positive control and 75% concentration were not significantly different. ANOVA results also showed that the calculated F value was 245.060 with a probability value of 0.033 (p <0.005), which means that the length of time of observation also affected the average mortality of *Aedes aegypti* larvae.

The results of the effectiveness of the ethanol extract of the herbaceous *Phyllanthus niruri* L (Table 1) against *Aedes aegypti* mosquito larvae showed that in negative control there was no larvae death, whereas in positive controls (given an abate of 1%) *Aedes aegypti* larvae mortality occurred. Abate is an organic phosphate compound containing phosphorotiate groups, abate is anticholinesterase which works to inhibit the enzyme cholinesterase in both vertebrates and invertebrates, causing interference with nerve activity due to accumulation of acetylcholin at the nerve endings. This has resulted in death [10]. Penetration of abate into the larvae takes place very quickly, organic phosphate poisoning in insects is followed by discomfort, hyperexcitation, tremors and convulsions, then muscle paralysis (paralysis), in mosquito larvae the death is caused by not being able to take air to breathe [11].

Based on observations in the positive control group when after the mosquito larvae were put into a container containing abate (2 minutes after being inserted), the mosquito larvae moved quickly, and moved above the water surface to look for oxygen. In the 4th minute, the movement starts slowly and finally dies in the 5th and 6th minutes.

The mortality of *Aedes aegypti* mosquito larvae in the ethanol extract of *Phyllanthus niruri* L herbal concentrations of 25%, 50% and 75% varies each time. At a concentration of 25% larval death begins at 30 minutes after the larvae are put into a container. In the 2-hour observation of mosquito larvae that died by 2 individuals and in the 6-hour observation had increased to 4,67 individuals. The peak mortality of larvae occurred at the 24-hour observation, which was 15,33 individuals. At a concentration of 50%, when the larvae are put into a container, the larvae are still moving as usual. However, in the 10th minute the larvae showed a rapid up and down movement to look for oxygen and at the 15th minute there was a larvae of dead mosquitoes. In the 25th minute the number of dead larvae was 3 individuals. Death of larvae increased every hour and at the 6th observation the number of dead larvae was 14,33 individuals, 12 hours observation was 18,00 individuals, the number of larvae died at 18 hours observation was 19,33 individuals and at 21 hours observation larvae in the container have experienced overall death.

At a concentration of 75%, when mosquito larvae are put into a container it still shows normal movement. At minute 4, the larvae are on the surface of the water, the movement starts slowly and dies. At the observation of 30 minutes after being put into a container the number of larvae of *Aedes aegypti* mosquitoes that died were 10 individuals. At 2 hours observation of larvae of dead mosquitoes had increased to 12 individuals and at 6 hours observation, the number of dead larvae was 18,33 individuals, at 12 hours observation the number of larvae that had died was 19,33 individuals. In the 13-hour observation the number of mosquito larvae in the container had died.

The mortality of *Aedes aegypti* mosquito larvae in this study is in line with the ever increasing concentration and time of observation. This is in accordance with the opinion of Kurniawati [12] that
if the concentration of *Tinospora cordifolia* extract is low, the effect on insects will be lower, on the contrary the administration of higher extract concentrations will also have a high effect because the working power of a compound is largely determined by the concentration which is given. The increased concentration of the extract causes an increase in the active ingredient content of the substance which functions as a pesticide capable of killing in large quantities [13].

The death of *Aedes aegypti* mosquito larvae in *Aedes aegypti* mosquito larvae at concentrations of 25%, 50% and 75% in this study was caused by the compound metabolite sekuder in the herb *Phyllanthus niruri* L. containing flavonoid compounds, saponins and alkaloids which are toxic to insects. Flavonoids are a type of phenol group and are commonly found in plants. Biologically flavonoids play an important role in plant pollination by insects. However, there are a number of flavonoids that have a bitter taste so that they can resist insects. When flavonoid compounds enter the mouth of an insect, it can cause weakness in the nerves and damage to spiracles so that the insect cannot breathe and eventually dies. In addition, the flavonoid group in the form of isoflavones also has an effect on insect reproduction, which inhibits the process of insect growth [14].

Saponins are toxic to cold-blooded animals, including mosquitoes. Saponin is a substance that when shaken with water will produce foam or foam and if hydrolyzed will produce sugar and sapogenin. The nature of sapogenin is hemolysis of blood, binding of cholesterol and toxins in insects. In addition, saponins can irritate the gastrointestinal mucosa and have a bitter taste so that it can reduce the appetite of larvae so that the larvae will starve to death. Therefore, it is dangerous for insects if saponins are given parental [15]. Alkaloids have metabolic properties with respect to one or several amino acids. The physiological activity is toxic and has a bitter taste. Other toxic effects can be more complex and dangerous to insects, which interfere with tyrosine activity which is an essential enzyme for hardening of the cuticle of the insect [16]. Alkaloids are active components that work in the nerves but can also cause digestive disorders because alkaloids can act as poisons through the mouth of the larvae.

### 3.2 LC$_{50}$ and LC$_{90}$ values

The results of calculations on the LC$_{50}$ and LC$_{90}$ values (Appendix 3) can be seen in Table 2. The results in Table 2 show that the LC$_{50}$ value is 2.644% with an upper limit of 1,040 and a lower limit of 3,674. This means that at a concentration of 2.644% the herbal extract of *Phyllanthus niruri* L is capable of killing 50% of *Aedes aegypti* mosquito larvae.

| Probability | Estimate | Lower Bound | Upper Bound |
|-------------|----------|-------------|-------------|
| 50          | 2,644    | 1,040       | 3,674       |
| 90          | 5,772    | 4,424       | 12,042      |

While the LC$_{90}$ value of 5.772% with an upper limit of 4,424 and a lower limit of 12,042. These results give a lower meaning at a concentration of 5.772% of the ethanol extract of the herb *Phyllanthus niruri* L capable of killing 90% of *Aedes aegypti* mosquito larvae. The smaller the LC$_{50}$ and LC$_{90}$, the smaller the extract concentration needed to kill mosquito larvae, so the better the effectiveness of the extract larvae sided [17].

### 4. Conclusions

Ethanol extract of the herb *Phyllanthus niruri* L concentration of 75% is effective in killing *Aedes aegypti* mosquito larvae. LC$_{50}$ value of 2.644% with an upper limit of 1,040 and a lower limit of 3,674. While the LC$_{90}$ value of 5.772% with an upper limit of 4,424 and a lower limit of 12,042.

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