ORIGINAL RESEARCH REPORT

Effectiveness of the Larvacide Ethanol Extract of Jackfruit (*Artocarpus heterophyllus* L.) against *Aedes aegypti* Larva

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ABSTRACT

Background: Dengue Hemorrhagic Fever (DHF) is a disease caused by the dengue virus, which enters the human bloodstream through the bite of a mosquito of the Aedes genus. The DHF control program is carried out by breaking the vector chain, the *Aedes aegypti* larvae. The underutilized jackfruit (*Artocarpus heterophyllus* L.) leaves contain secondary metabolites that have the potential to be larvicides. Secondary metabolites that have the potential as larvicides are flavonoids, saponins, tannins, alkaloids, steroids, and terpenoids. **Objective:** This study was to determine the effectiveness of jackfruit leaf extract as a larvicide in controlling *Aedes aegypti* vectors and to determine the most effective concentration of jackfruit leaf extract as an *Aedes aegypti* larvicide. **Material and Method:** The study was an analytic observational study using a cross-sectional design. The study was conducted from March 2022 to April 2022. Jackfruit leaf extraction was performed by percolation method using 96% ethanol solvent with flavonoid, saponin, tannin, alkaloid, and steroid terpenoid test. The data were analyzed with SPSS Anova (parametric) and Kruskal Wallis non-parametric. **Result:** In the extraction of 243 gr jackfruit leaves using the percolation method, the yield was 24.3%. Jackfruit leaf extract was effective as a larvicide against *Aedes aegypti* larvae, even at a concentration of 0.75%. The LC_{50} results for the total concentration obtained were 0.331% with strong toxicity (poisonous). Jackfruit leaf extract at a concentration of 2% yielded mortality value of 91.2 at 8 hours and at a concentration of 3% it yielded mortality value of 96 at 8 hours. **Conclusion:** Jackfruit leaf extract was effective as a larvicide against *Aedes aegypti* mosquito larvae.

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Highlights

1. Jackfruit leaf extraction was performed by percolation method using 96% ethanol solvent with flavonoid, saponin, tannin, alkaloid, and steroid terpenoid test.
2. Jackfruit leaf extract was effective as a larvicide against larvae of *Aedes aegypti*.

BACKGROUND

As a member of the Southeast Asia Regional Office (SEARO) of the World Health Organization, Indonesia holds a high rank in the incidence rate (IR) and case fatality rate (CFR) of dengue hemorrhagic fever (DHF) (Satoto, et al., 2020). Dengue Hemorrhagic Fever (DHF) is one of the diseases produced by the dengue virus, with a prevalence of 105.95 per 100,000 people in South Sulawesi Province in 2016-2017 (The Ministry of Health, 2014). Dengue fever outbreaks occur at high and varying incidences in Bandar Lampung each year. In the 2007-2017 period, the average incidence (per 100,000 population) was 103.97 (35.30-230.90) (Yushananta, 2021).

DHF outbreaks occur virtually annually in various locations. DHF is predicted to grow more, and its dissemination is becoming more broad (Harapan, et al., 2019). DHF transmission vector is widely used in residential and public locations, population density, population movement, and expanding urbanization (Dayani, 2020).

Dengue fever is a viral illness that is spread to humans by the bite of an infected mosquito (World Health Organization, 2019). *Aedes aegypti* and *Aedes albopictus* are the mosquito species that cause dengue (Mubbashir, et al., 2018). Since 1980 temephos has been used *en masse* for the control of the *Aedes aegypti* mosquito. For more than 25 years, insecticides including melatonin and temephos, have been used extensively in Indonesia to control *Aedes aegypti* mosquitoes, causing *Aedes aegypti* to grow resistant rapidly (Yanti, 2011).

DHF cases become high if the presence of mosquito larvae is found in the environment (Kesetyaningsih, et al., 2018). Larvae often reside and reproduce in a variety of locations, such as puddles or containers that may store water both inside and outside the home (Centers for Disease Control and Prevention, 2022). Mosquitoes infected with the *Aedes aegypty* virus and their bites will transmit dengue illness (Schaefer, et al., 2023).

Larvicides have several undesirable effects, including mosquito and larvae resistance. A larvicide is a type of insecticide used to control mosquitoes inside and outside the house. They eliminate mosquito larvae before they mature into adults. Some formulation function when mosquitos ingest them, while others work when they come into contact with the larvae (Centers for Disease Control and Prevention, 2020). Other safer alternatives can be used to reduce the side effects of using chemical insecticides. Larvicide development utilizes plants that have the potential to be sources of vegetable larvicides, such as jackfruit (*Artocarpus heterophyllus* L). At 4% concentration, it may damage or eradicate *Culex sp* larvae after 24 hours of treatment. Substances that are found in jackfruit leaves are including tannins (3.08%), flavonoids (0.92%), and saponins (1.36%) (Firdaus, et al., 2018).

OBJECTIVE

The objective of this study was to determine the concentration of jackfruit leaf extract that is effective as a larvicide for controlling *Aedes aegypti* vectors and the LC_{50} value of jackfruit leaf extract for a exterminating *Aedes aegypti* larvae.

MATERIAL AND METHOD

The study was an analytic observational study with a cross-sectional design. The study was conducted from March 2022 to April 2022. The extraction and testing of larvicides were carried out in the FMIPA Chemical Laboratory of the University of Lampung, Indonesia. Independent variables were defined as those values which influence other variables. Dependent variables were defined as those values which are influenced by other variables (Andrade, 2021). The independent variable used was the concentration of ethanol extracts and the dependent variable was the mortality of *Aedes aegypti* larvae.

For this experiment, the following implements were required: measuring cups, beakers, pipettes, volume pipettes, winnowers, blenders, stopwatches, analytical balances, cameras, knives, label paper, filter paper, plastic containers, rulers, pencils, stirring rods, funnels, Erlenmeyer, and spatulas. Jackfruit leaves (*Artocarpus heterophyllus* L.), *Aedes aegypti* mosquito larvae, 96% ethanol made in Indonesia by PT. Karya Muda Indochem (210114.001), distilled water, abate powder, HCL made in indonesia by Braco (2208195445), FeCl3 made in Indonesia by Merck (1.07174.1000), Mg powder made in Indonesia by Braco (263/MP/0617), Mayer reaction made in Indonesia by PT. Palapa Muda Perkasa (8085038811), 2 N hydrochloric acid made in Indonesia by PT. Smart Lab Indonesia (109502072001), and concentrated hydrochloric acid were the ingredients that were used for testing.

Ethanol extract of jackfruit leaves was prepared by washing the leaves, then picking and cutting them into small pieces to allow them to dry well. After drying, the leaves were put in a blender until smooth into simplisia and weighed a sample of 1000 grams of simplicya powder plus 10 liters of 96% ethanol, then poured slowly into the percolator until it was full of liquid. The percolator was closed and left for 3 hours. The percolate was allowed to drip at a rate of 1 minute per ml, and the liquid was repeatedly added and filtered until clear.

The results of this study were analyzed using the Shapiro-Wilk and ANOVA statistical tests. The hypothesis was examined statistically using IBM SPSS Statistics for Windows, version 25 (IBM Corp., Armonk, N.Y., USA). The Shapiro-Wilk test is used for valid and efficient normality tests on a small amount of samples. ANOVA is used to analyze independent and dependent variables, in this case to determine the efficacy of ethanol extracts as *Aedes aegypti* larvicides.

RESULT

The results of data analysis using the normality test Shapiro Wilk showed that the distribution was normal, and the value was significantly greater than ($p>0.05$). This means that each extract concentration of 0.75%, 1%, 2%, 3%, positive control, and negative control data was normally distributed so that it could be continued with the one-way ANOVA test.

Table 1 Treatment plan for 96% ethanol extract of jackfruit leaves on *Aedes aegypti* larvae.

Treatment	Concentration	Number of larva x repetition	Total
% Control (-)	Aquades	25 larva x 5	125 larva
% Control (+) *Temefos*	1%	25 larva x 5	125 larva
P1	0.75%	25 larva x 5	125 larva
P2	1%	25 larva x 5	125 larva
P3	2%	25 larva x 5	125 larva
P4	3%	25 larva x 5	125 larva
	Total		750 larva

Table 1 shows that the negative control group used distilled water and that the positive control, Temefos, is dissolved in distilled water until it reached a volume of 100 mL. The treatment group, on the other hand, used four different amounts (w/v) of jackfruit leaf extract solutions: 0.75%, 1%, 2%, and 3%. Each dose was mixed with distilled water until it had a volume of 100 mL and included 25 tails of *Aedes aegypti* larvae.

Table 2 Phytochemical screening test results of jackfruit leaf extract.

Qualitative Test	Results	Description
Tannins	The solution changes color to dark blue or greenish black	Positive
Flavonoids	The solution changes color to red, yellow, or orange	Positive
Saponins	The solution is green and a stable foam is formed	Positive
Alkaloids	The solution is green and a white precipitate is formed	Positive
Steroids	The solution turns purple	Positive
Terpenoids	The solution changes color to red	Positive

The findings of the phytochemical screening of jackfruit leaf extract (*Artocarpus heterophyllus* L.) are shown in Table 2. Table 2 also shows that jackfruit leaf ethanol extract contains tannins, flavonoids, saponins, alkaloids, steroids, and terpenoids (Nilakandhi, et al., 2023).

Table 3 Results of larvicide mortality effectiveness test.

Concentration (%)	Hourly mortality average					LC_{50}	P. Value
	1 hour	2 hours	5 hours	8 hours	9 hours		
K +	100	100	100	100	100		0.000
K -	0	0	0	0	0		0.000
0.75% extract	11.2	20.8	51.2	79.2	100	0.331 %.	0.000
1% extract	12	2.6	50.4	83.2	100		0.000
2% extract	12.8	26.4	60.8	91.2	100		0.000
3% extract	16	27.2	68.8	96	100		0.000

Data processing was done to determine the LC_{50} value of jackfruit leaves extract toxicity test against *Aedes aegypti* larvae. The results showed that the concentration of 0.331% was capable of killing 50% of the test larvae (Table 3).

Table 4. Results of least significant differences at 8 hours.

Treatment	0.75%	1%	2%	3%	K+	K-
0.75%		0.099	0.000	0.000	0.000	0.000
1%	0.000		0.002	0.000	0.000	0.000
2%	0.000	0.002		0.051	0.001	0.000
3%	0.000	0.000	0.051		0.099	0.000
K+	0.000	0.000	0.001	0.099		0.000
K-	0.000	0.000	0.000	0.000	0.000	

Statistical ANOVA data analysis of jackfruit leaf extract obtained a significant value of 0.000 which means that there is a significant difference so that the LSD (Least Significant Differences) further test can be carried out. According to the results of the LSD test, jackfruit leaf extract at 3% concentration for 8 hours was effective against *Aedes aegypti* larvae, which was equivalent to the positive control (temephos) ($p \geq 0.05$). This means the ethanol extract of jackfruit leaves with a concentration of 3% as a larvicide was not significantly different from the positive control with a significant value of 0.099.

DISCUSSION

Jackfruit leaves (*Artocarpus heterophyllus* L.) were dried before being used to make simplicia, resulting in an extremely fine powder (Nilakandhi, et al., 2023). Simplicia as much as 1000 grams was combined with 10 liters of 96% ethanol solvent to generate 243 grams of extract. Yield of ethanol extract from jackfruit leaves obtained 24.3%. Yield is the ratio between extracts obtained with simplicia. The higher the yield value, the more extracts obtained and the more desired analytes. In comparison between previous studies by Firdaus, et al (2018), the yield of ethanol extract of jackfruit leaves using ethanol solvent and using the maceration method had different results of 10.58%, while the yield of ethanol extract of jackfruit leaves using the percolation method was 24.3%. Simplicia may affect the amount of extract produced.

This study found that extract concentrations of 0.75%, 1%, 2%, and 3% produced larval mortality of respectively 79.2%, 83.2%, 91.2%, and 96% in 8 hours, while 1% temephos produced 100% larval mortality and negative control concentration produced 0% larval mortality. In comparison, previous research conducted by Firdaus, et al (2018) found that the fatality rate of *Culex sp*. larvae with the lowest average was discovered at a concentration of 0.25% (44%), and mortality at the maximum concentration was recorded at 1% concentration (72%). It can be determined that the ethanol extract of

jackfruit leaves was effective and harmful to the experimental animals. The lower LC_{50} value of a chemical substance suggests that the substance has a higher effectiveness in killing experimental animals because with these compounds it needs fewer concentrations to kill in long-term use. It can be concluded that the ethanol extract from jackfruit leaves was more effective in killing larvae with the lowest concentration of 0.75% (79.2%) and the highest mortality at a concentration of 3% (96%).

Then, probit analysis was performed in this study to estimate the efficiency of jackfruit leaf extract by observing the Lethal Concentration (LC_{50}) value. Probit LC_{50} analysis aims to determine the effectiveness of jackfruit leaf extract as a larvacide in controlling *Aedes aegypti* vectors and to determine the most effective concentration of jackfruit leaf extract as *Aedes aegypti* larvicide (Rahma et al., 2020). The results of the probit analysis showed the ability of jackfruit leaf ethanol extract of 0.331% by taking the extract using the percolation method. A previous study conducted by Firdaus, et al (2018) considered the potential of jackfruit leaf extract as a larvicide for *Culex sp.* mosquitoes containing alkaloids, flavonoids, saponins, steroids, and terpenoids. The LC_{50} value obtained was 0.382% by taking the extract using the maceration method. So, it can be concluded that the LC_{50} 0.331% of jackfruit leaf ethanol extract was better and had more toxic effectiveness because it used the percolation method of extracting ethanolic jackfruit leaves and the content of compounds in it were attracted by the solvent (Wigati, et al., 2023).

According to Rahmatullah, et al (2020), the LC_{50} value is very toxic in the range <1%, harmful 1-10%, moderately toxic 10-50%, slightly toxic 50-99%, and non-toxic in the range >100%. The concentration of larvicides is considered effective if it can cause the death of test larvae between 90-95% (Subramaniam et al., 2012). The results of this study showed that jackfruit leaf extract had lower killing power against *Aedes aegypti* larvae instar III and IV than positive controls.

Flavonoid compounds, saponins, tannins, alkaloids, steroids, and terpenoids have larvicidal activity against *Aedes aegypti* larvae (Rantina et al., 2022). In previous studies, saponins have a way of working as a stomach poison and inhibit the work of the cholinesterase enzyme in larvae and flavonoids act as respiratory poisons so that they can cause larval death, while tannins can reduce food intake and have an effect on the growth process of larvae so they become unable to develop and grow. Eventually, terpenoids can cause a decrease in the activity of digestive enzymes and affect the process of food absorption so that they can cause death in larvae, alkaloids, and steroids, although their benefits in the mechanism of killing larvae have not been known (Cania & Setyanimgrum, 2013).

Strength and limitations

This is the first study to use a percolation extraction method to study jackfruit leaf extract as a larvicide, therefore it can serve as a base for future studies on the same topic using different extraction techniques. The observation duration utilized to compute the larval death rate changed over time in this study, and only one species of mosquito larvae was evaluated.

CONCLUSION

Jackfruit leaf extract (*Artocarpus heterophyllus* L.) has larvicidal activity against *Aedes aegypti* larvae. It has a larvicidal efficacy class based on the LC_{50} value produced.

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Conflict of Interest

All authors have no conflict of interest.

Ethic Consideration

This research has been approved by the ethical committee of Fakultas Kedokteran Universitas Malahayati with ethics certification no. 1644/EC/KEPK-UNMAL/lll/2022 on 19-03-2022.

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Author Contribution
SM contributes to conception and design, critical revision of the article for important intellectual content, and final approval of the article. R contributes to conception of design, drafting of the article, analysis, interpretation of the data, critical revision of the article for important intellectual content and final approval of the article. DC contributes to conception and design and final approval of the article.

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