RUMPHIUS PATTIMURA BIOLOGICAL JOURNAL
2019, 1 (1): 12-17
E-ISSN : 2684-804X

BIOLARVACIDE ACTIVITY OF ETHANOL EXTRACT OF KEDONDONG STEM *(Spondias pinnata)* AGAINST *Aedes aegypti*

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Received: 6 February 2019 Accepted: 19 March 2019 Published: 25 March 2019

ABSTRACT

Aedes aegypti mosquito is a vector for Dengue Hemorrhagic Fever. One effort to prevent the spread of the disease is vector control. The use of insecticides is a form of vector control in an effort to control DHF. There are two broad categories of insecticides that are often used as household insecticides, namely insecticides that function to kill insects and insecticides that function to repel insects. Kedondong *(Spondias pinnata)* which is a family of anacardiaceae which generally grows well in tropical climates. Kedondong bark contains saponins, alkaloids, and flavonoids which are secondary metabolites involved in defense mechanisms against attack by many microorganisms. This study aims to determine the biolarvicidalactivity of the ethanol extract of kedondong stem bark in killing *Aedes aegypti mosquito larvae.* This study used 5 treatments and 3 replications. Each treatment contained 20 Aedes aegypti mosquito larvae. Observations were made for 24 hours with observation times of 0 hours, 6 hours, 12 hours, 18 hours and 24hours. The time calculation starts after the larvae are inserted into the experimental bottle. The results showed that kedondong bark extract was able to kill *Aedes aegypti mosquito larvae.* The effective dose of ethanol extract of kedondong stem bark in killing *Aedes aegypti* mosquito larvae is 20 mg/mL.

Keywords: *dengue hemorrhagic fever, kedondong, biolarvicida, aedes aegypti*

To cite this article:
Maniharapon, D., Ukratalo, A.M., Wisnanda, B. 2019. Biolarvacide activity of ethanol extract of kedondong stem (spondias pinnata) against aedes aegypti. *Rumphius Pattimura Biological Journal. 1 (1):12-17.* DOI https://doi.org/10.30598/rumphiusv1i1p012-017

INTRODUCTION

Indonesia is one of the largest tropical countries in the world. The tropical climate causes a variety of tropical diseases caused by mosquitoes such as malaria, dengue fever, filariasis, and chikungunya whichcause epidemics that occur in a wide spectrum and quickly. The main cause of the emergence of epidemics of various tropical diseases is the uncontrolled breeding andspread of mosquitoes as disease vectors (Minarni, 2010). Dengue Hemorrhagic Fever (DHF) is one of the communicablediseases that is still a global health problem, especially in developing countries, including Indonesia (Wurisastuti, 2012). Dengue Hemorrhagic Fever (DHF) is caused by the Dengue virus of the genus Flavivirus, family Flaviviridae and has 4 types of serotypes, namely: Den-1, Den-2, Den-3, and Den-4. These four dengue virus serotypes have been found in various regions in Indonesia. The structure of the antigens of the 4 serotypes is very similar to one another,

but the antibodies against each virus type cannot provide cross protection. Patients living in endemic areas may be infected with more than 1 serotype during their lifetime. The different genetic variations in the 4 serotypes are not only related to between virus types, but also withinthe virus type itself depending on the time and area of spread.

The mode of transmission of DHF is through the bite of the *Aedes aegypti* or *Aedes albopictus* mosquitoeswhich are the main and secondary vectors of DHF in Indonesia. Until now there has not been found a specific drug that can be used for the treatment of DHF, while the prevention of DHF is very dependent on vector control (Boesri et al, 2012). The use of insecticides is a form of vector control in an effort to control DHF. There are two broad categories of insecticides that are often used as household insecticides, namely insecticides that function to kill insects and insecticides thatfunction to repel insects. The use of vegetable pesticides or natural bioactive compounds derived from plants is currently being developed (Virgianti, 2015). Compounds that are larvicidal are compounds with activity to kill larvae by acting as stomach, contact or inhalation poisons (Mangindaan, 2013). Kedondong *(Spondias pinnata)* which is a family of anacardiaceae which generally grows well in tropical climates. Optimal growth and production, kedondong plants are planted in fertile, loose soil that contains lots of organic matter, isaerated and has good drainage, and has a pH of 5.5-6.5. Kedondong is also known in the treatment of infectious diseases such as bronchitis, ulcers, dysentery, diarrhea and skin diseases. Young leaves, flowers, roots and bark are used in traditionalmedicine (Gupta et al, 2010). Kedondong bark contains saponins, alkaloids, and flavonoids which are secondary metabolites involved in defense mechanisms against attack by many microorganisms.

Flavonoid compounds, alkaloids, saponins contained in kedondong stem bark are thought to damage larval membranes, inhibit endocrine work, produce chemical reactions that interfere with larval body metabolic processes, and interfere with the respiratory system in larvae which can ultimately reduce growth rates and cause death of mosquito larvae (Utomo et al, 2010). The advantages of vegetable pesticides compared to synthetic pesticides are the compounds contained therein. In a plant extract, apart from the main active compounds, there are usually many other less active compounds, but their presence can increase the overall extract activity (synergy). This allows insects not to easily become resistant, because the ability of insects to form a defense system against several different compounds simultaneously is smaller than a single insecticide compound.

METHODS

This research is an experimental study with a post test only control group design. This research design was chosen because no pre-test was carried out on the sample before treatment. Because randomization was carried out both in the experimental group and the control group; the groups were considered the same before treatment. In this way it is possible to measure the effect of treatment (intervention) on one experimental group by comparing it with another experimental group andthe control group. The implementation of this research was carried out at the Taxonomy Laboratory of the Biology and Chemistry Fundamentals Faculty of Mathematics and Natural Sciences, Pattimura University, Ambon.

Research design

This study used a completely randomized design (CRD) with five treatments and 3 timestest. The division of groups in this study is:

P1 : ethanol extract of kedondong stem bark dose of 0 mg/ml.

P2 : ethanol extract of kedondong stem bark dose of 2.5 mg/ml.

P3 : ethanol extract of kedondong stem bark dose of 5 mg/ml. P4 : Ethanol extract of kedondong stem bark dose of 10 mg/ml.P5 : ethanol extract of kedondong stem bark dose of 20 mg/ml

Tools and materials

The tools used in this study were analytical balance, pipette, measuring cup, plastic tray, 15 plastic containers (as containers), beaker glass, cloth (to protect the adult mosquitoes from flying out), blender or juicer, stir bar. glass, extractor(Maceration Equipment), evaporator, label paper, knife. The materials used in this study were: Ethanol, bark of kedondongstems; clean water or aquadest; Aedes aegypti larvae, Fish food for larvae food.

Population and Sample

Population: The population of this study were *Aedes aegypti* eggs obtained by the Laboratory for Control of Animal- Based Diseases (P2B2) of the Health Research and Development Agency (Balitbangkes) Banjarnegara. Inclusion Criteria:

- Instar healthy *Aedes aegypti* larvae that have reached instar IV.
- Larvae are actively moving.

Exclusion Criteria

- *Aedes aegypti* larvae that have not yet reached instar IV.
- Larvae that have turned into pupae or adult mosquitoes.
- Larvae that died before treatment.

Sample Size: Sample size was 20 instar IV larvae placed in each container with 3 replications for each test material. The total number of samples needed is 300 *Aedes aegypti eggs.* The sampling method in this study was simple random sampling of *Aedes aegypti larvae.* Even though the population is homogeneous, there are inclusion and exclusion criteria in determining the sample forresearch.

Procedures

Preparation of Mosquito Larvae: *Aedes aegypti* eggs were obtained from the Animal-Based Disease ControlLaboratory (P2B2) of the Health Research and Development Agency (Balitbangkes) Banjarnegara. *Aedes aegypti* eggs, hatched in a plastic container filled with ±1000cc clean water and fish food.Eggs that have hatched are left for up to 4 days so that the larvae reach intar III, then used for research.

Preparation of Test Material:

Kedondong stem bark is taken and air-dried at room temperature. After drying, grind it with a blender and the fine powder is weighed. The ethanol extract of kedondong stem bark wasmade using the maceration method. The steps taken are as follows: 1) Kedondong bark powder is then weighed using an analytical balance as much as 500 grams and then put into two Erlenmeyer pieces with a size of 1000 ml. Each erlenmeyer is filled with 250 gr of kedondong stem bark. 2) Enter 2000 ml of ethanol in each Erlenmeyer and leave for 24 hours. 3) After 24 hours, the solution was filtered using Whatman filter paper to obtain liquid extract fromthe kedondong stem bark. The extraction residue was repeated until the solution was clear, indicating that all the metabolites had been filtered out. 4) The liquid extract of the ethanol from the bark of the kedondong stem is then collected and evaporated using a rotary evaporator at 40°C to obtain a concentrated ethanol extract from the bark of the kedondong stem, and 5) The concentrated ethanol extract is then weighed and diluted with the appropriate distilled water. with the required dose.

Transfer of Larvae in Containers: 1) Larvae in trays are transferred to plastic containers. 2) Using a pipette, take 20 larvae and place them in each container. 3) After all the larvae are transferred to thecontainer, each group of containers is covered with a cloth. 4) Larvae were fed fish food during the study.

Larvicidal Activity Testing: A total of 20 A. aegypti mosquito larvae were transferred from the container into a beaker containing the extract (according to the dose) and Observations were made for 24 hours with observation times of 0 hours, 6 hours, 12 hours, 18 hours and 24 hours .8 Calculation of time begins after inserting the larvae into the test bottle. Observation of the life cycle, namely the test larvae given the extract were able to survive for a certain period of time but could not reach the next stage. The intended death effect is that the test larvae experience mortalitydue to the activity of the given larvicidal extract.

Data Collection: The data collected is by counting the number of dead larvae in each container. Counting of dead larvae was carried out during the observation, recorded in tabular form. Dead larvae are those that sink to thebottom of the container, do not move, leaving other larvae that can move clearly and do not respond to stimuli.

Data analysis

Observational data will be analyzed by Analysis Of Variance (ANOVA) using the SPSS 16.00 program. If thereis a significant difference, a further test will be carried out using the Least Significant Difference Test (LSD) at a confidence level of 0.05%.

RESULT AND DISCUSSION

The average results of observing the mortality of *Aedes aegypti* mosquito larvae at 0, 6, 12, 18 and 24 hoursin this study can be seen in Table 1.

Table 1. Average mortality of *Aedes aegypti* mosquito larvae

Time Observation (i-hour)	Mortality of *Aedes aegypti* mosquito larvae per each treatment					Total
	P1	P2	P3	P4	P5	
0	0.00 0.00 0.00 0.00 0.00	4.33 4.00	4.00 0.00	6.33 8.00	8.67 0.00	0.00 ± 0.00a
6	0.00 10.33	13.33 18.67	0, 33 15.00	17.67 20.00	**7.20±5.47b** 6.67	3.80 ± 2.34b
12	**10.27±8.22d**	**0.07±0.26a**	**8.60±6.61c**	Notes : Superscripts	13.33	7.27 ± 4.62c
18	with the same letters are not significantly different (P > 0.05).				20.00	12.47 ± 7.49d
24					20.00	14.60 ± 7.68e
Total					**12.00±8.11e**	**7.63 ± 7.50**

Table 1 shows that at P1 the average mortality of *Aedes aegypti* mosquito larvae of 0.07 ± 0.26 individuals. At P2, the average mortality of *Aedes aegypti* mosquito larvae was 7.20 ± 5.47individuals, the average mortality of *Aedes aegypti* mosquito larvae at P3 was 8.60 ± 6.61 individuals. At P4, the average mortality of *Aedes aegypti* mosquito larvae was 10.27 ± 8.22 individuals and at a dose of 20 mg/ml, the average mortality of *Aedes aegypti* mosquito larvae was 12.00 ± 8.11 individuals. At P5 it was also seen that at the 18th hour of observation all of *the Aedes aegypti* mosquito larvae had died. The average mortalityof mosquito larvae during the study can be seen in Figure 1.

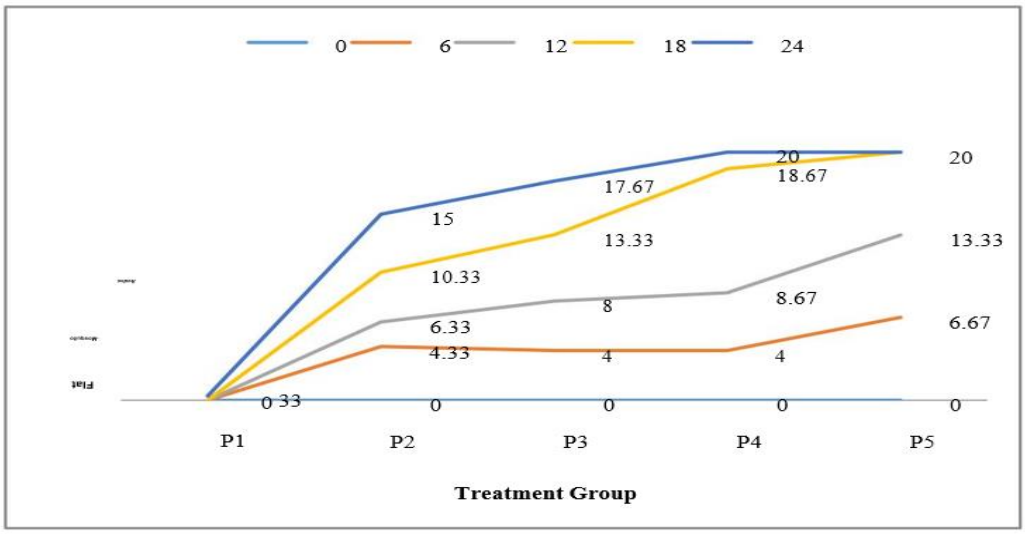

Figure 1. Average mortality of *Aedes aegypti* mosquito larvae.

Based on the results of the Analysis of Variance (ANOVA) using the SPSS 16 program, it was shown thatthe administration of ethanol extract from the bark of the kedondong bark had an effect on the death of *Aedes aegypti mosquito larvae.* Further test results using the Least Significant Difference Test (LSD) at a significant level of 0.05% showed that there was a significant effect on the average mortality of Aedes aegypti mosquito larvae *at* doses of 0 (control), 2.5 mg/ml, 5 mg /ml, 10 mg/ml and 20 mg/ml. The results of the analysis of the effect of observation time on the mortality of *Aedes aegypti* mosquito larvae also showed that there was a verysignificant effect between hours 0, 6, 12, 18 and 24. The increase in mortality of *Aedes aegypti* mosquito larvae at P2, P3, P4 and P5 was different at each observation time. At P2 the mortality of mosquito larvae increased at 18 hours of observation, namely as many as 10.33 individuals and peaked at 24 hours of observation, namely as many as 15.00 individuals. At P3, the increase in mortality of *Aedes aegypti* mosquito larvae varied where at 12 hours of observation the mortality of *Aedes aegypti* mosquito larvae was 8.00 individuals, 18 hours of observation were 13.33 individuals,24 observations were 17.67 individuals. At P4 at 12 hours of observation there was 8.67 individuals of larval mortality, at 18 hours of 18.67 individuals of observation and the peak of larval mortality *Aedes aegypti* mosquitoes at the 24 hour observation were 20.00 individuals. Whereas at P5, at 6 hours of observation there was a very significant mortality of *Aedes aegypti* mosquito larvae, namely 6.67 individuals, atthe 12th hour of observation there was an increase in mosquito larvae mortality of 13.33 individuals and at observations of 18 and 24 hours it became 20, 00 individuals. The occurrence of mortality of *Aedes aegypti* mosquito larvae in this study was in line with the

higher doses given to *Aedes aegypti mosquito larvae.*

The occurrence of death of *Aedes aegypti* larvae in this study was due to the large number of active compounds in the bark of kedondong stems which had direct contact with *Aedes aegypti larvae.* The higher theconcentration, the more active compounds received by *Aedes aegypti* larvae. The active compounds containedin the ethanol extract of kedondong stem bark are saponins, flavonoids, and tannins.Biologically, flavonoids play an important role in pollination of plants by insects. However, there are a number of flavonoids that have a bitter taste that can repel insects. If flavonoid compounds enter the insect's mouth, it cancause weakness in the nerves and damage to the spiracles so that the insects cannot breathe and eventually die (Syamsul et al, 2014). Saponins work as stomach poisons by inhibiting proteolytic enzymes which will cause a decrease in the activity of digestive enzymes and can also irritate the digestive tract mucosa in insects. Saponins also have detergent-like properties, so they are considered capable of increasing the penetration of toxins because they can dissolve lipophilic materials in water. Apart from flavonoids and saponins, other active compounds in kedondong stem bark that are thought to act as insecticides are tannins which function as contact poisons whichresult in the activation of the cell lysis system due to proteolytic enzymes in mosquito body cells. The tannin compounds contained in basil leaf extract are thought to reduce the activity of digestive enzymes such as amylase and protease, so that protein absorption can be disrupted and result in death in mosquitoes due to impaired nutrient absorption and decreased growth rate in mosquitoes. Tannin is a type of polyphenol that will inhibit the entry of food substances needed by insects so that the nutritional needs of insects are not met, eventually there will be metabolic and physiological disorders of cells which will cause cell damage (Syamsul et al, 2014). Reproductive physiology center located in the hypothalamus and pituitary to control the production of testosterone and spermatogenesis in males. Ties and activation of a specific receptor that functions to regulate

CONCLUSION

1. The ethanol extract of kedondong stem bark is able to kill Aedes aegypti mosquito larvae.
2. Effective dose of ethanol extract of kedondong bark in killing *Aedes aegypti* mosquito larvaeis 20 mg/mL.

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