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EFFECTIVENESS OF BERENUK LEAF EXTRACT (*Crescentia cujete* L.) AS AN OVICIDE FOR *Aedes aegypti* L.

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ABSTRACT

One of the preventions of Dengue Hemorrhagic Fever (DHF) disease can be done using insecticides against mosquito eggs or ovicides. The application of synthetic ovicide over a long period can cause the death of natural predators and environmental pollution, so an alternative botanical ovicide made from berenuk leaf (*Crescentia cujete* L.) extract is needed. The aim of the study was to analyze active compound content of berenuk leaf extract and analyze the effect and effective concentration of berenuk leaf extract on mosquito egg morphology and hatchability. The extract was prepared by maceration method using 96% ethanol solvent. The independent variables of the study include 0 ppm (K-); *Temephos* 1% (K+); extract concentration 6.25 ppm; 12.5 ppm; 25 ppm; and 50 ppm with four replicates in each treatment. The total egg samples used were 600 eggs, and observations were made at the 24th hour, 48th hour, 72nd hour, 96th hour, and 120th hour. The results of phytochemical screening using LC-MS showed that ethanol extract of berenuk leaves contained flavonoid compounds with the highest percentage of 3.0-3.7%. The observation showed that the higher the concentration of *C. cujete* leaf extract, the higher the percentage of unhatched eggs, and the longer the *Aedes. aegypti* eggs were exposed to *C. cujete* leaf extract, the lower the percentage of unhatched eggs. The results of observing *Ae. aegypti* egg morphology using SEM showed that in K (-) the condition was oval, while the eggs exposed to the extract had damage to the surface of their shells and the eggs looked deflated. The probit test results showed a lethal concentration (LC_{50}) of 6.51 ppm at the 96th hour of observation, which means *C. cujete* leaf extract at that concentration was effectively causes 50% egg hatching failure. Based on the results of the study, berenuk leaf extract has the potential as a botanical ovicide against *Ae. aegypti* L. eggs.

Keywords: Ovicide; *Aedes aegypti* L. eggs; *Crescentia cujete* L. leaf; secondary metabolites

ABSTRAK

Salah satu kaedah pencegahan Demam Denggi Berdarah (DHF) boleh dilakukan dengan menggunakan insektisid terhadap telur nyamuk atau ovisida. Penggunaan ovisida sintetik untuk jangka masa yang lama boleh menyebabkan kematian pemangsa semula jadi dan menyebabkan pencemaran alam sekitar. Oleh itu, ovisida botani alternatif yang diperbuat

daripada ekstrak daun berenuk (*Crescentia cujete* L.) diperlukan. Tujuan kajian ini adalah untuk menganalisis kandungan sebatian aktif ekstrak daun berenuk dan menganalisis pengaruh dan kepekatan berkesan ekstrak daun berenuk terhadap morfologi telur nyamuk dan kadar penetasannya. Ekstrak disediakan melalui kaedah maserasi menggunakan pelarut etanol 96%. Pembolehubah bebas kajian termasuk 0 ppm (K-); *Temephos* 1% (K+); kepekatan ekstrak 6.25 ppm; 12.5 ppm; 25 ppm; dan 50 ppm dengan empat replikasi dalam setiap rawatan. Jumlah keseluruhan telur nyamuk yang digunakan ialah 600 biji dan pemerhatian dilakukan pada jam ke-24, ke-48, ke-72, ke-96, dan ke-120. Hasil saringan fitokimia menggunakan LC-MS menunjukkan bahawa ekstrak etanol daun berenuk mengandungi sebatian flavonoid dengan peratusan tertinggi iaitu 3.0 hingga 3.7%. Hasil pemerhatian menunjukkan bahawa semakin tinggi kepekatan ekstrak daun *C. cujete*, semakin rendah peratusan telur yang tidak menetas, dan semakin lama telur *Aedes aegypti* terdedah kepada ekstrak daun *C. cujete*, semakin tinggi peratusan telur yang tidak menetas. Hasil pemerhatian morfologi telur *Ae. aegypti* menggunakan SEM menunjukkan bahawa pada K (-) keadaan adalah bujur, manakala telur yang terdedah kepada ekstrak mengalami kerosakan pada permukaan cangkerang dan telur kelihatan kempis. Ujian probit menunjukkan bahawa kepekatan maut (LC_{50}) sebanyak 6.51 ppm pada pemerhatian jam ke-96, bermakna ekstrak daun *C. cujete* pada kepekatan tersebut berkesan menyebabkan 50% kegagalan penetasan telur. Berdasarkan hasil kajian, ekstrak daun berenuk berpotensi menjadi ovisida botani terhadap telur *Ae. aegypti* L.

Kata kunci: Ovisida; telur *Aedes aegypti* L.; daun *Crescentia cujete* L.; metabolit sekunder

INTRODUCTION

Dengue fever (DHF) remains a global public health concern due to the rapid spread of the dengue virus, which is transmitted by the *Aedes aegypti* L. mosquito. More than 7.6 million cases of *dengue* fever and more than 3000 deaths were reported to WHO in April 2024 (WHO 2024). DHF cases in Indonesia totaled 114,720 cases in 2023 and increased to 119,709 cases in June 2024 (Kemenkes RI 2024). This is due to the transmission of *dengue* virus to humans through the bite of female *Ae. aegypti* mosquitoes. This condition is supported by high population mobility, extreme climate change, and population density in an area (Sogandi & Gunarto 2020).

Prevention that can be done as an effort to stop the spread of dengue disease is vector control (Maulidy et al. 2021). Control can generally be achieved through the maintenance of environmental hygiene and chemical control such as the use of insecticides, mosquito repellents, aerosols, and fogging (Rajendran et al. 2021). Using insecticides in the form of ovicides and larvicides is the most effective method of breaking the dengue transmission chain because it lasts longer than other control methods (Montenegro-Quiñonez et al. 2023). Ovicides are organophosphate-based insecticides that can kill or inhibit egg development (Qurota'ayun et al. 2022). Long-term use of synthetic-based ovicides can kill natural predators, create mosquito egg resistance, leave long-term residues, and cause environmental pollution (Subashini et al. 2017). Based on this, alternatives are needed to reduce the spread of the *Ae. aegypti* mosquito population with natural ingredients that are more environmentally friendly, one of which is botanical ovicides (Maretta et al. 2019). One of the plants that has potential as a botanical ovicide is a plant from the Bignoniaceae family, as shown in the research by Pravin et al. (2015) that the leaf extract of plants from the Bignoniaceae family, namely *Spathodea campanulata*, contains triterpenoid, alkaloid, tannin, flavonoid, and steroid compounds that can cause death in *Ae. aegypti* mosquito eggs.

Berenuk (*Crescentia cujete* L.) is a tropical plant of the Bignoniaceae family that is easily found in Indonesia. This plant is commonly known as a calabash tree which have a various medical property such as the fruit flesh and leaves which are used to treat illnesses such as colds, asthma, diabetes, and snake bites (Gonzales et al. 2023). The leaves also can be ground and used as a traditional compress for headaches and wounds (Arel & Ningsih 2022). Currently, berenuk plants grow wild and are only used by the community as street shade, so they have not been widely researched and developed (Atmodjo & Sidharta 2023). Some previous research on the use of berenuk leaves showed that the ethanol extract of berenuk leaves contains high levels of alkaloids and saponins, so it has the potential to become an active ingredient of plant pesticides (Wuandari 2017). Research on ectoparasites was also conducted by Roman et al. (2021), who showed that ethanol extract of berenuk leaves contains flavonoid and tannin compounds that have an acaricidal effect. Therefore, berenuk leaves may have the potential to be an alternative natural ingredient as a botanical ovicide.

Previous research related to qualitative phytochemical screening showed the presence of flavonoid, phenolic, steroid, and alkaloid compounds in ethanol extracts of *C. cujete* leaves (Arel et al. 2018). Other research related to phytochemical screening of ethanol extracts of *C. cujete* leaves using LC-MS has been conducted by Martha et al. (2023) showed only the identification of flavonoid compounds contained in *C. cujete* leaf extract. However, to date, no one has reported the total content of specific active compounds in the ethanolic extract of *C. cujete* leaves using the LC-MS test. Therefore, this study was conducted to determine the active compound content of *C. cujete* leaf extract and its potential as a botanical ovicide against *Ae. aegypti* egg hatchability, as well as to analyze the morphological condition of mosquito eggs after exposure to the ovicide. The implication of this study is to explore the potential of botanical ovicides as an environmentally friendly alternative to control the population of dengue fever vectors.

MATERIALS AND METHODS

Preparation and Extraction of *Crescentia cujete* L. Leaves

Crescentia cujete leaves were collected from the campus area of the State University of Malang, Indonesia. The leaves were washed and dried using a dehydrator at 50°C and then ground into simplisia powder. *Crescentia. cujete* leaf simplisia powder is ready to be used for extract preparation.

The extract was prepared by maceration method using 96% ethanol solvent. Simplisia was weighed up to 100 g and then placed in a 1-liter Erlenmeyer flask. Then 500 ml of 96% ethanol was added and homogenized using a shaker at 120 rpm for 2 x 24 hours and then filtered. The residue from the filtration was added to another 500 ml of 96% ethanol, shaken and filtered again. The results of the first and second filtration were mixed and then evaporated in a waterbath at 50°C to obtain a paste extract. Phytochemical screening was performed on the ethanolic extract of *C. cujete* leaves using LC-MS analysis.

Egg Collection of *Aedes aegypti* L.

Aedes aegypti mosquito eggs were collected from traps using ovitrap in the campus area of the State University of Malang, Indonesia. Ovitrap is an effective method used to detect the presence of mosquito populations based on the number of eggs collected (Wan-Norafikah et al. 2020). Ovitraps consist of sponges that are placed in a dark-colored bucket and then filled with well water until half of the sponge is submerged. The ovitrap was placed in a place out of direct sunlight and waited for about 3 to 5 days.

Ovicidal Testing of *Crescentia cujete* Leaf Extracts

The first step was to prepare a 100-ppm stock solution. *C. cujete* leaf extract as much as 10 mg was dissolved in 100 ml of well water. In addition, it was used to prepare a solution of ovicides with concentrations of 6.25 ppm (P1); 12.5 ppm (P2); 25 ppm (P3); and 50 ppm (P4) using serial dilution. The negative control (K-) used 100 ml of well water and the positive control (K+) used *temephos* 1% powder at 0.008 g/100 ml.

Aedes aegypti eggs attached to the ovitrap sponge were counted to 25 eggs, then the sponge was cut with a cutter. The results of the sponge cut with eggs were placed in six plastic cups containing ovicidal medium. Then plastic cups covered with gauze and placed at room temperature. Each treatment was repeated four times for a total of 600 eggs used in the study.

Observation of *Aedes aegypti* Eggs

Egg observations were made at 24th hour, 48th hour, 72nd hour, 96th hour, and 120th hour after application. At each observation time, the number of unhatched eggs was recorded and the percentage was calculated. Mosquito eggs that did not hatch by 120 hours after application were observed using a Scanning Electron Microscope (SEM). Egg observation by SEM aims to clearly identify the egg surface, texture, and shape of the egg after treatment.

Statistical Analysis

The data from the phytochemical screening of the ethanolic extract of *C. cujete* leaves were analyzed descriptively. The data from mosquito egg observations were analyzed using one-way ANOVA test. If the analysis results were significantly different, further tests were performed using Duncan's test. The analysis of the lethal concentration (LC_{50}) of *C. cujete* leaf extract as an ovicide was performed using the probit test. The morphological data of the mosquito eggs were analyzed descriptively.

RESULTS AND DISCUSSION

The results of phytochemical screening using LC-MS showed that the ethanolic extract of *C. cujete* leaves contained 84 active compounds belonging to the flavonoid, phenolic, steroid, and alkaloid compound groups (Table 1). Among the active compounds found in *C. cujete* leaf extract, there are compounds that have the highest percentage of 3.03 - 3.75% as shown in Table 1. The active compounds are kaempferol 3-(5"-feruloylapioside), quercetin 3,7-diglucoside, and kaempferol 3-(6"-caffeoylglucoside), which belong to the group of flavonoids. In addition, the ethanolic extract of *C. cujete* leaves also showed gallic acid compounds at 2.81%. Previous research on phytochemical screening using LC-MS on methanol, ethyl acetate, and chloroform extracts of *Carica pubescens* leaves by Rahayu et al. (2019) showed the content of kaempferol compounds in methanol extracts (3.50%), ethyl acetate (3.96%), and chloroform (4.51%), while gallic acid compounds were only detected in methanol extracts (5.69%) and ethyl acetate (4.81%). The difference in percentage was due to the difference in plant species and extraction solvents used in the study, ethyl acetate and chloroform being non-polar solvents. In the ethanolic extract of *C. cujete* leaves, quercetin compounds were detected at 2.57%. This compound was also detected in the ethanol extract of the stem bark of *C. cujete* with a percentage of 2.05% (Fatimah et al. 2020). The difference in percentage is due to differences in the plant parts extracted, the plant part used being the stem bark of *C. cujete*.

Table 1. Bioactive compounds identified in LC-MS analysis of ethanol extract of berenuk (*Crescentia cujete* L.) leaves

No.	Compound Name	Composition (%)	Class of Secondary Metabolite
1	kaempferol 3-(5"-feruloylapioside)	3,74975	Phenolic (Flavonoid)
2	quercetin 3,7-diglucoside	3,35583	Phenolic (Flavonoid)
3	kaempferol 3-(6"-caffeoylglucoside)	3,03107	Phenolic (Flavonoid)
4	chlorogenic acid	3,00425	Phenolic
5	quercitrin	2,97476	Phenolic (Flavonoid)
6	gallic acid	2,81446	Phenolic
7	p-coumaric acid	2,76828	Phenolic
8	liriodendrin	2,58465	Phenolic (Flavonoid)
9	quercetin 3-O-rhamnoside	2,58438	Phenolic (Flavonoid)
10	quercetin	2,57002	Phenolic (Flavonoid)
11	kaempferol-7-rhamnoside	2,47126	Phenolic (Flavonoid)
12	ferulic acid	2,47029	Phenolic
13	epigallocatechin 3-Ocinnamate	2,35286	Phenolic (Flavonoid)
14	vitexin	2,26675	Phenolic (Flavonoid)
15	5,7-dimethoxy-4-methylcoumarin	2,08197	Phenolic
16	2-hydroxy-3-methylanthraquinone	1,57184	Phenolic (Anthraquinone)
17	napabucasin	0,92881	Phenolic (Naphthoquinone)
18	β-sitosterol	0,51738	Steroid
19	β-skytanthine	0,31607	Alkaloid
20	Actinidine	0,25908	Alkaloid

Based on the results of the one-way ANOVA test, it shows that there is an effect of *C. cujete* leaf extract concentration on the hatchability of *Ae. aegypti* mosquito eggs. In addition, other tests were performed, namely the Duncan's test to determine the difference in the percentage of mosquito eggs that did not hatch between concentrations of *C. cujete* leaf extract by giving notations as in Table 2. The data on the percentage of mosquito eggs that did not hatch in Table 2 are presented graphically in Figure 1.

Table 2. Percentage of mosquito eggs that did not hatch after treatment

Treatment	Average Percentage of Unhatched Mosquito Eggs (%)±SE				
	24 hours	48 hours	72 hours	96 hours	120 hours
K+	100±0.0^{c}	100±0.0^{d}	100±0.0^{e}	100±0.0^{e}	100±0.0^{f}
K-	70±4.2^{a}	55±5.0^{a}	43±4.4^{a}	29±4.7^{a}	3±1.9^{a}
P1 (6.25 ppm)	76±1.6^{ab}	62±2.6^{a}	57±3.4^{b}	50±2.6^{b}	36±1.6^{b}
P2 (12.5 ppm)	83±3.4^{b}	73±3.4^{b}	68±3.7^{c}	61±4.1^{c}	47±1.9^{c}
P3 (25 ppm)	93±1.9^{c}	80±3.7^{b}	73±4.4^{c}	67±3.0^{c}	56±1.6^{d}
P4 (50 ppm)	97±1.9^{c}	90±2.6^{c}	86±2.6^{d}	81±3.0^{d}	70±3.8^{e}

Note: The same notation given to the average percentage of mosquito eggs that do not hatch shows that the meaning is not significantly different based on the Duncan test ($P<0.05$).

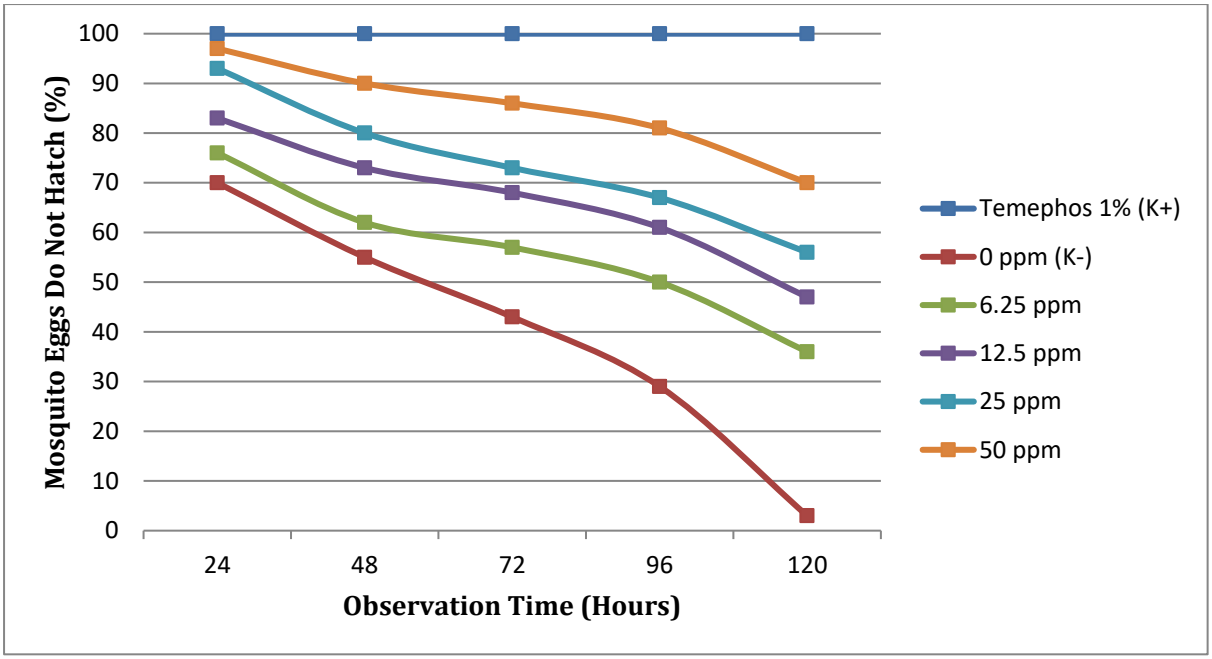


Figure 1. Percentage of eggs *Aedes aegypti* L. that did not hatch after treatment

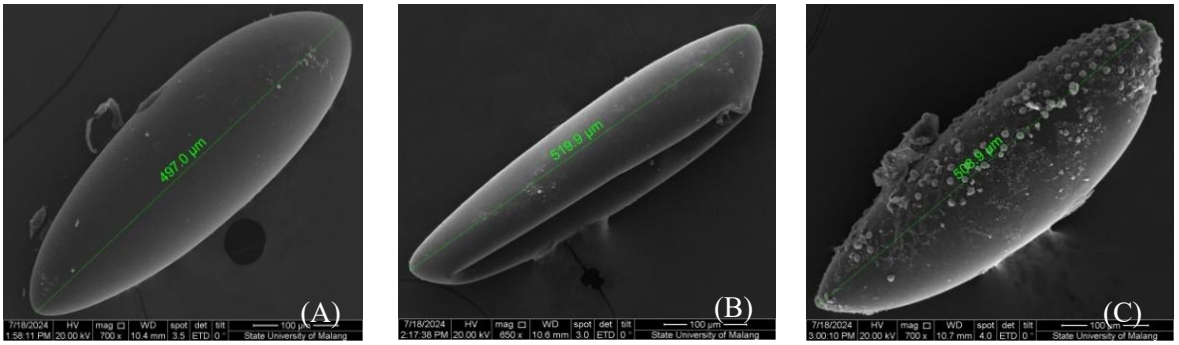
Based on research conducted on *Ae. aegypti* eggs using *C. cujete* leaf extract at concentrations of 6.25 ppm, 12.5 ppm, 25 ppm, and 50 ppm, it shows that all concentrations of *C. cujete* leaf extract cause hatchability failure of *Ae. aegypti* mosquito eggs. Egg hatchability is the ability of eggs to develop in the embryological process until the eggs hatch (Cahyanurani et al. 2023). Table 2 shows that the higher the concentration of *C. cujete* leaf extract, the lower the percentage of hatchability of mosquito eggs, so that *C. cujete* leaf extract is effectively used as an ovicide for *Ae. aegypti* mosquito eggs. This was demonstrated by comparing the difference in the percentage of unhatched eggs in the control and treatment groups from 24th to 120th hours of observation. In this study, well water was used as the ovicide medium and 120th hours of observation were required to obtain the maximum number of unhatched eggs. This is because *Ae. aegypti* mosquito eggs can hatch into larvae in approximately 1 to 5 days when submerged in rainwater or dug well water (Fahri et al. 2019). This is supported by the results of a previous study that showed differences in the hatching time of *Ae. aegypti* mosquito eggs in different water sources and found that the ability to hatch mosquito eggs was greater in dug well water, which takes 1-5 days, compared to rainwater, which takes 1-4 days (Prameswarie et al. 2023).

Based on Table 2, in the positive control, mosquito eggs did not hatch from the 24th until the 120th observation hour, thus having the highest percentage of unhatched mosquito eggs at 100%. This result is similar to a previous study using *temephos* as a positive control for *Ae. aegypti* mosquito ovicide, which showed that *temephos* can cause approximately 80% of mosquito eggs not to hatch at 120th hour (Yagoo et al. 2023). It is evident that *temephos* can penetrate the egg exochorion and cause the eggs of *Ae. aegypti* mosquitoes to fail to hatch. In the negative control, the percentage of eggs that failed to hatch by 120th hours after application was only 3%. There was an increase in the percentage of mosquito eggs that did not hatch at *C. cujete* leaf extract concentrations ranging from 6.25 ppm to 50 ppm, so the higher the concentration of *C. cujete* leaf extract ovicide, the higher the percentage of unhatched eggs, and the longer the *Ae. aegypti* eggs were exposed to *C. cujete* leaf extract, the lower the percentage of unhatched eggs.

Based on the graph in Figure 1, it shows that *C. cujete* leaf extract can be used as an ovicide because it is able to cause *Ae. aegypti* eggs not to hatch into larvae. The highest percentage of *Ae. aegypti* mosquito eggs that did not hatch after being treated at the 120th hour with a concentration of 50 ppm was 70%. Figure 1 shows that there was a decrease in the percentage of unhatched eggs in the negative control as well as the 6.25 ppm, 12.5 ppm, 25 ppm, and 50 ppm treatments from the first 24th hours to the 120th hour. The positive control, *temephos* 1%, showed 100% unhatched mosquito eggs from the 24th to the 120th hour of observation, which means that *temephos* can directly inhibit eggs from hatching into larvae at the 24th hour of observation. Figure 1 also shows that there is an increase in the percentage of *Ae. aegypti* mosquito eggs that do not hatch. This is because the higher the concentration of *C. cujete* leaf extract, the higher the content of secondary metabolites.

Active compounds in the ethanolic extract of *C. cujete* leaves, such as flavonoids, are compounds with a high degree of polarity. Flavonoid compounds can enter the mosquito eggshell through polygonal dots found on the outer layer of the eggshell so that they can damage the embryo through the egg chorion pore (Muhajir et al. 2023). Flavonoids can also enter the egg through the mosquito egg microfilament and diffuse into the egg membrane, disrupting metabolic processes and causing embryos to fail to develop and hatch into larvae (Sari 2018). Flavonoid compounds that enter mosquito eggs cause fluid to escape from the egg or dehydrate the egg (Martini et al. 2018). In addition, there are phenolic compounds, steroids, and alkaloids found in the leaves of berenuk. Phenolics and steroids are known to be toxic compounds that can inhibit the development of *Ae. aegypti* eggs (Wahyuni et al. 2023). The alkaloid compound can cause eggs to fail to hatch by breaking down the egg cell wall, damaging *Ae. aegypti* eggs (Aditama et al. 2022).

Morphology of mosquito eggs is observed by Scanning Electron Microscope (SEM) at the 120th hour observation, the differences between normal *Ae. aegypti* eggs, positive controls and eggs treated with botanical ovicides will be seen as shown in (Figure 2). Based on Figure 2, the morphological condition of *Ae. aegypti* eggs in the negative control treatment are oval round, so they can still hatch. In contrast, the condition of eggs in the treatment exposed to *temephos* compounds showed damage to the eggshell with a deflated shell. Furthermore, in the picture of mosquito eggs after treatment with botanical ovicides of berenuk leaf extract (Figure 2), there are differences in the morphological condition of the egg surface. In Figures C to F, the condition of unhatched mosquito eggs shows that there is damage to the exochorion surface of *Ae. aegypti* eggs, the shape of the egg becomes increasingly deformed, and the eggshell looks increasingly wrinkled as the concentration of botanical ovicides increases. This is consistent with a previous study conducted by Pravin & Mohanraj (2019), where SEM results showed that *Ae. aegypti* eggs were damaged after treatment with hexane extract of *Spathodea campanulata* leaves.



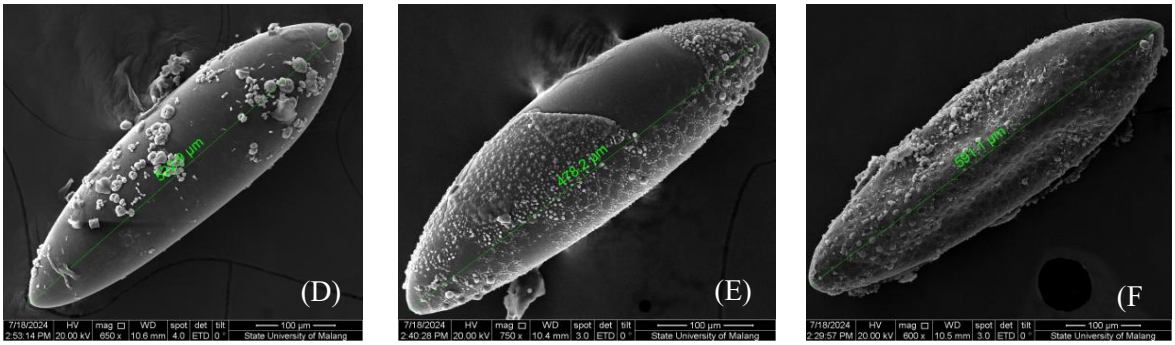


Figure 2. (A) Negative control eggs *Aedes aegypti* L.; (B) Positive control eggs of *Aedes aegypti* L.; Eggs after being treated with ovicide from ethanol extract of berenuk (*Crescentia cujete* L.) leaves; (C) 6.25 ppm; (D) 12.5 ppm; (E) 25 ppm; (F) 50 ppm

The lethal concentration (LC_{50}) of *C. cujete* leaf extract as *Ae. aegypti* egg ovicide is the concentration of leaf extract that effectively causes 50% egg hatching failure. The data obtained from the probit test conducted with SPSS software. The results of the probit test are given in Table 3. Based on the results of probit test, it shows that ethanol extract of *C. cujete* leaves effectively causes *Ae. aegypti* mosquito eggs unhatched. This is because the higher the concentration (Figure 1) of ethanol extract of *C. cujete* leaf ovicide, the higher the bioactive compounds contained in it. The lethal concentration value (LC_{50}) is found at the 96th observation hour, which is 6.51 ppm, so the longer the observation time required, the higher the concentration of *C. cujete* leaf ethanol extract ovicide needed to reach the effective concentration.

Table 3. LC_{50} value of berenuk (*Crescentia cujete* L.) leaf extract on the hatchability of mosquito eggs

Observation Time	**Lethal Concentration (LC_{50}) in ppm**
96 hours	6.51

Previous research on *Ae. aegypti* larvicides from ethanol extracts of *C. cujete* fruit flesh and skin obtained lethal concentration values (LC_{50}) of 3112.767 ppm for fruit flesh and 3415.007 ppm for fruit skin at the 96th observation hour (Hoeriah 2023). The difference in the effective concentration value compared to the effective concentration value in this study is due to the differences in the research object and the extracted plant parts, the object being the larvae of *Ae. aegypti* mosquitoes and the plant parts being the flesh and skin of *C. cujete* fruit. Previously conducted *Ae. aegypti* ovicide research using the essential leaf oil of *Tetradenia riparia* showed an LC_{50} value of 1570.505 ppm at the 24th hour of observation (Rabelo et al. 2024). It is known that *T. riparia* belongs to the same order as *C. cujete*, namely the Lamiales. However, there is the difference in the effective concentration value when compared to the effective concentration value in this study is due to differences in plant species, as well as extraction methods and solvents, that the material used for ovicides is the result of essential oil extraction from *T. riparia* leaves.

Another research related to *Anopheles stephensi* egg ovicides from methanol extracts of *Pajanelia longifolia* leaves showed a lethal concentration value (LC_{50}) of 1223.95 ppm at 48th hours of observation (Sowmyashree et al. 2023). *P. longifolia* belongs to the same family

as *C. cujete*, namely the Bignoniaceae. The difference in the effective concentration values is due to the differences in the research objects, plant species, extraction methods, and solvents. The object used in the study was *Anopheles stephensi* mosquito eggs, and the material used for ovicides was the result of methanol extraction from *P. longifolia* leaves. The difference in the lethal concentration (LC_{50}) values may be due to the content of different secondary metabolite compounds (Zuraida 2018). Each plant has different secondary metabolite compounds that can affect the effective concentration value. This is because the production of secondary metabolites in plants is not continuous, but only used for specific purposes such as attracting other organisms, protection against pathogens, and adaptation to the environment (Dalimunthe & Rachmawan 2017).

CONCLUSION

This study concludes that the extract of berenuk leaves (*Crescentia cujete* L.) is effective in inhibiting the hatching of *Ae. aegypti* mosquito eggs and have the potential as a botanical ovicide material that can control the population of Dengue Hemorrhagic Fever vectors naturally and safely for the environment. In general, this study shows that flavonoids are compounds with the highest percentage of 3.0-3.7% contained in the ethanolic extract of berenuk leaves. The highest percentage of *Ae. aegypti* eggs that unhatched was eggs that had been treated for 120 hours with a concentration of 50 ppm, which was 70%. Under SEM it can be seen that the eggs were damaged on the surface of their exochorions and egg shells that looked increasingly wrinkled as the concentration of ovicide increased. Further studies on this extract of berenuk leaves need to be carried out by testing its efficacy in the field.

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AUTHORS DECLARATIONS

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Conflict of Interest
The authors declare that there is no conflict of interest.

Ethics Declarations
There are no ethical issues required in this research.

Data Availability Statements
The data obtained from research or that has been analyzed is presented in this article.

Author's Contributions
Nurul Izzatin Niswah (NIN) contributed to conducting the research, analyzing the data, and writing the draft manuscript. Sofia Ery Rahayu (SER) contributed to conceptualizing the research, supervising the research, reviewing and editing the draft manuscript. Frida Kunti Setiowati (FKS) contributed to reviewing tha data analysis and draft manuscript.

REFERENCES

Aditama, W., Nasrullah, Khairunissa, Zulfikar, Arianto, B. & Darmiati. 2022. Perbedaan ekstrak biji dan daun alpukat (*Persea americana* Mill) sebagai ovisida terhadap nyamuk *Aedes aegypti*. *Buletin Kesehatan Lingkungan Masyarakat* 41(4): 174-180.

Arel, A. & Ningsih, W. 2022. Isolasi senyawa ekstrak etil asetat daun tumbuhan berenuk (*Crescentia cujete* L.). *Forte Jurnal* 2(1): 67-73.

Arel, A., Wardi, E.S. & Oktaviani, Y. 2018. Profil metabolit sekunder ekstrak daun berenuk (*Crescentia cujete* L.) dan uji sitotoksik dengan metode *Brine Shrimp Lethality Test*. *Jurnal Katalisator* 3(2): 82-88.

Atmodjo, P.K. & Sidharta, B.R. 2023. The secondary metabolite and antibiotic activity of calabash leaf and fruit (*Crescentia cujete* L.) ethanol extract. *SENAPAS Proceedings* 1(1): 322-326.

Cahyanurani, A.B., Ramadhani, I., Suprihadi, Widodo, A. & Arifin, M.Z. 2023. Kajian pembenihan ikan baung (*Hemibagrus nemurus*) yang dipijahkan secara semi alami. *Jurnal Perikanan* 13(1): 51-61.

Dalimunthe, C.I. & Rachmawan, A. 2017. Prospek pemanfaatan metabolit sekunder tumbuhan sebagai pestisida nabati untuk pengendalian patogen pada tanaman karet. *Warta Perkaretan* 36(1): 15-28.

Fahri, S., Ariyani, S., Yamistada, G. & Manulang, E.S. 2019. Hatchability of the eggs *Aedes* spp. in clean and polluted water. *KnE Life Sciences* 2019: 134-140.

Fatimah, Martha, R.D. & Kusumawati, A. 2020. Deteksi dan identifikasi senyawa flavonoid ekstrak etanol kulit batang tanaman majapahit (*Crescentia cujete*) dengan LCMS. *CHEESA: Chemical Engineering Research Articles* 3(2): 88-98.

Gonzales, A.L., Sevilla, U.T.A. & Tsai, P. 2023. Pharmacological activities of bioactive compounds from *Crescentia cujete* L. plant - a review. *Biointerface Research in Applied Chemistry* 13(2): 1-24.

Hoeriah, V. 2023. *Efektivitas Ekstrak Etanol Buah Berenuk (Crescentia cujete Linnaeus.) Sebagai Larvasida Aedes aegypti*. Malang: Universitas Negeri Malang.

Kemenkes, RI. 2024. Sehat negeriku: waspada DBD di musim kemarau. https://sehatnegeriku.kemkes.go.id/baca/rilis-media/20240616/0045767/waspada-dbd-di-musim-kemarau/ [16 Juni 2024].

Maretta, G., Kuswanto, E. & Septikayani, N.I. 2019. Efektivitas ekstrak daun patikan kebo (*Euphorbia hirta* L.) sebagai ovisida terhadap nyamuk Demam Berdarah *Dengue* (*Aedes aegypti*). *BIOSFER: Jurnal Tadris Biologi* 10(1): 1-9.

Martha, R.D., Fatimah, Danar & Parbuntari, H. 2023. Identification of flavonoid compounds in ethanol extract of majapahit plant (*Crescentia cujete*) leaves and their potential as anticancer. *CHEESA: Chemical Engineering Research Articles* 6(1): 34-48.

Martini, Astriana, N., Yuliawati, S., Hestiningsih, R., Mawarni, A. & Purwantisari, S. 2018. Keefektifan ekstrak daun kecubung (*Datura metel* L.) dalam menghambat penetasan dan siklus hidup *Aedes aegypti* L. *Jurnal Entomologi Indonesia* 15(1): 50-56.

Maulidy, F.R., Wydiamala, E. & Biworo, A. 2021. Aktivitas ekstrak etanol daun pucuk merah (*Syzygium myrtifolium* Walp.) sebagai ovisida dan *Insect Growth Regulator* terhadap *Aedes aegypti*. *Homeostasis* 4(2): 335-344.

Montenegro-Quiñonez, C.A., Louis, V.R., Horstick, O., Velayudhan, R., Dambach, P. & Runge-Ranzinger, S. 2023. Interventions against *Aedes*/*dengue* at the household level: A systematic review and meta-analysis. *EBioMedicine* 93: 104660.

Muhajir, N.F., Nadifah, F., Afridayanti, N. & Arisandi, D. 2023. *Perasan Buah Pare (Momordica charantia) Sebagai Ovisida Aedes aegypti*. Surabaya: Seminar Nasional Kesehatan UNIPA.

Prameswarie, T., Ramayanti, I., Ghifari, A., Hartanti, M.D., Anggina, D.N., Silvana, R. & Ismail. 2023. *Aedes aegypti* hatchability and larval development based on three different types of water. *Majalah Kesehatan Indonesia* 4(1): 27-32.

Pravin, Y. & Mohanraj, R.S. 2019. Bio efficicay of *Spathodea campanulata* P. Beauv. (Bignoniaceae) hexane leaf extract against *Dengue* and Zika virus vector *Aedes aegypti* (Diptera: Culicidae). *International Journal of Advanced Research (IJAR)* 7(2): 657-672.

Qurota'ayun, S.D., Rosa, E., Pratami, G.D. & Kanedi, M. 2022. Potential of pepper leaf (*Piper nigrum* L.) ethanol extract as ovicide for *Aedes aegypti*. *Jurnal Sains Natural* 12(4): 170-175.

Rabelo, E.M., Martins, R.L., Rodrigues, A.B., de Sousa, R.S., Farias, A.L.F., Galardo, A.K.R., Cantuaria, P.C., Tomazi, R. & de Almeida, S.S.M.S. 2024.Composition and bioactivity from the essential oil of *Tetradenia riparia* in pathogenic bacteria, *Aedes aegypti* L. (Diptera: Culicidae), and toxicological evaluation of *Mus musculus* (Rodentia: Muridae). *Preprints* 2024: 2024030105.

Rahayu, S.E., Sulisetijono & Lestari, U. 2019. Phytochemical screening, antioxidant activity, and total phenol profile of *Carica pubescens* leaves from Cangar, Batu-East Java, Indonesia. *IOP Conf. Series: Earth and Environmental Science* 276(012022): 1-7.

Rajendran, D., Adnan, F.N., Besar, A.U.A., Yusoff, M. & Zuharah, W.F. 2021. Status of insecticide resistance on *Aedes aegypti* (L.) and *Aedes albopictus* (Skuse) in Kampar, Perak, Malaysia. *Serangga* 26(2): 245-254.

Roman, B.K., Laut, M.M. & Almet, J. 2021. Aktivitas bioinsektisida ekstrak daun maja (*Crescentia cujete* Linn.) terhadap *Rhipicephalus sanguineus* dari anjing lokal. *Jurnal Kajian Veteriner* 9(3): 203-212.

Sari, A.N. 2018. *Efektivitas Daun Kemangi (Ocimum sanctum L.) Sebagai Ovisida Terhadap Nyamuk Aedes aegypti*. Lampung: FITK UINRIL.

Sogandi, S. & Gunarto, F. 2020. Efek larvasida fraksi etil asetat daun bangun-bangun (*Plectranthus amboinicus*) terhadap mortalitas larva *Aedes aegypti*. *Aspirator: Journal of Vector-borne Disease Studies* 12(1): 27-36.

Sowmyashree, K., Chalannavar, R.K. & Nityasree. B.R. 2023. The efficacy of *Pajanelia longifolia* (Willd.) K. Schum leaf extracts against the malaria vector *Anopheles stephensi* Liston (Diptera: Culicidae). *Plant Science Today* 10(2): 316-324.

Subashini, K., Sivakami, R. & Jeyansankar, A. 2017. Phytochemical screening and ovicidal activity of *Scutellaria violacea* (Lamiaceae) leaf extract against vector mosquitoes (Diptera: Culicidae). *International Journal of Advance Research in Biological Sciences* 4(3): 152-158.

Wahyuni, D., Swandono, H.U., Mawardika, H. & Prana, M.Y. 2023. Karakterisasi dan potensi ekstrak daun paitan (*Tithonia diversifolia*) sebagai penolak nyamuk *Aedes aegypti*. *Bioscientist: Jurnal Ilmiah Biologi* 11(2): 1150-1160.

Wan-Norafikah, O., Khairul-Hazim, K., Nurul-Najiyah, M., Siti-Nurdiyana, M., Nur-Syaahidah, K. & Atiqah-Nazirah, R. 2020. Dispersal of *Aedes aegypti* Skuse (Diptera: Culicidae) in a university campus in Selangor, Malaysia. *Serangga* 25(2): 123-131.

WHO (World Health Organization). 2024. Disease outbreak news: *Dengue* – global situation. https://www.who.int/emergencies/disease-outbreak-news/item/2024-DON518. [30 Mei 2024]

Wuandari, M.S. 2017. Efektivitas daun majapahit (*Crescentia cujete*) sebagai pestisida nabati *Spodoptera litura* pada sawi (*Brassica juncea*). *Jurnal Prodi Biologi* 6(4): 245-254.

Yagoo, A., Milton, M.C.J., Vilvest, J., Johnson, I. & Balakrishna, K. 2023. Mosquito larvicidal, pupicidal, and ovicidal effects of the different extracts of the leaves of *Peltophorum pterocarpum* against *Aedes aegypti* and *Culex quinquefasciatus*. *Future Journal of Pharmaceutical Sciences* 9(32): 1-7.

Zuraida. 2018. Analisis toksisitas beberapa tumbuhan hutan dengan metode *Brine Shrimp Lethality Test* (BSLT). *Journal of Forest Products Research* 36(3): 239-246.