

The Effectivity of Ethanolic Extract from Papaya Leaves (*Carica papaya* L.) as an Alternative Larvacide to *Aedes* spp

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Abstract

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BACKGROUND: Dengue haemorrhagic fever (DHF) is a disease found in most tropical and subtropical regions of the world, including Indonesia. One of the problems of vector control programs is insecticides resistance to *Aedes* spp.

AIM: The objective of this study is to determine the effectiveness of an alternative larvacide using papaya leaves (*Carica papaya* L.).

METHODS: To obtain an ethanolic extract of *C. papaya* leaf (EECP), the dried of *C. papaya* leaf was macerated with ethanol 70%. Phytochemical compounds were screened qualitatively. Twenty-five larvae were entered into each cup that had been mixed with five concentrations of EECP i.e., EECPI (100-), EECPII (150-), EECPIII (200-), EECPIV (250), EECPV (300 ppm), 1% of Temephos (T), and water (A). Alkaloid carpain, saponin, flavonoid, tannin, glycosides and triterpenoid/steroid were traced in EECP. The mortality of larvae at 180, 360, 1440 and 2880 minutes were observed. The lethal concentration (LC50) and lethal time (LT50) were measured. Probit analysis was used to determine the concentration of killing larvae.

RESULTS: The mortality of larvae was found at 360 minutes only in EECPV. Then after 1440 minutes, all extracts shown the increasing of larvae mortality. LC50 and LT50 values were 215,96 ppm and 2,369 minutes of each.

CONCLUSION: EECP has larvicidal activity to *Aedes* spp.

Introduction

Dengue infection is a disease caused by the dengue virus found in most tropical and subtropical regions of the world. It is endemic especially in Southeast Asia, South Asia, Central America, South America and the Caribbean Islands [1]. The virus is transmitted to humans through the bites of female *Aedes aegypti* and *Aedes albopictus* [2]. The World Health Organization (WHO) estimates the incidence of dengue fever (DF) has increased over the past 50

years with attacks occurred in more than 100 million people each year and causes around 30,000 deaths, especially children [3]. The incidence of Dengue Hemorrhagic Fever (DHF) has also increased fourfold over the past three decades and there are now 2.5 billion people at risk for this disease [4]. Asia ranks first in the number of people with dengue fever annually. Between 1968 and 2009, WHO noted Indonesia as the country with the highest DHF cases in Southeast Asia with an incidence of 68.2% per 100,000 of the population [5]. According to the Indonesian Ministry of Health 2018, the incidence rate (IR) of dengue hemorrhagic fever in Indonesia in 2010

to 2017 was very fluctuating, in 2010 amounting to 65.70 per 100,000 population, 2011 down significantly to 27.67 per 100,000 population then in 2012 increase to 37.27 per 100,000 population and 2013 it became 45.85 per 100,000 population, in 2014 it down to 39.80 per 100,000 population and 2015 increase again to 50.75 per 100,000 population and 2016 to 78.85 per 100,000 population until 2017 down significantly to 26.10 per 100,000 population. In 2017 there were 68,407 dengue cases with 493 deaths. This number down quite dramatically from the previous year 204,171 cases with 1,598 deaths [6].

In the North Sumatera region, DHF is still a public health problem. The Incidence Rate (IR) in North Sumatera showed an increase in the last 5 years: 18.5% per 100,000 population, 19.8% per 100,000 population, 21.2% per 100,000 population, 24.1% per 100,000 population, and 61.4% per 100,000 population in 2012, 2013, 2014, 2015 and 2016, respectively. The IR rate of DF in North Sumatera in 2016 was above the national indicator [7].

Dengue control program must be intensified, especially at the District/City level and at the Primary Health Centres [5]. With the evidence of a continuous increase in DHF cases in the last few years, it is necessary to consider other options for vector control of the vectors.

Since 1972, the government has been using malathion for fogging to prevent dengue transmission. However, the toxin residues produced by this insecticide may accumulate in humans and interfere with blood metabolism and acetylcholinesterase enzyme (AChE) disorder causing neurological manifestations like paresthesia, tremor, balance disorders to seizures [8]. Long-term effects include carcinogenic (formation of cancerous tissue in the body); mutagenic (genetic damage for future generations), teratogenic (birth of disabled children from poisoned mothers) and environmental pollution [9]. The resistance of *Aedes* spp. to temephos, 1% has also been widely reported in Latin America (Brazil, Cuba, Argentina, Peru, and Colombia), Thailand, Banjarmasin, and Indonesia [10], [13].

Due to the negative effects of the long-term use of chemical insecticides, it is important to study natural insecticides derived from plants as an alternative to larvacide [14]. Natural larvacide, including cyanide, saponins, tannins, flavonoids, alkaloids, steroids, and essential oils have been reported to have larvicidal effect [15]. The advantages of using these natural ingredients include lower risk for soil and water pollution due to rapid degradation or decomposition by sunlight, air, humidity and other natural components, and lower toxicity in mammals [16].

Young and fresh papaya leaves are rich in flavonoid compounds, alkaloids carpaines and other polyphenol ingredients [17]. These compounds can be

potentially used as natural larvicides without harming the environment [18]. Hayatie et al. described the phytochemical constituents in *Carica papaya* L, such as alkaloid carpaines, tannins and flavonoids, have a lethal effect in larvae [19]. Alkaloid carpaines compounds have nervous toxins which kill *Aedes aegypti* larvae, however, are safe in humans [20]. Also, the saponin compounds reduce the digestive tract wall stress-causing larval death [21].

The effectiveness of plants as larvicides are generally assessed by the lethal concentration (LC) and lethal time (LT) [22]. WHO recommends the LC50 or LC90 values as the benchmarks in determining the effectiveness of a larvacide [23]. Kurniawan et al. showed that the ethanol extract of papaya leaves could kill the third instar larvae of *Aedes aegypti* with LC50 of 0.37% and LT50 at 1981 minutes [24]. When methanol extract of papaya leaves is combined with spinosad bacteria, the LC50 was 76.36 ppm on *A. aegypti* instar III and LC50 of 92.78 ppm on *A. aegypti* instar IV [25]. Others have also shown the effect of California papaya leaf extract on mortality of instar III and IV *Aedes aegypti* larvae with LC50 of 0.395% and LC90 of 0.625% [26]. These extracts have also been shown to be effective on other mosquitoes like *Anopheles* spp., and *Culex quinquefasciatus* larvae [27], [28].

Indonesia is the third-largest papaya producer in the world. Therefore, the use of papaya leaf as a source of natural insecticide is reasonable and affordable [29]. We aimed to evaluate the effectiveness of papaya leaves as natural larvicides on *Aedes* spp. instar III / IV larvae.

Material and Methods

This was an experimental study with a post-test control group design. Samples were 700 larvae of *Aedes* spp. mosquitoes, whereas each of 25 mosquito larvae was allocated in each tube with 200 ml of water in a 250 ml container according to the WHO 2005 provisions [30]. Samples in the study must meet the inclusion criteria that larvae of living *Aedes* spp. mosquitoes are at stages of instar III / IV, and *Aedes* spp. mosquito larvae are actively moving at observation.

Tools for producing papaya leaf extract include scales, tin cans for papaya leaf bases during the drying process, freeze dryer, macerator tool to form liquid extract, cotton and filter paper as filters placed in a macerator, bottles for the papaya leaf extract storage containers, and a rotavapor.

Tools to evaluate the effectiveness include 250 ml plastic cup containing 200 ml of water, pipette larvae to pick up the larvae, nylon gauze to close the

glass of larval growth, glass cups as a container to measure the volume of water, timer to measure the length of time of research and a loop to observe the movement of larvae.

The 250 ml plastic cups were divided into 7 groups to evaluate the effectiveness of 70% ethanol extract in *Aedes* spp. larvae. Of which five cups was for papaya leaf extract with concentrations of 100 ppm; 150 ppm; 200 ppm; 250 ppm; 300 ppm; one plastic cup for negative control containing distilled water (placebo, no treatment), and one plastic cup was for positive control containing temephos 1%.

Carica papaya leaf extract

The method for collecting young papaya leaves was done purposively without comparing with the same plants from other regions. Papaya leaves from the researchers' home garden, Medan Tembung, Medan City were collected, then cleaned from the dirt, washed with water until clean, afterwards dried in a freeze dryer at temperature of 50°C. The dried papaya leaves are called simplicia. Simplicia is considered dry if the material could be broken by bare hands. Simplicia was then soaked in 200 ml of 70% ethanol for 2 days then carefully placed into the macerator. The results of maceration were obtained from filtering the maceration process which was accommodated with a one-liter plastic bottle. The maceration process was repeated by adding 100 ml of 70% ethanol to the pulp then stirring and leave stand for 24 hours while continue to do stirring occasionally. The results of the second maceration was combined with the first one then stirred until evenly distributed and evaporated with a rotary evaporator so that dense extracts were obtained [31].

Phytochemical screening

The phytochemical of papaya leaf extract was screened to determine the content of secondary metabolites or active compounds in papaya leaf extract qualitatively. Compounds assessed including alkaloid group compounds such as glycosides, saponins, tannins, and flavonoids.

Larvicidal effect

Twenty-five larvae were added into each cup that has been mixed with various concentration of papaya leaf extract, negative control and positive control. The numbers of dead larvae were then counted at 180, 360, 1440, and 2880 minutes.

Data analysis

Data were not normally distributed. Kruskal-Wallis test was conducted to see the difference in the mortality of *Aedes* spp. larvae between the test

groups and followed by post hoc analysis using the Mann-Whitney test to determine the correlation value of each pair and toxicity test with probit analysis for assessing the effectiveness of papaya leaf extract against *Aedes* spp. larvae.

This study was approved by the Ethical Committee of Faculty of Medicine University of Sumatera Utara (No.617/TGL/KEPK FK USU-RSUP HAM/2018).

Results

Phytochemical of papaya leaves used in this study is described in (Table 1).

Table 1: Phytochemical screening of ethanolic extract of C. papaya leaf (EECP)

Phytochemical Test	Result	Indicator
Alkaloid Carpain	+	Formed orange deposits (Dragendorff's reagent) Formed white sediment (Mayer's reagent) Formed yellow deposits (Hager's reagent)
Flavonoid	+	Yellow fluorescence
Tannin	+	Formed in dark blue or greenish black
Saponin	+	There is foam that lasts for 10 minutes
Glicosides	+	Formed blue or green
Steroid/Triterpenoid	+	A brownish or violet ring is formed A greenish blue ring is formed
Essential Oil	-	It does not smell distinctive and there are no stains on filter paper

The average percentage of larval mortality from various observation times shows that found a significant difference in each concentration at the time observation (Table 2).

Table 2: The average percentage of larvae mortality Aedes spp. larvae between the test groups

Group	The Percentage of Larvae Mortality (Mean ± SD)				p value
	180'	360'	1440'	2880'	
Concentration 150 ppm	0 ± 0	0 ± 0	13 ± 8,2	19 ± 5,0	0.004
Concentration 150 ppm	0 ± 0	0 ± 0	19 ± 5,0	32 ± 5,6	0.002
Concentration 150 ppm	0 ± 0	0 ± 0	22 ± 2,3	52 ± 5,6	0.002
Concentration 150 ppm	0 ± 0	0 ± 0	65 ± 4,0	70 ± 4	0.003
Concentration 150 ppm	0 ± 0	1,0 ± 2,0	76 ± 6,5	85 ± 2,0	0.003
Temephos 1%	100 ± 0	100 ± 0	100 ± 0	100 ± 0	1
Aqua	0 ± 0	0 ± 0	0 ± 0	0 ± 0	1
p value	0.00	0.01	0.00	0.00	

The probit analysis in this study showed LC50 of 215.96 ppm and LT50 of 2369.64 minutes (Table 3).

Table 3: Lethal concentration 50 (LC50) and lethal time 50 (LT50)

	LC ₅₀ Concentration (ppm)	LT ₅₀ Time (minute)
Value	215.96	2369.64

Discussion

This study determined the effectiveness of papaya leaf extract from papaya leaves of Bangkok type as an alternative larvacide [16]. Qualitative phytochemical screening was carried out to determine the content of secondary metabolites from papaya leaf extract, which showed alkaloid carpaines compounds, flavonoids, saponins, tannins, glycosides and triterpenoids / steroids [32]. This finding is similar with those reported by Begum et al., Malathi et al., and Choundary et al., [29], [33], [34].

The alkaloids found in papaya leaves were alkaloids carpaines, that are compounds act by inhibiting the activity of the AChE. AChE affects the transmission of nerve impulses which causes disruption of muscle coordination, convulsions, respiratory failure and death [35]. Flavonoids work as a respiratory inhibitor or as a respiratory toxin. Flavonoids have a mechanism of action by entering the body of the larvae through the respiratory system which then causes wilting on the nerves and damage to the spinal and consequently insects will be unable to breathe and eventually die [36].

Saponins are stomach poison for cold-blooded animals, including mosquitoes. Saponins decrease the surface permeability membrane of the larval digestive tract. Hence the wall of the larvae's digestive tract becomes corrosive. Saponins inhibit the action of enzymes resulting in decreased digestive activity and the use of protein for insects [37]. Tanin causes difficulty for insects to digest food so there will be a decrease in growth [38]. Glycoside is a secondary metabolite in plants that is poisonous to stomach. It works by restraining appetite from the mosquito larvae [39]. Triterpenoids/steroid binds to free sterols in food digestion where the decreasing number of free sterols will interfere with the skin turn over the process in insects. Triterpenoid can also cause a decrease in digestive enzyme activity and affect the food absorption process. In this study, we have shown that all concentrations of papaya leaf extracts have a mortality effect on *Aedes* spp. larvae starting at 360 minutes although the significant effect was only seen at minutes 1440 and 2880. Nevertheless, their effects are much slower compared to temephos 1% which has shown an effect as early as at 180 minutes. Temephos 1% caused 100% mortality on larvae and water gave survival to the larve. This lower effectiveness of papaya leaf extract has also been seen in other studies with different amount of concentration and dose [40].

Our *post hoc* analysis also showed no significant difference in the mortality of *Aedes* spp. larvae at concentrations of 100 ppm to 300 ppm in minutes 180 to 360 but significant differences in all concentrations except for concentrations 100 ppm and 250 ppm were seen at minutes 360 to 1440 and from minutes 1440 to 2880. This result explained that the

concentration is related to the length of time of exposure and begin to appear effective at a concentration of 200 ppm, which starts at 360 minutes to 2880 minutes and increasing with the length of time of exposure. The toxic concentrations accumulated with the increased exposure time before giving an effect to the larvae.

Kurniawan et al. showed a higher LC50 level than of that found in this study but with a shorter LT50 [24]. This indicates that concentration is inversely proportional to the exposure time. Kadafi et al. used a California-type of papaya leaf had an LC50 higher than in this study [26]. This might be explained by the differences in the composition of the active ingredients which influence the larval mortality [41]. While Refai et al., used 96% ethanol as solvents and had an LC50 value 20 times fold higher than in this study [14]. This difference can be influenced by the selection of improper solvents that affect the extraction process of active compounds in this plant, thus affecting the numbers of larval mortality [31]. The use of suitable types of solvents affects the effectiveness of larvacides in papaya leaves used [42].

We have shown that papaya leaf extracts have a fatal effect on *Aedes* spp. larvae. However, further study is still needed to determine the accurate dose of these extract as a larvacide. The effect of these extracts on the larvae of other species still also needs to be studied. Differences in species factors may affect the larval mortality against the toxic insecticide power [43]. One study has shown the same fatal effect on *Anopheles* spp. although the LC50 was higher [27]. Biological factors such as the geographical location of the original plant, storing methods, and the age of plants may also give vary influence [41]. Furthermore, other factors including temperature, air humidity, water pH may affect the body resistance of each larva [44], [46].

In conclusion, lethal concentration 50 from the ethanol extract of papaya leaves valued at 215.964 ppm or 0.021% with LT50 of 2369,642 minutes are effective as *Aedes* spp. instar III and IV larvacides.

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