

An Analysed Of Effect Papaya Leaf Extract The Concentration Aedes Aegypti Mosquito Larva

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ArticleInfo	ABSTRACT
Keywords: Papaya leaf extract, Aedes Aegypti, Dengue Fever.	The high number of dengue fever cases in Indonesia will reach an increase of 30% in 2022, which can cause death, which is caused by the Aedes aegypti mosquito vector. Control and eradication of the Aedes aegypti mosquito is generally carried out using chemical insecticides. However, continued use of chemical insecticides can cause resistance in vector mosquitoes and have negative impacts on the environment. Papaya leaves (<i>Carica papaya</i> L.) have long been known to have various health benefits, including as a traditional medicine for dengue fever which contains active compounds such as flavonoids, alkaloids, saponins and the enzyme papain. The aim of this research was to analyze the effect of papaya leaf extract concentration (<i>Carica papaya</i> L.) on the mortality of Aedes aegypti mosquito larvae. This type of research is a laboratory experiment, carried out at the FK UNPRI Laboratory. The population in this study was 30 Aedes Aegypti mosquito larvae and the samples used were extracts from papaya leaves (<i>Carica Papaya</i> L) with concentrations of 15% and 85%. The sample collection technique uses purpose sampling technique. Data were analyzed using the Kruskal-Wallis test. The results can be concluded that papaya leaf extract (<i>carica papaya</i> L) at a concentration of 85% is proven to be effective against the death of Aedes aegypti larvae. ($p = 0.379$) with the LD50 equation within a 2 hour minimum period to kill Aedes aegypti larvae. The higher the dose of papaya leaf extract will result in a greater number of larval deaths.
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INTRODUCTION

The high number of dengue fever cases in Indonesia will reach an increase of 30% in 2022, which can cause death, which is caused by the Aedes aegypti mosquito vector. Control and eradication of the Aedes aegypti mosquito is generally carried out using chemical insecticides. The use of chemical insecticides, apart from having respiratory problems as a side effect, will also pollute the environment. The large number of cases of dengue fever requires appropriate management to reduce it. Since 1976, Indonesia has been using synthetic abate products (temephos) to control mosquitoes. In 1980, this synthetic abate (temephos) was designated as part of the Aedes aegypti mosquito control program in Indonesia. However, there is resistance from various species of mosquitoes which are

disease vectors. Reports of resistance of *Aedes aegypti* larvae to abate (temephos) have been found in several countries such as Brazil, Bolivia, Argentina, Cuba, the Caribbean and Thailand (1)

The results of a 2015 survey conducted by the East Java Provincial Health Service for the Indonesian Ministry of Health reported that cases of Dengue Hemorrhagic Fever (DHF) in the East Java area had reached 1,817 cases (Ministry of Health, 2015). The recent dengue fever outbreak reported to WHO in October 2012, namely in Portugal, was reported with a total of 18 confirmed cases and 191 possible new cases reported. According to WHO data, from January to April 2004, in Indonesia there were 58,301 locally registered cases of dengue fever and 658 deaths. The Ministry of Health, through the Director General of Disease Control and Environmental Health (P2PL), revealed that up to November 2012, there had been 404 cases of death due to dengue fever in 31 provinces.(3)

Apart from the impact, resistance to controlling mosquito nests using chemicals such as abate can cause environmental pollution because it contains chemicals which nature takes a long time to break down.(1) Therefore, to reduce the negative impact of using chemical (synthetic) insecticides, it is necessary to develop safer alternatives so that the development of the mosquito life cycle can be hampered and they cannot develop to adulthood. One way is to use natural insecticides.(2)

Papaya leaves (*Carica papaya* L.) have long been known to have various health benefits, including as a traditional medicine for dengue fever. Papaya leaves contain active compounds such as flavonoids, alkaloids, saponins and papain enzymes which have the potential to act as natural larvicides to kill *Aedes aegypti* mosquito larvae. Some of these substances contain substances that can kill mosquito larvae. Papaya leaves also contain saponin, which is toxic to cold-blooded animals, namely by destroying red blood cells through a hemolysis reaction. Other ingredients contained in papaya leaves are alkaloids (carpain) and papain which also functions as a repellent and even kills mosquitoes.(2)

Literature Review

Papaya Leaves

The papaya plant is a plant originating from America which has the scientific name *Carica Papaya* L., while in Indonesia the papaya plant is called variously, including gedang (Sundanese), kates (Java), peute, betik, ralempaya, punti wood (Sumatra), banana. Malacca, Bandas, Manjan (Kalimantan), Kalaujawa, Padu (Nusa Tenggara), Kapalay, Kaliki, and Unti Java (Sulawesi). Apart from that, there are also foreign names for this papaya plant, including papaw tree, papaya, papaya, melonenbaum, and fan mu gua (1).

Classification of Papaya Plants (*carica Papaya* L.):

Kingdom	: Plantae	Class	: Magnolipside
Subkingdom	: Viridiplantae	Superoder	: Rosanae
Infrakingdom	: Sreptophyta	Orders	: Brassicales
Superdivision	: Embryophyta	Family	: Caricaeae
Division	: Tracheophyta	Genus	: <i>Carica</i>
Subdivision	: Spermatophyta	Species	: <i>Carica papaya</i> L.



Figure 1. Papaya Plant (*Carica Papaya L.*)

Papaya Plant Morphology (*Carica Papaya L.*)

Papaya plants have physical characteristics, including the tree does not have branches, the stem is round and hollow, does not have cambium, there are bumps from leaf stalks that have fallen off, the leaves are collected at the end of the stem and the shape is shared by fingers, papaya fruit is shaped to elongated depending on the type, the green color shows Young fruit with a yellowish or orange color is old fruit, the fruit has a large hollow in the middle, the fruit stalk is short, and the seeds are black and covered in a thin film (Muhlisah, 2002:58)

The flowers of the papaya plant are compound flowers which are arranged on a stalk or flower axis (Pedunculus). This plant has three types of flowers, namely male flowers (masculus), female flowers (feminus), and perfect flowers (hermaphrodites) (Kalie, 2008: 11).

Extraction

The extraction process is made from several processes using several suitable solvents. This process can use a solvent that can search for most of the secondary metabolites contained in the simplicia leaf powder (Rivai et al., 2014). The extraction process can be stopped when there is a balance between the concentration of compounds contained in the solvent and the concentration contained in the plant and this extraction process is specifically aimed at materials originating from plants (Mukhriani, 2014). Techniques for extracting active ingredients found in plants, namely: (Mukhriani, 2014)

1. Conventional Extraction Techniques

a) Maceration

This extraction method is the most widely used because this method is quite simple, both for small and large activities. Put the existing plant powder and solvent in a tightly closed container at room temperature. After the extraction process has been completed carried out, separating the solvent from the sample using filtration. The negative effects obtained from this technique are that it takes longer, a lot of solvent is also needed and it is possible that some compounds in the plant can be lost.

b) Percolation

In this method, the sample powder is slowly wetted using a percolator (a cylindrical container with a tap underneath) adding solvent to the sample powder and slowly letting it drip to the bottom. This method also takes a lot of time and uses a lot of solvent.

c) *Ultrasound – Assisted Solvent Extraction*

There is another way of maceration method by utilizing a high frequency signal of 20 kHz called Ultrasound–Assisted Solvent Extraction. An ultrasonic and ultrasound container containing sample powder is prepared, mechanical pressure is required on the cells, to produce cavities in the sample. Increasing the solubility of compounds in solvents and increasing extraction yields can cause cell damage.

Aedes Aegypti mosquito

Aedes sppis the vector of Dengue Fever and Dengue Hemorrhagic Fever. *Aedes spp* has spread in tropical countries and there are around 50 million dengue fever infections in more than 100 countries every year. In Indonesia there are two vectors, namely *Aedes Aegypti* as the main vector and *Aedes Albopictus* as a potential vector. These vectors are spread throughout Indonesia, except for areas with an altitude of more than 1000 meters above sea level. (Jacob et al, 2014). Female *Aedes Aegypti* mosquitoes like to lay their eggs on the surface of the water on the inside walls of places that contain little water. The water must be clear and not exposed to direct sunlight. The water place chosen is the water place inside and near the house.

Larvae of *Ae. Aegypti* is usually found in drums, jars, barrels or bathtubs in family homes in Indonesia where cleanliness is not maintained. In areas with wells with salt water or drinking water supplies are not available regularly, such as coastal areas, residents often store water in drums with a capacity of 200 liters. The size of the drum and the length of time the water is stored in it result in lots of mosquitoes coming from inside the drum. On the other hand, the water in barrel jars and bathtubs is more often used up because the capacity of the jars is only 50 liters, which is enough for drinking supplies for 1-2 days. Therefore, there are fewer mosquitoes from that place. Likewise, with a bathtub with a capacity of 200 liters, the water quickly runs out for bathing and washing and is then filled again with new water. Female mosquitoes prefer loosely covered places rather than open places as a place to lay their eggs.

Anthropophilic is the nature of the *Aedes Aegypti* mosquito (likes humans) and only female mosquitoes bite. Female mosquitoes usually bite inside the house, sometimes outside the house, in a slightly dark place. At night mosquitoes rest in the house on hanging objects, such as clothes, mosquito nets, on walls and near their breeding places. The *Aedes Aegypti* mosquito has the habit of repeatedly biting (multiple bitters), namely biting several people in turn in a short time. This is because the *Aedes Aegypti* mosquito is very sensitive and easily disturbed. This really helps *Aedes Aegypti* in transferring the dengue virus to several people at once so that there are reports of Dengue Fever sufferers in one house. (Soedarmo, 2009).

Morphology

Aedes Aegypti Adults are small with a black base color. Black scaly proboscis, short palpi with black tip with silver white scales. The occiput has wide, white scales located lengthwise. The femur has white scales on the posterior surface and the basal, anterior and middle half of the white scales are elongated. The tibia is all black. The rear tarsi have a white ring on the first to fourth basal segments and the fifth segment is white. Wings measuring 2.5 – 3.0 mm, black scales.



Figure 2. Adult *Aedes Aegypti* mosquito

Life cycle

The *Aedes Aegypti* L. mosquito undergoes complete metamorphosis from egg, first instar larva, second instar larva, third instar larva, fourth instar larva, pupa, to imago. The description of the complete metamorphosis of mosquitoes from egg to adult is as follows:

1. Egg

The *Aedes Aegypti* L. mosquito has eggs that are very small and black in color. These eggs are usually located in parts that are not directly adjacent to the ground, but close to the surface of the water, for example in a tub with clear water. (Wahyuni, 2009. Eggs can survive for months at temperatures - 2°C to 4°C. However, if the humidity is too low, the eggs will hatch within 4 days. Under optimal conditions, the development of eggs until they become adults takes approximately 9 days. Adult female mosquitoes that start sucking human blood, 3 days later are able to lay 100 eggs. 24 hours later the female mosquito sucks blood again and then lays eggs. (Soedarmo, 2009)



Figure 3. *Aedes Aegypti* Mosquito Eggs

2. Larvae

The *Aedes Aegypti* L mosquito larva has a cylindrical shape and its body consists of 3 parts, namely the head (cephal), chest (thorax) and stomach (abdomen). During its development, the larva undergoes 4 changes of skin (molting) from instar larva I to IV and pupa. The *Aedes Aegypti* L mosquito larva consists of a head, thorax and

abdomen. On the head there is a pair of antennae and a compound head, as well as a prominent mouth brush. The abdomen consists of 9 distinct segments, and the last segment is equipped with an air tube (siphon) to take in oxygen and is equipped with pecten in the last segment which is short and bulging. On the abdominal segment there is no fan-shaped hair (palmatus hair). On each side of the 8th abdominal segment there are 8 to 21 comb scales or 1 to 3 in a row and are thorn-shaped. On the thorax there are long spines with a curved shape and a pair of hairs on the head. Larvae obtain food with the help of a mouth brush which functions to produce a flow of water that can carry food into the mouth

3. Pupae

The pupa is the last stage that is in the water and does not need food because it is a resting phase. The pupa has segmentation on its abdomen (resembling a paddle) so it looks like a comma. The fused head and thorax are equipped with a pair of respiratory trumpets. Pupae have great buoyancy. Usually the pupa rests on the surface of the water in a static position but can swim well. The pupal phase takes 2 - 5 days to become an adult mosquito. The total cycle that can be completed is 9 to 12 days. In the pupal phase there is no difference between males and females. In general, male mosquitoes hatch first compared to others female mosquitoes then come out of the water and develop into mosquitoes.



Figure 4. *Aedes Aegypti* Pupa Stage 4. Imago (Adult Mosquito)

The adult *Aedes Aegypti* L mosquito has a characteristic black body with white stripes all over its body. The habitat of the *Aedes Aegypti* L mosquito is in the wild and around the house, and can even be found in public places. This mosquito has the ability to fly up to 100 meters. Blood is a source of protein to mature eggs, therefore, those who actively bite or suck blood from morning to evening are female mosquitoes. The blood sucked by female mosquitoes contains protein which can help the egg maturation process. After sucking blood, the female *Aedes Aegypti* L mosquito will look for a place to rest, while the male needs to fulfill the nutrients in the body by sucking the juice of plant flowers which contain sugar. The female *Aedes Aegypti* L mosquito has a piercing-sucking mouthpart and prefers humans (anthropophagus), while the male mosquito has weak mouthparts so it cannot penetrate human skin, therefore male mosquitoes prefer plant fluids (phytophagus). . The

lifespan of the female *Aedes Aegypti* L mosquito ranges from 2 weeks to 3 months or 1.5 months depending on the temperature and humidity of the surrounding air.

METHODS

This research is experimental research by providing treatment to samples in the form of *Aedes Aegypti* mosquito larvae in the laboratory. The research used three repetitions with concentrations of 15% and 85%. The treatment that will be carried out is *Aedes Aegypti* mosquito larvae given papaya leaf extract (*Carica Papaya* L) with distilled water as a negative control. The research was carried out at the FKKGIK Parasitology Laboratory, Prima Indonesia University for the process of making papaya leaf extract (*Carica papaya* L) and testing for anti-larvicide *Aedes Aegypti*. The research was conducted in January-February 2022.

The sample in this study was papaya leaves (*Carica Papaya* L) purchased at the Kabanjahe traditional market, North Sumatra. The data collection method is carried out in a purposeful manner sampling Using extracts from papaya leaves (*Carica Papaya* L). Rotary Evaporator, measuring cup, beaker glass, macerator, water bath, stirring rod, electric scale, extraction bottle, 250ml plastic cup, stick, spoon. Papaya leaves (*Carica papaya* L.), 96% ethanol, distilled water, *Aedes aegypti* larvae.

Preparation of papaya leaf extract (*Carica papaya* L.)

Choose papaya leaves (*Carica papaya* L.) that are still fresh, wash them clean, then cut them into pieces, and dry them in the air. It is not recommended to dry them in the sun. After the dry papaya leaves, blend them until they become powder. A total of 500 grams of papaya leaf simplicia powder (*Carica papaya* L.) was weighed then macerated with 5L of 96% ethanol at room temperature for 3 days then filtered. Then the dregs were remacerated with 3.75L of 96% ethanol at room temperature for 3 days then filtered. The filtrate obtained was then evaporated using a vacuum rotary evaporator at a temperature of 50°C, speed of 70 rpm, and pressure of 0.7 bar to obtain a thick extract.

Hatching of *Aedes aegypti* Mosquito Eggs

Aedes aegypti mosquito eggs purchased from ITB University were hatched by mosquitoes at the FKKGIK Parasitology Laboratory, Prima Indonesia University, which lasted for 3 days. Method:

1. Eggs in paper are cut as needed and then placed in a container filled with water
2. Give mosquito larvae pellets as food when they hatch
3. Place it in a closed and safe place during the hatching process.

Papaya leaf extract (*Carica papaya* L.) with a concentration of 0% as control, 15% and 85% was poured into each plastic cup. The treatment uses papaya leaf extract (*Carica papaya* L.) which is given in 100 ml in a plastic cup. Then as many as 20 *Aedes Aegypti* mosquito larvae were put into each plastic cup containing papaya leaf extract (*Carica Papaya* L.) using a larvae filter/spoon. Each treatment was repeated 3 times so that 15 experimental units were obtained. Observations were carried out for 48 hours and then the number of dead larvae was counted. The data obtained will be analyzed using the Kruskal-Wallis test.

RESEARCH RESULTS

Research Results

Data obtained from research conducted is as follows:

Table 1. Treatment Results of Papaya Leaf Extract against Aedes larvae

Times	Control			Extract aedes larvae					
				15%			85%		
	I	II	III	I	II	III	I	II	III
30 minutes	0	0	0	0	0	0	0	0	0
1 hours	0	0	0	0	0	0	0	0	0
2 hours	0	0	0	5	5	0	15	5	5
4 hours	0	0	0	15	20	5	25	15	20
8 hours	0	0	0	25	30	15	40	25	25
12 hours	0	0	0	30	45	30	50	45	40
24 hours	0	0	0	35	50	50	70	65	60
48 hours	0	0	0	45	60	60	85	75	70

The data from the table above shows the death of *Aedes aegypti* larvae due to the effects of papaya leaf extract larvicide (*carica papaya* L.) which was repeated three times with 48 hour observations. In the control group there was no death of the larvae. In the control group given in the first 2 hours, the effectiveness of the larvicide was not that strong, this was shown from the first repetition, namely from a total of 30 larvae, concentrations of 15% and 85% showed that the larvicide effect began to appear significant in the first 2 hours.

Based on the results of this research, it was found that the benefits of Papaya leaf extract (*carica papaya* L.) are the effectiveness of papaya leaves (*carica papaya* L.). Data from this research are presented in percent of the number of larvae that die per unit of observation time. The percentage of dead larvae was then analyzed using non-parametric statistical analysis due to the small number of samples. The statistical analysis used in this research is the Kruskal-Wallis test. The results of this analysis are presented in the table below

Table 2. Comparison of the Percent of Dead Larvae for Each

Times	Percent of Dead Larvae [Median (Range)]			Mark P
	Control	Papaya Leaf Extract		
		15%	85%	
30 minutes	0	0	0	1
1 Hours	0	0	0	1
2 Hours	0	10 (0)	5 (10)	0.036
4 Hours	0	20 (15)	20 (10)	0.053
8 Hours	0	40 (15)	25 (15)	0.037
12 Hours	0	50 (10)	45 (10)	0.031
24 Hours	0	60 (20)	65 (10)	0.028
48 Hours	0	75 (10)	75 (15)	0.014

Based on the table above, it can be seen that the percentage of larval death from each extract concentration is statistically different between each concentration after 2 hours of observation. This can be seen from the P value of each sample group being smaller than 0.05. The analysis then continued by comparing the minimum period required for each concentration of papaya leaf extract to kill *Aedes aegypti* mosquito larvae using Kruskal Wallis analysis. The results of this analysis are presented in the table below.

Table 3. Comparison of Minimum Periods for Killing Larvae in Leaf Extracts papaya

Extract	Minimum Period to kill larvae	Mark P
	Median (Range)	
15%	2 (2)	0.379
8%	2 (0)	

Based on the data in the table above, it can be seen that there are no statistically significant differences in the various concentrations of papaya leaf extract in the minimum period required to kill *Aedes aegypti* mosquito larvae, this is reflected in the P value which is greater than 0.05. Then the analysis continued to predict the LD50 value of papaya leaf extract 2 hours after administering the extract to *Aedes aegypti* mosquito larvae, using simple linear regression analysis. The results of this analysis are displayed in the table below.

Discussion

From the results of the normality test using Kolmogorov - Smirnov it was found that the data was normally distributed. However, in the homogeneity test using the Levene test it was found that the data was not homogeneous. Because one of the conditions for the bivariate one-way ANOVA test was not met, an alternative Kruskal Wallis test was carried out. From the results of the Kruskal Wallis test, it was found that the p value of the data was <0.05, where there was a significant relationship between the concentration of papaya leaf extract and the number of larval deaths. The greater the concentration of papaya leaf extract, the greater the number of larval deaths. Then the Mann Whitney test was used to compare the negative control group, namely those using distilled water, with the positive control group and the papaya leaf extract group and the results showed that the p value of the data was significantly different when compared with the negative control group, namely the group using distilled water.

The LD50 acute toxicity test of a drug is defined as a single dose of a substance that is statistically expected to kill 50% of experimental animals (Radji and Harmita, 2008). This test is carried out by administering the chemical substance being tested once or several times within a period of 24 hours, then observed for 14 days (Hendriani, 2007). The acute toxicity test aims to observe the toxic effects of a compound that can occur over a long period of time. short after giving it in a certain dose. At least four dose levels are recommended in acute toxicity testing, these doses range from low doses that do not or almost kill all test animals to the highest doses that can kill all or almost all test animals (Fadli, 2015). The purpose of acute toxicity testing of a traditional medicine is to determine

the potential for acute toxicity (LD50) assessing various clinical symptoms, spectrum of toxic effects, and mechanisms of death (MOH RI, 1989) in Angelina et al (2008).

Based on the results of data analysis, it is known that papaya leaf extract significantly influences the death of *Aedes aegypti* mosquito larvae, which can be seen from the results of observations, the dead larvae have the characteristics of not moving when touched using a pipette, the body of the larvae is white or pale yellow, and its body shape is elongated. This is due to the larvicidal effect of papaya leaf extract (*Carica papaya*) which is the influence of the content of secondary metabolite compounds contained therein. It can be seen from the results of phytochemical tests carried out that papaya leaf extract contains secondary metabolites such as alkaloids, flavonoids, saponins and tannins.⁶

Flavonoids work as powerful respiratory inhibitors or as respiratory poisons. Flavonoids have a way of working, namely by entering the larva's body through the respiratory system which will then cause wilting of the nerves and damage to the respiratory system and result in the larvae not being able to breathe and eventually dying. The changing position of the larva's body from normal can also be caused by flavonoid compounds due to the way it enters through the siphon, causing damage so that the larva must align its position with the surface of the water to make it easier to take in oxygen.⁷

In this study, researchers increased the dose of papaya leaf extract to 85%, resulting in an increase in the number of larval deaths. at a concentration of 85% it was proven to be effective against the death of *Aedes aegypti* larvae ($p = 0.379$) with the LD50 equation within 2 hours of the minimum period to kill *Aedes aegypti* larvae. The description of the results when compared with previous research conducted by La Taha and Nur Inang in 2018 using the highest dose of 20% with observations for 12 hours can be concluded that the higher the dose of papaya leaf extract will result in a greater number of larval deaths. The results can be concluded that papaya leaf extract (*carica papaya* L)

CONCLUSIONS

The conclusion From the research that has been carried out it can be concluded that: There is an effect produced by papaya leaves (*carica papaya* L) as a larvicide because there is a total death of *Aedes aegypti* larvae with an index value ($P= 0.379$). Concentration of papaya leaf extract (*Carica papaya* L) To inhibit the development of *Aedes aegypti* mosquito larvae using the LD50 equation carried out in the first 2 hours, 85% papaya leaf extract is needed.

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