Concentration and Time Exposure Determination of Methanol Extract from Carica papaya Leaves in The Larvicidal Activity Against Aedes aegypti Larvae

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Abstract
Dengue Haemorrhagic Fever (DHF) is a disease caused by dengue virus which is transmitted through Aedes aegypti mosquito bite. Efforts to control the widespread of the vectors have been made using biological agents and also chemical compound. Chemicals known as a standard protocol have raised concerns about resistance and harmfulness to the environment. Hence, the present study was aimed to explore the larvicidal activity of papaya (Carica papaya) leaf extract against Aedes aegypti larvae in regards to the optimum concentration and time exposure. Preparation the obtained extract was diluted to make a serial concentration. These solutions were made by pipetting 0.65 mL, 1.25 mL, 2.5 mL, 5.0 mL, and 10.0 mL of extract into 10.0 mL volumetric flasks and dilute with distilled water. The test solution was poured into a glass jar contained 90 mL of distilled water and filled with 20 third instar larvae. Each experiment was replicated four times. The larval mortality was recorded in 24h and calculated as a percentage of total larvae used in the experiment. The table above shows the value of LC50 And LT50 from toxicity assay of papaya leaf extract. According to the LT50 value, it can be seen that the lowest LT50 of 1,006h occurred at the concentration of 11000 ppm. Moreover, calculated LC50 is 4929,344 ppm. Based on these results, papaya leaves have the ability to Aedes aegypti larvaside so that it can help in breaking the chain of development of Aedes aegypti.

***Keywords*:** *Larvicidal Activity, Papaya Leaf, Methanol Extract, Aedes aegypti*.

1. Introduction

Indonesia is a tropical country and rich in biodiversity, including the abundant type of medicinal plants. However, the usage of this particular medication has been abandoned and replaced by modern and chemical drug. The tropical climate in Indonesia not only gives many advantages as the richness of plant that can grow but also becomes an optimal breeding place

for disease vectors which are mostly insects like a mosquito. The presence of mosquitoes is often felt to interfere with human life from its itching bite to its role as a harmful diseases vector causing elephantiasis, malaria, dengue fever, and many more. Mosquitoes are divided into three subfamilies which are Toxorhynchitinae, Culicinae, and Anophelinae, while blood-sucking mosquitoes that are considered as disease vectors are Aedes, Culex, Anopheles, and Mansonia (Rickard, 1960). *Aedes aegypti* is a well-known vector for Dengue Hemorrhagic Fever (DHF) disease, including in Indonesia. In 2016, the incidence of DHF in Surabaya reached 938 cases with 503 of male patients and 435 of female patients, while the case of death was 7 people, with CFR 0.75% (Dinas Kesehatan Kota Surabaya, 2018). Thus, controlling this vector will result in significant benefit to disease prevention.

Efforts have been made to restrain the vector population by means of a biological agent (natural enemies) or chemical compound. Nowadays, the standard protocol of vector control is chemicals as a repellent lotion or liquid sprayer used in the breeding site of the larvae (Kardinan, 2007). The latter way has already generated drawback as resistance occurred due to long term exposure to the organism. Then it necessitates the development of biolarvicide from plant origin material which also eco-friendly. Aforementioned of Indonesian richness in the medicinal plant has benefitted the quest of a larvicidal agent, one of the most potentials is *Carica papaya*. It has been reported that papain and carpaine alkaloid contained in papaya leaf could lyse an essential protein for the growth of larvae causing growing disruption or even death. Moreover, natural product has more advantage to the environment because of its biodegradable property and selectiveness toward pathogenic larvae. The present study explored the larvicidal potency of papaya leaf extract against *Aedes aegypti* larvae in regards to the optimum concentration and time exposure.

2. Methodology

2.1 Carica papaya Extraction

Papaya leaves with dark green colour were sorted out then washed to remove dirt. Subsequently, it was dried under the sun but covered by the black fabric. During the drying process, the leaves are turned back and forth, ensuring both sides of the leaf has dried evenly. Then, the dried leaves are powdered using a blender. The dry powder (100 grams) was extracted with an excess of methanol (450 mL) for five days, in a glass jar with a watertight cover, stirred each day slowly to maintain the homogenity. The solvent was squeezed and filtered out using Whatman filter paper number 42. In order to make a concentrated methanol extract, the extract was evaporated for roughly two hours in a rotary evaporator apparatus with a temperature of 60℃ and rotation of 80 rpm. The obtained extract was weighed and stored in a desiccator.

2.2 Larvicidal Assay

Aedes aegypti Larvae Preparation

Mosquito larvae were obtained from the *Aedes aegypti* egg rafts reared in 1000 mL beaker glass containing distilled water two days before the examination. Once the eggs were hatched out into first instar larvae, they were fed with fish feed called pellet until moulted two times

into third instar larvae. During the larvicidal assay, third instar larvae were exposed to a serial concentration of each fraction.

Extract Preparation

The obtained extract was diluted to make a serial concentration. These solutions were made by pipetting 0.65 mL, 1.25 mL, 2.5 mL, 5.0 mL, and 10.0 mL of extract into 10.0 mL volumetric flasks and dilute with distilled water.

Toxicity Assay

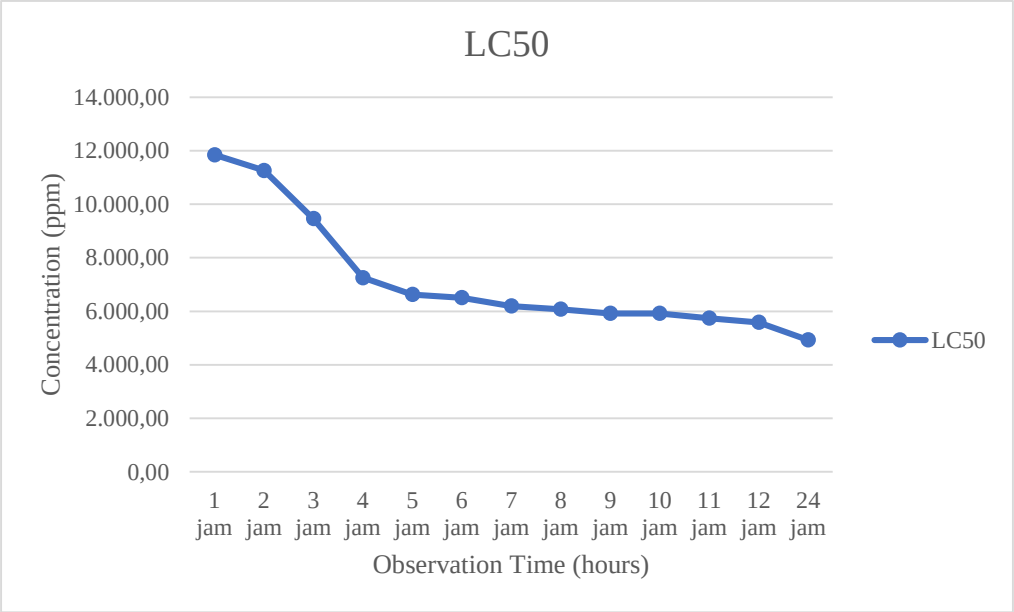
The test solution was poured into a glass jar contained 90 mL of distilled water and filled with 20 third instar larvae. Each experiment was replicated four times and compared to the negative and positive control. The larval mortality was recorded in 24h and calculated as a percentage of total larvae used in the experiment.

$$\text{Mortality rate} = \frac{\text{Death larvae}}{\text{Total larvae}} \times 100\%$$

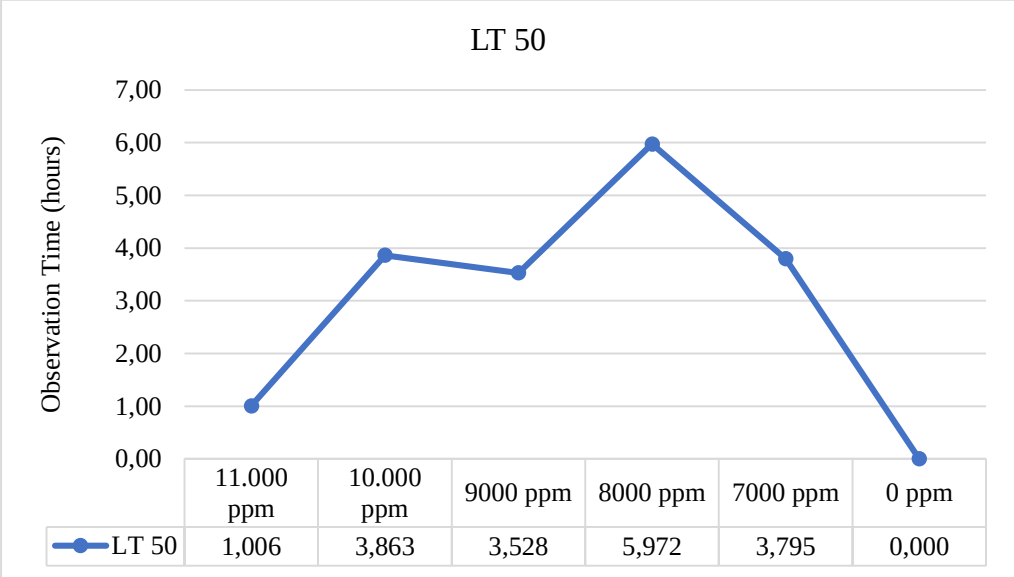
3. Result and Discussion

Table 1. Observation Result of Toxicity Test of Papaya Leaf Extract Against Aedes aegypti Larvae

Conc.	Amount of Death Larva in Interval Time													LT50
	1	2	3	4	5	6	7	8	9	10	11	12	24	
11.000 ppm	7	10	12	15	15	15	15	15	16	16	16	16	20	1,006
10.000 ppm	3	5	9	12	15	15	16	16	16	16	17	17	20	3,863
9000 ppm	1	2	10	14	15	15	16	16	16	16	16	16	18	3,528
8000 ppm	1	1	9	11	11	12	12	13	14	14	15	15	19	5,972
7000 ppm	0	1	7	14	16	16	17	17	17	17	17	18	18	3,795
0 ppm	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LC 50	11845	11258	9457	7252	6628	6507	6195	6073	5921	5921	5740	5587	4929	



Picture 1. Chart LC50



Picture 2. Chart LT50

The table above shows the value of LC50 And LT50 from toxicity assay of papaya leaf extract. According to the LT50 value, it can be seen that the lowest LT50 of 1.006h occurred at the concentration of 11000 ppm. Moreover, calculated LC50 is 4929.344 ppm.

The growth of *Aedes aegypti* mosquitoes phases through several stages such as the egg, larval and the pupa phase, each of these phases occur in the water for 2, 7, and 2 days respectively until transforming to become a matured one. During the larval stage, *Aedes aegypti* experiences 4 instars marked by moulting process influenced by food availability, temperature, species (Sudarwati, Tri Puji Lestari. 2016). Larvae can survive by hanging on the surface of the

water as long as they do not get disturbance from wobble, loud noise or predators. It suggests that the larval stage is fragile and the most feasible point to interfere in the life-cycle of the mosquito using larvicide agent like papaya leaf extract.

The result showed that papaya leaf extract possessing larvicidal activity toward *Aedes aegypti* larvae as it can be seen from the mortality rate. In table 1, the highest concentration of 11000 ppm could kill 50% of the mosquito population in 1,006 hours while at 8000 ppm yielded 5,972 hours. Furthermore, in order to kill half of the population in 24h, the calculated concentration is 4929,344 ppm. This is due to the direct contact of the toxic metabolic compounds from the extract with the part or whole body of the larvae (Djojosumarto, 2000). The compositions that act as extracts contained in papaya leaf extracts are flavonoids, saponins, tannins, and papain.

Processing data with LC50 and LT50 to study optimally killing Aedes aegypti larvae. LC50 is called Lethal Concentration 50 and LT50 is called Lethal Time 50, where the dose of LC 50 is the main component in determining the toxicity of an insecticide, while LT 50 is the time to calculate larval mortality for the test sample (Adibah & Dharmana, 2017). Where the optimal concentration to kill *Aedes aegypti* larvae in an interval of 24 hours is 4929,344 ppm. Based on these results, papaya leaves have the ability to *Aedes aegypti* larvaside so that it can help in breaking the chain of development of *Aedes aegypti*

4. Conclusion

Carica papaya leaves extract has biolarvicidal potency against *Aedes aegypti* larvae is 1000 ppm could kill 50% of the mosquito population in 1,006 hours while at 8000 ppm yielded 5,972 hours. Furthermore, in order to kill half of the population in 24h, the calculated concentration is 4929,344 ppm.

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