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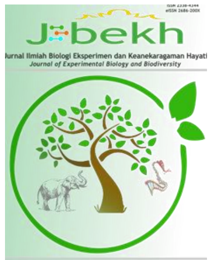

Ovicidal Potential of Ethanol Extract of Cherry Leaves (*Muntingia calabura* L.) against *Aedes aegypti* Mosquito

Potensi ovisida Ekstrak Etanol Daun Kersen (*Muntingia calabura* L.) terhadap Nyamuk *Aedes aegypti*

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Abstrak

Demam berdarah adalah salah satu penyakit virus yang ditularkan melalui artropoda dan masih merupakan masalah kesehatan yang signifikan di seluruh dunia. Virus dengue ditularkan nyamuk *Aedes aegypti* dari satu manusia ke manusia lainnya melalui hisapan darah. Nyamuk ini biasanya hidup dan bertelur di habitat yang dekat dengan genangan air. Upaya pengendalian *Ae. aegypti* yang biasa dilakukan adalah menggunakan bahan kimia alami dan sintetis. Senyawa untuk mengendalikan telur *Ae. aegy*pti disebut ovisida. Salah satu bahan alam yang diduga mengandung kimia alami potensial untuk ovisida adalah daun kersen (*Muntingia calabura* L.). Penelitian ini bertujuan mengetahui potensi ekstrak etanol daun kersen (*M. calabura* L.) sebagai ovisida terhadap *Ae. aegypti*. Ada 5 perlakuan dan 4 kali ulangan yang digunakan. Hasil penelitian ini menunjukkan bahwa ekstrak etanol daun kersen (*M. calabura* L.) memiliki potensi sebagai insektisida Ae aegypti pada konsentrasi 1% dan waktu pemaparan 72 jam.

Kata kunci: Ovisida, daun kersen, *Aedes aegypti*, Demam Berdarah *Dengue* (DBD)

Abstract

Dengue fever is one of the viral diseases transmitted through arthropods and is still a significant health problem worldwide. Dengue virus is transmitted by *Aedes aegypti* mosquitoes from one human to another through blood sucking. These mosquitoes usually live and lay eggs in habitats close to stagnant water. The usual efforts to control *Ae. aegypti* are using natural and synthetic chemicals. Compounds to control *Ae. aegypti* eggs are called ovicides. One of the natural ingredients that is thought to contain natural chemicals that have the potential to be used as an ovicide is cherry leaves (*Muntingia calabura* L.). This study aims to determine the potential of the ethanol extract of cherry leaves (*M. calabura* L.) as an ovicide against *Ae. aegypti*. Five treatments and four replications were used. The results of this study indicate that the ethanol extract of cherry leaves (*M. calabura* L.) can potentially be an insecticide for *Ae. aegypti* at a concentration of 1% and an exposure time of 72 hours.

Keywords: Ovicide, cherry leaves, *Aedes aegypti*, dengue fever

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INTRODUCTION

Dengue hemorrhagic fever (DHF) is one of the most dangerous mosquito-borne viral diseases. WHO data in 2022 revealed that DHF mainly attacks tropical countries, with the number of incidents reaching 390 million cases each year. The world's countries with a DHF risk are 70% in the Asian hemisphere, including Indonesia. The population of the DHF vector mosquito, *Aedes aegypti*, is highly susceptible to developing because of its short life cycle [1]. One of the provinces in Indonesia with relatively high cases of Dengue Fever (DHF) is Lampung Province. In 2020-2021, the number of DHF cases in this province was recorded at 6,372 cases [2].

The population and distribution of *Ae. aegypti* can be controlled using various efforts, including synthetic chemical and biological. Synthetic chemical control is more widely used because it is more practical than biological control. However, synthetic chemical control is known to pollute and damage the environment if carried out continuously. Using synthetic chemicals can cause residues that can cause disease vectors to become resistant to insecticides [3].

To prevent these problems, developing environmentally friendly natural compounds as a substitute for synthetic compounds is necessary. There are several natural compounds in plants that can control the population of *Ae. aegypti* starting from eggs (ovicides), larvae (larvicides), and adult mosquitoes (adulticides) [4]. Secondary plant metabolites that can be developed into ovicides are tannins, saponins, and flavonoids [5]. These compounds can inhibit egg hatching by disrupting egg permeability, inhibiting the exchange of fluids inside and outside the cell. When the potential of the fluid outside the cell is higher than inside the cell, the fluid inside the cell can come out, and the cell shrinks [6].

One of the plants suspected of having potential for ovicide is kersen (*M. calabura* L). The utilization of the ethanol extract of kersen leaves (*M. calabura* L.) as a larvicide has been conducted by previous researchers. The study used ethanol extract of acacia leaves for comparison with the effectiveness of ethanol extract of kersen leaves as a biolarvicide [7]. Research on certain plant extracts for ovicide has also been widely conducted. However, research on using kersen leaf extract as an ovicide has not been widely conducted. Therefore, this study was conducted.

This study aimed to determine the secondary metabolite compounds contained in the ethanol extract of cherry leaves and the potential of the ethanol extract of cherry leaves as an ovicide for *Ae. aegypti*; and to determine the morphological changes in *Ae. aegypti* eggs after administration of ethanol extract of cherry leaves.

METHODS

This study consisted of five stages: cherry leaf extraction, phytochemical test of cherry leaf ethanol extract, ovicidal potential test of cherry leaf ethanol extract, and data analysis.

Preparation of Equipment and Materials

The equipment and materials used in this study include a blender, an analytical balance, a stirring rod, an Erlenmeyer flask, a beaker, a funnel, a dropper, a measuring cup, an oven, a microscope, a rotary evaporator, a dark bottle, cherry leaves (*M. calabura* L.), and *Ae. aegypti* mosquito eggs, 96% ethanol, and distilled water.

Extraction of Cherry Plant Leaves

The cherry leaves used in this study were old leaves. One kilogram of cherry leaves was washed with running water until clean of dirt. Furthermore, the leaves were dried for approximately two days, then oven-dried at 50 °C for approximately four days until completely dry. Then, the leaves were ground using a blender until they became powder. Then, 500 grams of leaf powder was macerated by soaking it in 3 liters of 96% ethanol solution for three days. After that, the filtrate from the maceration was filtered and evaporated using a rotary evaporator to obtain the ethanol extract of cherry leaves [8].

Qualitative Phytochemical Test

Phytochemical tests were conducted to determine the content of secondary metabolites in the ethanol extract of cherry leaves. Qualitative phytochemical tests followed Harborne's (1987) and Robinson's (1995) procedures.

Tannin test: 0.5 g sample + distilled water + 2 drops of 10% $FeCl_3$ solution. Positive results are indicated by a change in the color of the sample to blackish blue.

Saponin test: 1 mL sample + warm water, then shaken for 30 seconds. The presence of foam indicates positive results.

Flavonoid test: Extract sample + ethanol + n-hexane. Then, residue + 10 ml 80% ethanol + 0.5 g Mg + 0.5 M HCl. Positive results are indicated by a change in the color of the sample to pink or purple.

Steroid test: 0.5 mL sample + 0.5 mL glacial acetic acid + 0.5 mL H_2SO_4. Positive results are indicated by a change in the color of the sample to blue or purple.

Terpenoid test: 0.5 mL sample + 0.5 mL glacial acetic acid + 0.5 mL H_2SO_4. Positive results are indicated by a change in the color of the sample to red or yellow.

Alkaloid test: 0.5 mL sample + 5 drops of chloroform liquid + 5 drops of Mayer's reagent (1 g of KCl dissolved in 20 mL of distilled water, and 0.271 g of $HgCl_2$ was added until dissolved). Positive results are indicated by a change in the color of the sample to brownish white.

Ovicidal Potential Test

The ovicidal test of the ethanol extract of cherry leaves was conducted by dividing the extract into several concentrations, namely 0.125%, 0.25%, 0.5%, and 1%. With a completely randomized design, mosquito eggs were divided into five groups each: group 1 was the control (given % extract), groups 2, 3, 4, and 5 were given cherry leaf extract of 0.125%, 0.25%, 0.5%, and 1%, respectively. The number of replications in this study was 4 times, obtained from the calculation using the following formula [9].

$$t\,(r-1) \geq 15$$
$$5\,(r-1) \geq 15$$
$$5r - r \geq 15$$
$$5r \geq 20$$
$$r \geq 4$$

t = number of treatment groups
r = number of replications

Ethanol extract of cherry leaves was put into each container according to the specified concentration, then 25 mL. *Ae. aegypti* eggs were added to the container. *Ae. aegypti* eggs were left for 24, 48, and 72 hours. Then the eggs were observed, the number of unhatched eggs was counted, and changes in egg morphology were recorded after treatment [10].

Data Analysis

The number of unhatched eggs was analyzed using one-way ANOVA. The LSD post-hoc test was used to determine the differences in effects between treatments. Observations of changes in egg morphology were analyzed descriptively and presented in tables and figures.

RESULTS AND DISCUSSION

Phytochemical Content of Cherry Leaves

The results of the phytochemical test of the ethanol extract of cherry leaves (*M. calabura* L.) are presented in Table 1. Five secondary metabolites were identified: tannins, saponins, flavonoids, alkaloids, and steroids. At the same time, the terpenoid test showed negative results. These results are from previous studies that show that the phytochemical compounds of the ethanol extract of cherry leaves are alkaloids, flavonoids, saponins, steroids, and tannins [11]. Other studies also showed the same results, where the ethanol extract of the leaves of this plant contains flavonoids, tannins, alkaloids, saponins, and steroids [12].

Factors that affect the extraction results are the type of solvent used during the maceration process. The type of solvent affects the amount of secondary metabolite compounds dissolved. Ethanol solvent (96%) can bind polar secondary metabolites. Polar phytochemical compounds include tannins, saponins, and alkaloids, while steroid and terpenoid compounds are non-polar [9]. This caused terpenoid compounds not to be detected in this study because 96% ethanol solvent cannot bind non-polar compounds. In addition, terpenoid compounds are also susceptible to modification due to oxidative reactions.

Table 1. Results of phytochemical test of ethanol extract of cherry leaves

No.	Secondary metabolite	Observation	Results
1.	Tanin	The color changes to blackish blue	+
2.	Saponin	Foam is formed	+
3.	Flavonoid	The color of the solution changes to yellow	+
4.	Alkaloid	The color changes to brownish white	+
5.	Steroid	Color changes to blue	+
6.	Terpenoid	No color change	-

Notes :
(+) : Secondary metabolite compounds present in the extract
(-) : No secondary metabolite present

Flavonoids are compounds that are thought to play the most crucial role in inhibiting the hatching power of *Ae. aegypti* mosquito eggs are damaged by damaging the eggshell layer so that the embryo is damaged. In addition, this compound also prevents the exchange of oxygen and carbon dioxide between the egg embryo and the environment. The extract covers the polygonal points on the egg's surface. Closing the polygonal points disrupts the egg embryo's respiration and metabolism, so the embryo development is disrupted and dies [11]. Tannin is also one of the compounds that can reduce cell permeability, inhibit the process of egg cell division, damage the egg cell wall, denature proteins, inhibit nucleic acid synthesis, and activate enzyme activity [12].

The rate of hatching of *Ae. aegypti* The work of the secondary metabolite compound saponin influences Aegypti eggs. Saponin damages the egg layer so that toxic compounds can enter and damage the cells. This condition causes the fluid in the cell to come out due to the potential of the extracellular fluid being higher than the potential of the intracellular fluid in the egg. The release of cell fluid also causes the egg cells to become asymmetrical and shriveled, thus affecting the typical morphology of the egg. In addition, eggs tend not to develop due to insufficient nutrition and minerals [13].

In addition to tannins, saponins, and flavonoids, the ethanol extract of cherry leaves (*M. calabura* L.) also contains alkaloid and steroid compounds. Alkaloid compounds play a role in degrading cell membranes and damaging the central nervous system. Meanwhile, steroid compounds can interfere with embryo development [14].

Number of *Ae. aegypti* eggs that did not hatch

The results of the inhibition test of cherry leaf extract on the hatching of *Ae. aegypti* eggs are presented in Figure 1. The number of mosquito eggs that did not hatch increased with increasing extract concentration. At concentrations of 0.125% and 0.25%, it could inhibit the hatching of *Ae. aegypti* eggs by up to 85.3% and 94%. Concentrations of 0.5% and 1% inhibited hatching more, namely 98.3% and 99.3%. In addition to concentration, the duration of eggs exposed to the extract also affected the egg hatching process. The number of Ae. aegypti eggs that did not hatch at 24 hours, 48 hours, and 72 hours of extract exposure also differed. The highest number of *Ae. aegypti* eggs that did not hatch were at 72 hours of exposure. In the control, the number of *Ae. aegypti* eggs that did not hatch decreased with increasing exposure time. This was because the time for the active substances to enter the eggs was insufficient [15].

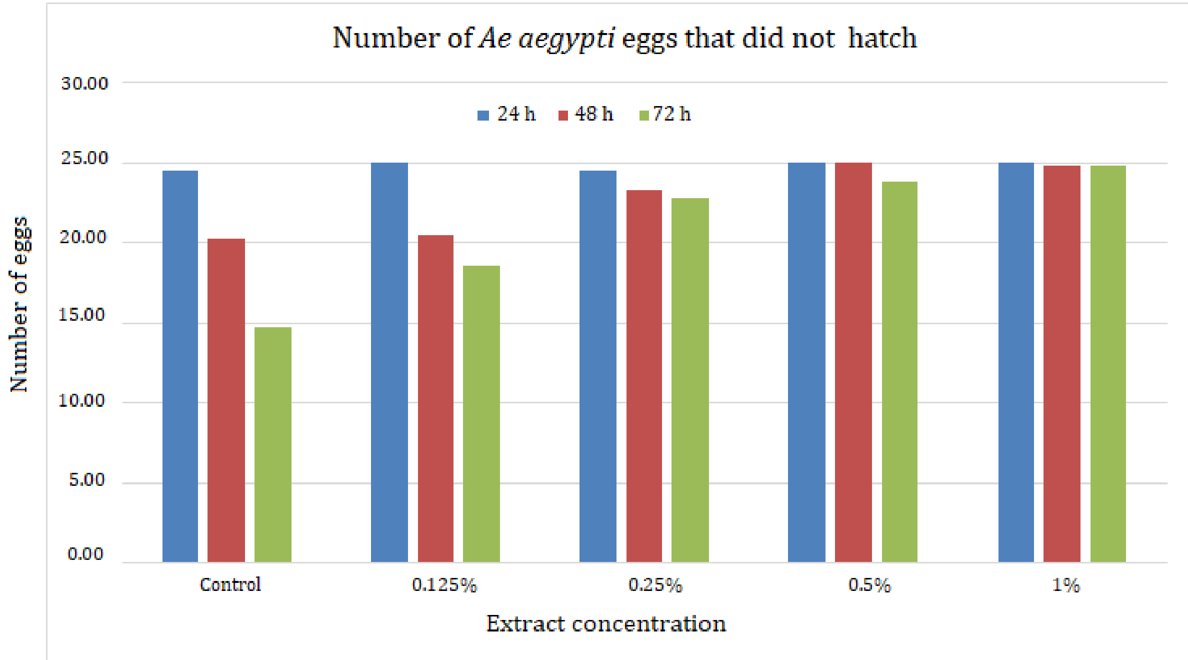

Figure 1. Number of *Ae aegypti* eggs that did not hatch after being treated with cherry leaves extract

Post hoc test data of the effect of the ethanol extract of cherry leaves on *Ae. aegypti* eggs after 72 hours are presented in Table 2. The results showed that all concentrations were different from the control. At concentrations of 1%, 0.5%, 0.25%, and 0.125%, the average number of unhatched eggs was 24,75; 23,75; 22,75; and 18,5 eggs, respectively. The best exposure time that effectively inhibited the hatching of *Ae. aegypti* mosquito eggs were 72 hours.

Table 2. Ovicidal potential of ethanol extract of cherry leaves against *Ae. aegypti* in 72 h

Extract concentration	N	Number of not-hatching eggs (Mean ± SD)
0% (kontrol)	4	14,75±4,34 (a)
0,125%	4	18,5±1,29 (b)
0,25%	4	22,75±0,95 (c)
0,5%	4	23,75±1,89 (d)
1%	4	24,75±0,5 (e)

Morphological Change of the Eggs

Ethanol extract of cherry leaves also affects the typical morphology of *Ae. aegypti* mosquito eggs (Table 3). The extract changes the shape of the eggs to be asymmetrical, the shell is damaged or torn, and the color of the shell becomes brighter. It is suspected that secondary metabolites in cherry leaves can disrupt permeability and allow toxins to enter the eggs. As a result, the morphology of the egg shell becomes wrinkled. In addition, the toxic compound can damage and tear the eggshell. The picture of mosquito egg damage due to cherry leaf extract is presented in Figure 2.

Table 3. Effect of cherry leaves extract on egg morphology

Extract concentration	Eggs morphology	Notes
0,125%	Eggs are brownish, asymmetrical, and damaged at the tips.	Severely damaged
0,25%	Eggs are asymmetrical and damaged at the ends	Moderately damaged
0,5%	The egg changes shape to become round	Moderately damaged
1%	The anterior part of the egg is damaged, and the egg turns brownish.	Severely damaged

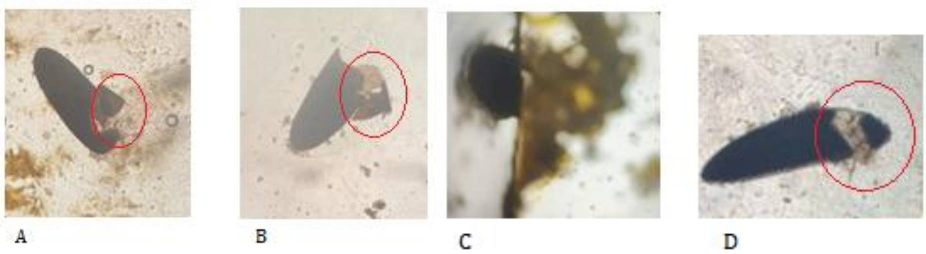

Figure 2. Morphological damage of *Ae. aegypti* eggs after being given cherry leaves extract. A. Tip of the egg damaged due to 0.125% extract; B. Tip of the egg damaged due to 0.25% extract; C. Eggs change shape due to 0.5% extract; D. Eggs change shape and color at the tip due to 1% extract.

The morphology of *Ae. aegypti* eggs can be damaged because the eggs experience severe dehydration. This dehydration is caused by fluid release from the cells because the fluid's potential outside the cell is higher than inside. As a result, the morphology of *Ae. aegypti* is asymmetrical [16].

CONCLUSION

Ethanol extract of cherry leaves (*M. calabura* L.) contains tannin, saponin, flavonoid, alkaloid, and steroid compounds with potential as *Ae. aegypti* ovicide, where the concentration of 1% is the most effective, with an average number of eggs that did not hatch reaching 99.3%, causing the most severe damage to egg morphology.

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