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Larvicidal activity of methanol fractions from *Carica papaya* leaves extract against *Aedes aegypti*

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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 compounds. Chemicals known as a standard protocol have raised concerns about resistance and dengirus 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 from toxicity assay of papaya leaf extract. According 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*.

***Key words**: Larvicidal activity, Papaya leaf, Methanol extract, Aedes aegypti, Dengue hemorrhagic fever*

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 breed-

ing 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 is also eco-friendly. A forementioned 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. This is a research grant from Ristekdikti through Research of Beginner Lecturers.

Materials and Methods

Carica papaya extraction

Fresh leaves of *Carica papaya* were shade-dried and powdered. The dry powder (150 g) was immersed with an excess of n-hexane (450 mL) for three days, in a glass jar with a watertight cover, stirred each day slowly to maintain the homogeneity. The solvent was squeezed and filtered out using Whatman filter paper number 42. The mascerated leaf powder then shade-dry and soak again using methanol as described previously. The crude extracts were filtered with the same method and stored in a bottle. In order to make a concentrated methanol extract, the extract was evaporated for two hours in a rotary evaporator apparatus with a temperature of 60 ºC and rotation of 80 rpm. The obtained extract was weighed and stored in a desiccator before being used for the fractions identification by the column chromatography method.

Fraction identification

The column for chromatography was prepared by inserting cotton in the bottom of the column tube. The mobile phase eluent was a mixture of n-hexane and ethyl acetate in a ratio of 8:2. Silica gel as a stationary phase (7 g) was dissolved in the mixture of eluent and load it into the column. Then add the eluent to the half of the column and let it rest for 24 hours. Put 1 g of extract into the column and run until it reaches down the bottom. Collect the typical fraction in one vial while keep adding the eluent continuously till the column is clear. The obtained fraction was evaporated to remove the solvent and tested with TLC method.

Larvicidal assay

Aedes aegypti was obtained from the egg rafts which were reared in beaker glass containing distilled water two days before the assay. 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. These solutions were made by pipetting 0.65 mL, 1.25 mL, 2.5 mL, 5.0 mL, and 10.0 mL of each fraction into 10.0 mL volumetric flasks and dilute with distilled water to obtain concentrations of 7000 ppm, 8000 ppm, 9000 ppm, 10000 ppm, and 11000 ppm respectively. Eventually, 20 larvae were transferred gently to the test solution. Each experiment was replicated six times. The larval mortality was in the 12 hours and calculated as a LC_{50} of concentration to total larvae used in the experiment.

Results and Discussion

After going through the multistage maceration utilizing n-hexane followed by methanol, from 150 gram of dry leaf powder, the extraction process yields 5.12 g methanol extract or equal as 3.14 with green-yellowish colour. Following six hours running in the column, 100 vials of different extract (Figure 1) were collected based on the similarity of its colour. Then, they were classified into 10 common fractions, which were identified by the RF value and colour of the TLC's spot colour under UV lamp with the wavelength of 366 nm, as shown in Figure 2.

Fig. 1. Fractions in vial

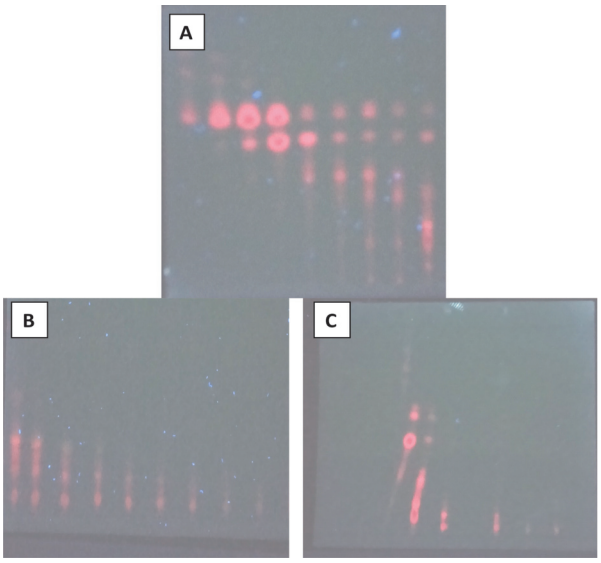

Fig. 2. Spot Colour of TLC Plate Under 366 nm UV Lamp; A)Fraction 1 until 11; B) Fraction 12 until 13 ;C) Fraction 14 until 100

This study was conducted through three pivotal stages. The first one is the extraction of *Carica papaya* leaves. Multistage maceration was chosen in order to separate unwanted compound thus higher proportion of desired metabolites obtained at the second stage. The leaf is well-known having a high amount of chlorophyll which could be extracted by adding n-hexane during the first stage of the maceration process. N-hexane, as a non-polar solvent, could easily dissolve chlorophyll without disrupting the compound that we are looking for, which mostly polar (Departemen Kesehatan Republik Indonesia, 1986; Zein, 2005). Subsequently, a high polarity solvent of methanol was used to withdraw the secondary metabolites that have larvicidal activity like alkaloid, flavonoid, and tannin. As an extract consists of multi-compound, then column chromatography was used to separate them. This second stage of the study worked based on the "like dissolve like" rule of thumb in which a polar solute will be easily dissolved to the polar solvent and vice versa (Nur *et al.*, 2014; Tuntun, 2018). Inside the column, there is an interaction between the mobile and stationary phase so that the multi-compound will be separated based on its polarity as they are going through down the column.

Larvicidal assay toward *Aedes aegypti*

As can be seen in figure 3, fraction number 1 and 7 showed the highest number of larval death though number 7 reach the same amount with the concentration of 10000ppm onwards. By contrast, number 4 and 10 did not possess larvicidal activity at all. While fraction number 2, 6, 8, 9 appear having slight difference, however, the highest concentration of all those fractions caused the highest death of larvae.

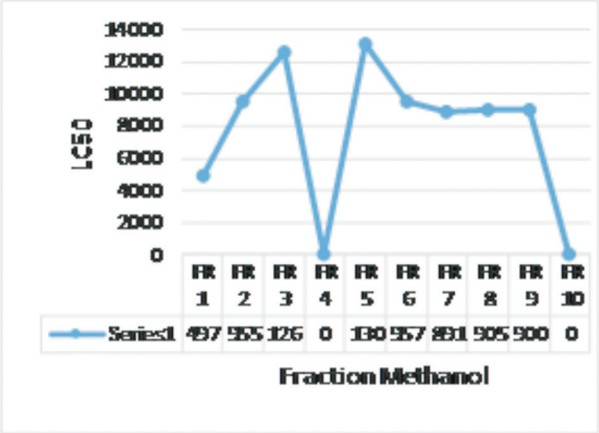

Fig. 3. Larvicidal result of methanol fraction toward *Aedes aegypti* larvae

As can be seen from Figure 3, 100 vials of fractions were narrowed down to only 10 fractions named FR1 to FR10 respectively. This classification was made depending on the number of spots as well as RF value by means of the TLC method. Later on, these 10 fractions underwent larvicidal activity against *Aedes aegypti* and resulting FR1 as the most active one. FR1 is known to possess the LC50 value with the lowest concentration of 4970 ppm. Moreover, at the highest concentration of 11000 ppm FR1 has almost the same larvae mortality rate as the control positive, indicating that this fraction has no significant different of larvicidal activity from a chemical compound though it is not even better.

In summary, we have demonstrated that methanol extract of *Carica papaya* which underwent through the fractionation process showed a robust larvicidal activity. Here, we can say that the fractionation is an integral and crucial part to obtain the desired compound as it is proved that not all of the fraction cause larvae death. However, more complex development steps are still needed to make eco-friendly larvicide optimally ready to use in the community.

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