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Original Article

Larvacidal Activity of the Mulberry (*Morus alba* L.) Leaf Extract Against Larvae of *Aedes aegypti*

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ABSTRACT

Dengue Haemorrhagic Fever (DHF) is one of the major public health problems in Indonesia. As the population density increases, the number of sufferers increases. *Aedes aegypti* mosquitoes are vectors for the disease. The absence of drugs make the best prevention effort by eradicating mosquito nests, killing larvae and adult mosquitoes. Mulberry leaves (*Morus alba L.)* may be used as larvacides in the presence of chemical compounds of flavonoids and saponins that inhibit feeding and disrupt the process of insect metabolism. The purpose of this research has to determine the effect of mulberry leaf extract (*Morus alba L.)*, to determine the larvicide effect of mulberry leaf extract (*Morus alba L.*) and to determine the concentration of mulberry leaf extract (*Morus alba L.)* which is optimal in killing third instar *Aedes aegypti* larvae. This research used Randomized Design Group (RDG) method with treatment consisted 4 concentrations (0.25%, 0.5%, 0.75%, and 1%), negative control and positive control (ABATE) with 6 repetitions. The results of probit analysis showed that LC_{50} values were 1.124% and LC_{90} was 4.413%. From the one way ANOVA test at each concentration of 0.25%, 0.5%, 0.75%, and 1%,the F count result is 208.331, the value was greater than F table which is 2.53 and the significant value is 0.000 (sig <0.05) then mulberry leaf extract (*Morus alba L.)* has a affected to eliminated of *Aedes aegypti* larvae. Conclusion from the results of the one way ANOVA test of mulberry leaf extract (*Morus alba L.)* was affected to eliminated third instar *Aedes aegypti* larvae.

Keywords: Larvacidal; *Aedes aegypti*; Mulberry Leaf Extract; Vector; Natural Product

Highlights: This research proved the effectiveness of mulberry leaf extract (Morus alba L.) against third instar of Aedes aegypti larvae and has potential as alternative products to synthetic insecticide.

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INTRODUCTION

Indonesia is one of tropical country in the world. Tropical condition cause vector borndisease grow rapidly such as malaria, dengue fever, filariasis, and chikungunya disease. *Aedes aegypti* is a very important disease vector, transmitting the arbovirus causing dengue hemorrhagic fever and chikungunya in human. At present, no effective vaccine is available for dengue, therefore, the only way of reducing the incident of this disease is by controlling the vector, mosquitos, which frequently depends on applications of synthetic insecticides. Eradication of mosquitos borne disease is to break the chain of life cycle of mosquitos that consist four steps; eliminating the cause of the disease, isolation of the patient, preventing mosquitos bite, and vector control[1]. Vector control effort have been carried out various ways that is mechanics, biology and chemistry[2.] However, the use of these chemicals insecticides has enormous negative impact such as environmental pollution, predatory mortality, targeted insect resistance, and causing various dangerous disease in human.

Based on the research of concerning larvae effect from various natural compound, many research showed that saponin and flavonoid from medicinal plans have effect of larvicides[3]. More important fact is the plant extract are sometimes more effective than the synthetic pesticide and phytochemical have the major role in mosquito control programme[4]. In this sense, substances extracted from plants present a great perspective for the control of *Aedes Aegypti* and other vectors of vector born disease.

Many biological effects including free radical scavenging activity have been reported for flavonoids, which are generally attributed to their structural features. The flavonoid content in mulberry leaves was ranging from 26.41 ± 1.14 mg to 31.28 ± 2.12 mg which are effective as larvacide[5]. Some research found the efficacy of using natural product for larvicidal against *Aedes aegypti* larvae, such as *Acacia nilotica, Baccharis reticularia, Bauhinia pulchella, Bauhinia ungulate, Cinnamomum osmophoeum, Cunninghamia konishii, Curcuma longa, Eucalyptus camaldulensis, Eucalyptus nitens, Mentha spicata,* and many more species been identified as promising larvacide[6].

Based on this fact, an alternative larvicidesderived from natural compounds needed to reduce the use of chemical insecticides and discoveries of other potential natural product been done based on the active ingredients which impacted the longevity of *Aedes agypti* larvae. This research aimed to determine the larvicide effect of mulberry leaf extract (*Morus alba L.*) and to discovered the efficacy of mulberry leaf extract (*Morus alba L.)* as natural product against *Aedes aegypti* larvae.

MATERIALS AND METHODS

This research was an analytic experimental study in accordance as described by World Health Organization (WHO) guidelines for laboratory and field testing of mosquito larvicides. This study was conducted in the Laboratory of Parasitology Laboratory of Institute of Health Bhakti Wiyata Kediri.

1. Preparation of test materials

Aedes aegypti mosquito eggs were obtained from the Public Health Office of East Java. The larvae were cultured and maintained in the Laboratory at 27ºC and 85% of relative humidity. The mosquito eggs then placed in plastic tray filled with water as for the maintenance of the larvae. Mosquitos' eggs will hatch into larvae within 1-2 days. Hatching eggs into larvae

are separated by using larval pipettes for colonization and fed by chicken's liver. After the third phase instar larvae, the larvae are removed by using a larval pipette into a plastic cup containing extract with different concentrations in each cup.

2. Mulberry leaf extract Preparation

Mulberry leaf extract made in accordance with the method of maceration for 24 hour using ethanol 96% as solvent.

Mulberry leaves were purchased from Kayon flower market, Surabaya, Indonesia. After remove any materials and cleaning under tap water, the Mulberry leaves were stored in an oven and dried in the sunlight and then stored at room temperature until further use. The 500 g of the plant sample powdered were soaked in ethanol and chloroform separately for 24 hrs. The maceration product then filtered and concentrated under 40ºC using rotary evaporator and produced 31 ml mulberry extract.

Ethanol extract of mulberry extract dilute by aquadest to 0.25%, 0.5%, 0.75%, and 1%. As for positive control is abate containing 0.01% temephos, and tap water as negative control.

3. Larvicidal Activity of mulberry extract

The larvicidal activity was assesed by the procedur of WHO and Pesticide Commission. According to WHO procedure, concentration is considered to have an effect when causing death test larvae of 10-95% which will be used to find the value of lethal concentration. Meanwhile, according to the Pesticide Commission, the use of larvicides is said to be effective if it can kill 90-100% test larvae.

4. Bioassay Experiment

For the bioassay test, larvae were taken into five batch, 25 larvae *Aedes aegypti* of eachbatch, in 100 ml desired concentration of mulberry extract (0.25%, 0.5%, 0.75%, and 1%). The negative control wastap water and 0.01% temephos as positive control. After the adding the larvae, the glass dishes were kept in laboratory at room temperature. The number of larvae death were counted after 24 hours of exposure, and the percentage of larvae mortality was reported from the average of six replicate. Dead larvae were removed as soon as possible in order to prevent decomposition, which may cause rapid death of remaining larvae. The mean of death of each treatment group in each unit of observation time was tested by using Probit analysis until LC_{50} value was obtained.

RESULTS AND DISCUSSION

Plant extracts exert a multitude of biological activities on pests including larvicide, repellent, insect growth regulator, and more[7,8,9]. This may be because different phytochemicals found in plants can work synergistically to induce such reactions. Plant pesticides are biodegradable and rarely become resistance to pests duel to the synergistic action of complex biomolecules, thereby reducing the long-term environmental impacts of their use[10,11].

Several studies found the potential compound from natural products have larvicidal activity. A review conducted by Wuillda *et al*, revealed about 86 compounds were settled as potentially larvicidal, and wide variety of compounds have been found, such as acetogenins, alkaloids, naphthoquinones, lignans, quassinoids, flavonoids, fatty acids, monoterpenes, sesquiterpenes, and others [12].

Roots, bark and leaves of *Morus alba L.* are used for various health benefit and the presence of precious phytochemicals (coumarins, flavonoids, phenols) of *Morus*

alba L. leaves possess pharmacological importance. Concentrations of total phenolic compounds of *Morus alba L.* like tannins, alkaloids and saponins were within safe range [13].

The 24hr bioassay is major tool for evaluating the toxicity and have been applying by many researcher. The mosquito larvae exposed under mulberry leaf extract showed significant behavioral changes were observed within 30 minutes of exposure. The most obvious sign of behavioral changed was inability to come on the surface, restlessness, and led to death. No such behavioral change were observed in control group.

This research was conducted in Laboratory of Parasitology Laboratory of Institute of Health Bhakti Wiyata Kediri. The result study are presented in the following Table 1 and the analysis was present on Table 2.

Table 1. Mortality Data of *Aedes aegypti* larvae after 24 hour exposure Mulberry Leaf Extract

Concentration (%)	Total Larvae	Repeatition						Mean	Mortality
		1	2	3	4	5	6	X	%
0.25%	25	12	16	14	18	18	18	16	64
0.5%	25	13	14	15	17	17	17	15,5	62
0.75%	25	14	14	16	17	17	18	16	64
1%	25	14	15	14	17	18	18	16	64
Postive control	25	16	16	17	19	18	19	17,5	70
Negative control	25	0	0	0	0	0	0	0	0

Tablel 2. Analysis Probit

Concentration (%)	Percentage of Larvae Death	LC_{50} (%)	LC_{90} (%)
0.25%	64%	1.124% (0.154-1.744)	4.413 (3.613-5.939)
0.5%	62%		
0.75%	64%		
1%	64%		

Result of experiment conducted for evaluating the larvicidal efficacy of Mulberry Leaf Extract showed that is toxic to *Aedes aegypti* larvae. Lethal concentration of mulberry leaf extract were 1.124% (LC_{50}) and 4.413% (LC_{90}). Based on the results of this study, it can be seen that the extract can be used as larvacide. This occurs because the mulberry leaf extract contain active compounds such as alkaloids, soponin, flavonoids and other chemicals that can affect the nervous system, digestion and breathing in larvae[7,14]. Mortality of mosquito larvae showed no big difference value from all concentration,it indicates that the extract is toxic[15,16]. In this study the temperature, pH and humidity are still at normal limits, so the possibility of mosquito larvae in this study died caused by external influences.

Variation of mosquito larvae mortality caused by the variety of

sensitivity and resistance of each larva to the material active in the extract[17,18]. The death of the larvae is caused by the inability of the larvae to detoxify the toxic compounds thatenter the body[19,20]. Based on the results of the observations during the larvae test exhibited anxiety symptoms characterized by upward motion movements on the test medium, while the larvae control showed aresting state on the surface forming angels [16,17,21].

The difference in the percentage of larval mortality is duel to the diffusion speed of extracts entering into different cells so thatat low concentrations the larvae can still tolerate these toxic compounds, whereas athigh concentrations the larvae can not tolerate the entry of these toxic compounds[22]. The interaction of toxic substances a biological system is determined by the concentration and length of time. Toxic substances that play a role in lethal larvae are alkaloids, saponins, and flavonoids. Alkaloids that enter the body of the larvae through absorption and degrade the skin cell membrane, besides alkaloids can also interfere with the larva nervous system work[14,15].

Alkaloid compounds act as larvicides by inhibiting the feeding power of the larvae (antifeedant), so the larvae will experience nutritional deficiencies and eventually die[18]. Based on the results of these studies the alkaloids contained in the leaves of elasticity serves as a poison or poisoning stomach. The alkaloid can also be used as an insecticides. The alkaloid compound inhibits the work of acetylcholinesterase enzyme that serves in continuing stimulation to the nervous system, so transmission of excitement does not occur[23,24]. Another active compound contained in the mulberry extract is saponins[25]. Saponins result in decreased activity of digestive enzymes and the absorption of food in insects. In addition, saponins also damage the larvae and causing the death of larvae[3,25,26]. *Morus Alba* extract and its other compounds usually flavonoids have antioxidant properties by scavenging free radicals and protect many organs from oxidative stress [5,13].

CONCLUSIONS

As the leaf extract of Mulberry is toxic for *Aedes aegypti* larvae even at low doses, the plant may eventuallyprove to be useful larvicide. The plant can be eco-friendly and may served as suitable alternative to synthetic insecticides as they are relatively safe, inexpensive and available in many areas of the world.

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CONFLICT OF INTEREST

The authors confirm that there is no conflict of interest.

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