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ARTICLE

THE EFFECTIVENESS OF *Eucheuma cottonii* EXTRACT AGAINST DENGUE VECTOR LARVAE AS AN EVALUATION FOR NATURAL LARVICIDE DEVELOPMENT

[*Efektivitas Ekstrak Eucheuma cottonii Terhadap Larva Vektor Dengue Sebagai Evaluasi Untuk Pengembangan Larvisida Alami*]

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ABSRACT

Dengue fever (DHF) is a tropical disease transmitted by the *Aedes aegypti* mosquito. Prolonged use of synthetic chemical larvicides such as Abate® has led to resistance and environmental impacts. Therefore, an alternative natural larvicide that is more environmentally sustainable is needed. *Eucheuma cottonii* is a red alga known to contain bioactive compounds such as flavonoids, alkaloids, terpenoids, saponins, and tannins that can potentially inhibit mosquito larvae development. This study aims to evaluate the effectiveness of the ethanol extract of *E. cottonii* on the mortality of *Aedes aegypti* instar III larvae as a basis for the development of natural biolarvicides. The study used a completely randomized design (CRD) with six treatments: four extract concentrations (50 ppm, 100 ppm, 200 ppm, and 300 ppm), one positive control (Abate®), and one negative control (water). Each test unit contained 20 third-instar larvae with four replicates. Observations were made 4, 8, 24, 48, and 72 hours after treatment. The highest larval mortality was achieved at a 200 ppm concentration of 13.25 ± 3.94. The LC_{50} value at 24 hours was 42.35 ppm, while the LT_{50} reached 52.77 hours, indicating a slow and less effective larvicidal effect at the test concentration. Although the ethanol extract of *E. cottonii* contains bioactive compounds, its effectiveness as a larvicide against *Aedes aegypti* larvae is still relatively low. These results indicate that *E. cottonii* is not optimally used alone as a bio-larvicide, but still has the potential to be further developed through improved extraction methods, increased concentrations, or combination formulations. This study provides a foundation for creating sustainable and environmentally friendly plant-based larvicides.

Keywords: *Aedes aegypti,* biolarvicide, DBD, ethanol, *Eucheuma cottonii*

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ABSTRAK

Demam Berdarah Dengue (DBD) merupakan penyakit tropis yang ditularkan oleh nyamuk Aedes aegypti. Penggunaan larvasida berbahan kimia sintetis seperti Abate® secara berkepanjangan menimbulkan resistensi dan dampak lingkungan. Oleh karena itu, diperlukan alternatif larvasida alami yang lebih ramah lingkungan. Eucheuma cottonii merupakan alga merah yang diketahui mengandung senyawa bioaktif seperti flavonoid, alkaloid, terpenoid, saponin, dan tanin yang berpotensi menghambat perkembangan larva nyamuk. Penelitian ini bertujuan untuk mengevaluasi efektivitas ekstrak etanol E. cottonii terhadap mortalitas larva Aedes aegypti instar III sebagai dasar pengembangan biolarvasida alami. Penelitian menggunakan Rancangan Acak Lengkap (RAL) dengan enam perlakuan: empat konsentrasi ekstrak (50 ppm, 100 ppm, 200 ppm, dan 300 ppm), satu kontrol positif (Abate®), dan satu kontrol negatif (air). Setiap unit uji berisi 20 larva instar III dengan empat kali ulangan. Pengamatan dilakukan pada 4, 8, 24, 48, dan 72 jam setelah perlakuan. Mortalitas tertinggi larva dicapai pada konsentrasi 200 ppm sebesar 13,25 ± 3,94. Nilai LC_{50} pada 24 jam adalah 42,35 ppm, sedangkan LT_{50} mencapai 52,77 jam, menunjukkan pengaruh larvasida yang lambat dan kurang efektif pada konsentrasi uji. Meskipun ekstrak etanol E. cottonii memiliki kandungan senyawa bioaktif, efektivitasnya sebagai larvasida terhadap larva Aedes aegypti masih tergolong rendah. Hasil ini menunjukkan bahwa E. cottonii belum optimal digunakan secara tunggal sebagai biolarvasida, namun tetap memiliki potensi untuk dikembangkan lebih lanjut melalui peningkatan metode ekstraksi, peningkatan konsentrasi, atau formulasi kombinasi. Penelitian ini memberikan landasan awal untuk pengembangan larvasida nabati yang berkelanjutan dan ramah lingkungan.

Kata kunci: *Aedes aegypti, biolarvasida, DBD, etanol, Eucheuma cottonii*

INTRODUCTION

Dengue fever (DHF) is still one of the environmental health problems, especially in Indonesia. Over time, DHF tends to increase the number of sufferers and widen the spread area. The Ministry of Health of the Republic of Indonesia noted that in 2016, there were 201,885 dengue patients in all regions of Indonesia. In contrast, as many as 1,585 people died due to dengue virus attacks that move into the human body through *Aedes aegypti* mosquito bites (Kemenkes RI, 2017). According to Nurafif and Hardi (2015), Dengue fever occurs due to dengue virus infection by the *Ae. Aegypti mosquito, which causes acute fever for 2-7 days with two or more manifestations such as headache, retro-orbital pain, myalgia, arthralgia, skin rash, bleeding manifestations, leukopenia*, and thrombocytopenia (100,000 cells per mm3 or less). DHF disease will be mild if a person experiences several symptoms of DHF disease and immediately seeks medical attention to obtain further medical action, so as not to endanger the patient's life.

DHF disease can be prevented by eradicating the larvae of infectious mosquitoes, known as Mosquito Nest Eradication - Dengue Hemorrhagic Fever in Indonesia. Another effective mosquito larvicide control method is applying a substance that kills larvae to suspected breeding sites. This method of mosquito larvae control is usually known as larvicide (Verawaty *et al*., 2019). Seaweed is one type of algae that can be used as a natural ingredient for making larvicides or biolarvicides because it contains flavonoids, saponins, and other compounds that can inhibit and eradicate the development of *Ae. aegypti* mosquito larvae. One type of seaweed that can be used as a biolarvicide for *Ae. aegypti mosquito larvae are E. cottonii. E. cottonii* is a multicellular red alga that is thought to have secondary metabolite compounds such as alkaloids, flavonoids, triterpenoids, and steroids (Khurniasari, 2004; Noshirma & Willa, 2016). These compounds are assumed to inhibit the development of mosquito larvae during the larval, pupal, and adult stages, together with the classes of active compounds include terpenes, alkaloids and amides, steroids, flavonoids, furanochromones, phenylpropanoids and phenol derivatives, lignans and neolignans, naphthoquinones, fatty acids and their derivatives (Silvério *et al.,* 2020), but the exact concentration that could inhibit the larval stage to the adult stage is still unknown. The number of larvae that died due to the effect of *E. cottonii* seaweed extract was the parameter tested in this study (LC_{50} and LT_{50} values). This study is expected to provide benefits and information on the effectiveness of *E. cottonii* as a candidate for biolarvicide to suppress the population of *Ae. aegypti* mosquitoes.

MATERIALS AND METHODS

Material

The tools used are sample storage containers/plastic bottles, plastic bags, a blender, an evaporator, an oven, a beaker, a glass, a newspaper, plastic trays, label paper, a stopwatch, and stationery. The materials needed are 5 kg of *E. cottonii* seaweed, 480 instar III larvae of *Ae. aegypti,* tampah, 96% ethanol solvent, well water, fish pellets, rotary vacuum evaporator, hot plate, and microscope.

Preparation of *Eucheuma cottonii* Seaweed Samples.

E. cottonii seaweed was obtained from Hybridharmayoga Village, Ketapang District, South Lampung. Samples were identified by species name at the Botany Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, University of Lampung. Seaweed that has been taken and then washed in running water until it is clean. After that, the drying process was carried out by drying and also placed on a tampah for small seaweed for ± 1 week at room temperature. Then, continue drying the seaweed using an oven at 37-38°C until the seaweed's moisture content is reduced and completely dry. After drying, the seaweed was blended until smooth like powder to be used in the next stage (Muawanah *et al*, 2016).

Extraction of *Eucheuma cottonii* Seaweed Samples

Five hundred grams of *E. cottonii* seaweed was mashed and mixed with 2 L of 96% ethanol solvent into a glass beaker to macerate for 3 × 24 hours. Next, filtering was carried out to obtain ethanol maserat, which would later be evaporated for 2 hours using a rotary vacuum evaporator. The extraction results are then heated using an oven (Thermo-Scientific) at around 37 – 38 °C to obtain a paste-shaped maserat result. A methanol-free test was conducted to ensure that methanol was no longer present in the extract. This was done by adding concentrated sulfuric acid to the extract as an indicator of methanol content. After that, the macerate can be stored in a container bottle (Prabha *et al*, 2013).

Sample Preparation of *Aedes aegypti* Mosquito Eggs

Ae. aegypti mosquito eggs were obtained from Loka Penelitian dan Pengembangan Kesehatan (Litbangkes) Pangandaran. The eggs inside the filter paper were placed on a plastic tray containing well water. The eggs were fed with crushed fish at intervals of one or two days. Mosquito eggs were reared until they reached the third instar larval stage. Third instar larvae are characterised by 4-5 mm long larvae, spinae, and blackish brown siphons. When the eggs had developed to the third instar stage, the larvae were transferred to plastic cups for further treatment (Garcia-Luna *et al*, 2019).

Larvicidal Test of *Eucheuma cottonii* Seaweed Extract against Instar III Larvae of *Aedes aegypti* Mosquitoes.

Six plastic cups, each filled with 20 third-instar larvae of *Ae. aegypti* larvae were prepared and each plastic cup was given *E. cottonii* extract with different concentrations of 50 ppm, 100 ppm, 200 ppm, 300 ppm, positive control with 1% Abate®, and negative control with well water (Hari & Mathhew, 2018). For a 50 ppm *Eucheuma cottonii* extract solution, 50 mg *of Eucheuma cottonii* paste is dissolved in 1 liter of distilled water and used as a stock solution. For a 100 ppm solution, 100 mg of *Eucheuma cottonii* paste is dissolved in 1 liter of distilled water. For a 200 ppm solution, 200 mg is dissolved, and so on. This treatment was carried out in as many as four replicates. Then, observations were made for 4, 8, 12, 24, 48, and 72 hours.

Data Analysis

The data obtained were then analysed using probit analysis to determine the effective dose given to the third instar larvae of *Ae. aegypti* as larvicide. Probit analysis was performed using SPSS version 22.

RESULTS

The results of larvicidal testing of *E. cottonii* extract against instar III larvae of *Ae. aegypti* showed that the extract began to work after 8 hours of treatment. The most considerable average larval mortality was at a concentration of 200 ppm, with a total mortality of 13.25 larvae, and a larval mortality percentage of 66%. The detailed graph of the percentage of larval mortality is presented in Table 1.

Table 1. Mortality of *Aedes aegypti* larvae after administration of *E. cottonii* extract during observation (*Mortalitas larva Aedes aegypti setelah pemberian ekstrak E. cottonii selama pengamatan*).

Observation Time (Hour) (*Waktu Pengamatan*) (Jam)	**Number of Larvae Killed** (*Jumlah Larva Mati*) **(n = 20)**					
	50 ppm	**100 ppm**	**200 ppm**	**300 ppm**	**Negative Control** (*Kontrol Negatif*)	**Positive Control** (*Positive Control*)
4	0,00 ± 0,00	0,00 ± 0,00	0,00 ± 0,00	0,00 ± 0,00	0,00 ± 0,00	20,00 ± 0,00
8	0,75 ± 0,96	0,00 ± 0,00	1,50 ± 1,73	1,00 ± 0,82	0,00 ± 0,00	20,00 ± 0,00
12	2,50 ± 0,58	2,00 ± 0,82	2,75 ± 2,06	3,50 ± 1,73	0,00 ± 0,00	20,00 ± 0,00
24	4,00 ± 0,82	2,00 ± 0,82	4,75 ± 3,20	5,75 ± 2,87	0,00 ± 0,00	20,00 ± 0,00
48	7,75 ± 2,99	7,00 ± 6,78	7,75 ± 5,50	9,75 ± 2,06	0,00 ± 0,00	20,00 ± 0,00
72	13,00 ± 3,37	11.50 ± 3,37	13,25 ± 3,94	11,50 ± 3,10	0,00 ± 0,00	20,00 ± 0,00

The percentage of mortality of instar III larvae of *Ae. aegypti* mosquitoes against *E. cottonii* extract at different observation times show that the 200 ppm concentration has the highest percentage of 66% (13,25 at 72 hours observation time). Furthermore, the data obtained were analysed using Probit Analysis to obtain the 50% Lethal Concentration (LC) and Lethal Time (LT) values presented in Table 2.

Table 2. Results of Probit Analysis of LC_{50} of *Eucheuma cottonii* Seaweed Extract against Instar III Larvae of *Aedes aegypti* at Different Observation Times (*Nilai LC_{50} hasil analisis probit ekstrak rumput laut Eucheuma cottonii terhadap larva instar III Aedes aegypti pada waktu pengamatan yang berbeda*).

Observation Time (Hours) (*Waktu Pengamatan*) (*Jam*)	**LC_{50} (ppm)**
4	0,243894
8	0,000332
12	1,543183
24	**63, 885340**
48	726.1678
72	**42. 358551**

Based on the results of probit analysis, the Lethal Concentration value of the extract used after 72 hours of treatment was 42.358551 (42,36) as the LC50 value. At the same time, the Lethal Concentration value at the 24-hour observation time is 63.885340 (63,88) ppm. After the LC*50* value is known, the 50% Lethal Time (LT) value is also analysed, which can be observed in Table 2.

Table 3. Results of Probit Analysis of LT50 of *Eucheuma cottonii* Seaweed Extract Against Instar III Larvae of *Aedes aegypti* at Different Concentrations (*Nilai LT_{50} hasil analisis probit ekstrak rumput laut Eucheuma cottonii terhadap larva instar III Aedes aegypti pada waktu pengamatan yang berbeda*).

Treatment (*Perlakuan*) **(ppm)**	**LT50 (Hours)** (*Jam*)
Negative Control *(Kontrol Negatif)*	0
Positive Control *(Kontrol Positive*)	0
50 ppm	54.753
100 ppm	65.362
200 ppm	**52.773**
300 ppm	65.350

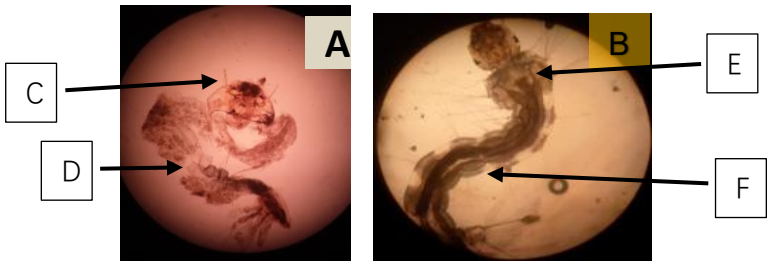

Figure 1. A) Morphology of third instar larvae after 72 hours of treatment; B) Morphology of third instar larvae in the negative control. Part C shows the damaged head of the larva, while part D shows the damaged thorax and abdomen, compared to parts E and F in the negative control (distilled water), which are normal (*(A) Morfologi larva instar ketiga setelah 72 jam perawatan; B) Morfologi larva instar ketiga dalam kontrol negatif. Bagian C menunjukkan kepala larva yang rusak, sedangkan bagian D menunjukkan toraks dan perut yang rusak, dibandingkan dengan bagian E dan F pada kontrol negatif (air suling), yang normal*).

Based on the results of probit analysis, treatment with *E. cottonii* seaweed extract at a concentration of 200 ppm causes the fastest death with Lethal Time (LT50) 52.773 (52,77) hours. At the same time, the longest Lethal Time (LT50) value is at a concentration of 100 ppm for 65.362 (65,36) hours. *E. cottonii* extract against instar III larvae of *Ae. aegypti* showed morphological changes such as damage to parts of the larval body as shown in Figure 2A. In the negative control (Figure 2B), the larval body parts were still complete, and no damage was found.

Based on the results presented above, the administration of *E. cottonii* ethanol extract to the third instar larvae of *Ae. aegypti* mosquitoes have the most significant average larval mortality at a concentration of 200 ppm, namely 13.25 larvae. The 200 ppm concentration had an average larval mortality of 1.75 times that of the 300 ppm concentration. The cause is thought to be due to the formation of resistant properties in the third instar larvae of *Ae. aegypti* against toxins or microorganisms given at a concentration of 300 ppm (Hutabarat, 2020). The toxin in this case is the ethanol extract of *E. cottonii,* which acts as a larvicide. An increase in concentration causes a detoxification event or disposal of toxins in the body of insects such as mosquitoes or flies.

Furthermore, the percentage mortality of *Ae. aegypti* larvae of *E. cottonii* extract with a concentration of 200 ppm was higher than the other concentrations (Table 1). This shows that *E. cottonii* extract influences the death of instar III larvae of *Ae. aegypti because it contains compounds that are effective* as larvicides. Based on research conducted by Afif *et al* (2015), *E. cottonii* contains steroid compounds that have benefits and compounds that are toxic to *Ae. aegypti* mosquito larvae. Steroids will attack parts of the larval body and inhibit larval development so that the larvae experience death when given *E. cottonii* extract.

Then, in the probit analysis (Table 1), the Lethal Concentration (LC) of the extract used after 72 hours of treatment was 42.358551. In a study conducted by Putra *et al* (2021) using *E. cottonii* extract and tested on *Artemia salina* larvae, an LC50 value of 62.62 ppm was obtained. In addition, research conducted by Afif *et al* (2015) using *E. cottonii* to test its toxicity using 1-butanol solvent showed the acquisition of an LC50 value of 70.32 ppm. The difference in variation in the value of lethal concentration can occur in each treatment because the compounds in the extracts are different.

Furthermore, the morphological changes in the larval body shown in Figure 1A after 72 hours of treatment are caused by *E. cottonii* extract, containing various active compounds such as steroids, alkaloids, saponins, triterpenoids, and flavonoids that play a role in inhibiting the development of *Ae. aegypti* larvae. These steroid compounds inhibit larval growth because steroids are toxic to *Ae. aegypti* larvae. In addition to steroids, alkaloid compounds can disrupt the impulse system to muscle cells. Thus causing damage to the larval body (Bisyaroh, 2020). In addition, there are also tannin compounds that can interfere with the work system of cell metabolism. Thus, the larvae will lack nutrients and die. Tannins can also bind to proteins that play a role in larval growth. Other research conducted by Setyaningsih & Swastika (2016) states that alkaloid and triterpenoid compounds can inhibit larval feeding power. This will cause abnormal growth in larvae and cause death (Tandi, 2010). Research conducted by Prakoso *et al*. (2017) revealed that flavonoid compounds act as respiratory poisons or respiratory inhibitors that cause inhibition of the airway of the *Ae. aegypti* mosquito. This compound works by entering the respiratory tract and making the mosquito's respiratory muscles weak and withered. This causes the mosquito to be unable to breathe and results in death.

DISCUSSION

Detoxification events can occur due to an increase in the number of esterase enzymes that play a role in the mechanism of insect resistance to larvicides. This causes the originally toxic compound to have a non-toxic effect on the target organism and form an immune system that is resistant to toxins (Sutarto & Syani, 2018). The formation of this immune system causes mosquitoes not to die and form new immune populations. Thus, the mortality rate of third instar of *Ae. aegypti larvae decrease* when the concentration of the extract is too high (Widyastuti & Ikawati, 2016). Eucheuma cottonii extract is applied as a larvicide based on larval population selection and concentration. Larvae will experience resistance if the concentration is too high. Thus, the larvae will remain alive. Conversely, larvae that are susceptible to insecticides and at the appropriate extract concentration will die. This is what causes the level of larval resistance to larvicides to vary (Sinaga *et al*., 2016).

This study was conducted up to 72 hours of observation. Based on the results of probit analysis, the Lethal Concentration value of the extract used after 72 hours of treatment was 42.35 ppm as the LC50 value. At the same time, the lowest Lethal Concentration value is at the observation time of 4 hours with an LC50 value of 0.24 ppm. In research conducted by Putra *et al*. (2021) using *Eucheuma cottoni* extract and tested on *Artemia salina* larvae, an LC50 value of 62.62 ppm was obtained. In addition, research conducted by Afif (2015) using *Eucheuma cottonii* to test its toxicity using 1-butanol solvent showed the acquisition of an LC50 value of 70.32 ppm. The results of death concentrations that are not much different are found in research conducted by Muawanah *et al*. (2016), showing an LC50 value of 72.49 ppm. Differences in variations in the value of lethal concentration can occur in each treatment because the compounds contained in the extracts are different. This affects the LC value obtained after probit analysis (Bureni *et al*., 2018). Based on the results of probit analysis, treatment with *Eucheuma cottonii* seaweed extract at a concentration of 200 ppm causes the fastest death with Lethal Time (LT50) 52.773 hours. In comparison, the longest Lethal Time (LT50) value is at a concentration of 100 ppm for 65.36 hours. Various studies have been conducted to determine the LT50 value of *Aedes aegypti* mosquito larvae using various plant and algae extracts. Some previous studies on the mortality of *Aedes aegypti* mosquito larvae using Moringa leaf extract explained that the LT50 value obtained to cause death to the larvae was 18.98 hours (Yasi, 2018). Waskito & Cahyati (2018) mentioned that bay leaf extract requires time for 27.46

hours to kill *Aedes aegypti* mosquito larvae effectively. The difference in time of death (LT) in killing *Aedes aegypti* mosquito larvae is due to differences in extracts and concentration variations used. The lower LT50 value indicates that the chemical compounds contained in the extract are increasingly toxic due to the rapid rate of infection (Nurhaifah & Sukesi, 2015).

Larvae were given *Eucheuma cottonii* extract after 72 hours of treatment, which showed damage to the head (caput), thorax, and abdomen. At the same time, the siphon did not show any damage. The results of research conducted by Mufadal (2015) stated that *Eucheuma cottonii* contains steroid compounds that are toxic. The steroid compounds contained most in *Eucheuma cottonii* are fucosterol type steroids. This steroid compound inhibits larval growth because steroids are toxic to *Aedes aegypti* larvae. In addition to steroids, *Eucheuma cottonii* also contains several other compounds that inhibit growth and damage larval body parts, such as alkaloids, saponins, triterpenoids, and flavonoids. The mechanism of action of alkaloids in Eucheuma cottonii as larvicides is by accumulating acetylcholine compounds in the larval body through the process of inhibiting the work of the Acetylcholinesterase enzyme. This will cause disruption of the impulse system to muscle cells (Bisyaroh, 2020). The same thing is explained in the research of Kurniawan *et al*. (2015) that after disruption of the nervous system, *Aedes aegypti* mosquitoes will experience convulsions, then paralysis, and finally die.

The state of *Aedes aegypti* larvae in the study of Adrianto *et al*. (2014) after being treated using citrus plant extracts caused the larval body to become whiter and the disappearance of black lines on the abdomen, and the larval head was almost detached due to the alkaloid compounds contained in the extract. Thus, alkaloids play a role in damaging cells and the nervous system in larvae, as seen in Figure 9, where the head and body of larvae containing the nervous system are destroyed or damaged after being given *Eucheuma cottonii* seaweed extract. In Figure 2, it can be seen that the abdomen is damaged due to alkaloid compounds that have properties that are stomach poisons. This alkaloid compound will cause changes in the color of the larvae from dark to transparent. The body movements of the larvae are also slower and tend to bend when stimulated (Bisyaroh, 2020). Other research by Setyaningsih & Swastika (2016) states that alkaloid and triterpenoid compounds can inhibit larval feeding power.

In addition, there are also tannin compounds that can interfere with the cell's metabolic system. Thus, the larvae will lack nutrients and die. Tannins can also bind to proteins that play a role in larval growth. This will cause abnormal growth in the larvae and cause death (Tandi, 2010). Other compounds contained in *Eucheuma cottonii* extract, such as saponins, also inhibit the development of *Aedes aegypti* larvae. Saponins have the property of damaging the cuticular membrane in larvae which results in a decrease in the work activity of digestive enzymes and food absorption in insects. The saponin content in *Eucheuma cottonii* is thought to irritate the mucosa of the larval digestive tract. In addition, the waxy layer on the insect's body that functions to protect its outer body can be eroded and damaged (Figure 2). This will cause the insect to lose a lot of body fluids and result in death (Minarni *et al*., 2013).

Not only the digestive system and nervous system, *Eucheuma cottonii* extract also contains flavonoid active compounds that cause damage to the respiratory system. Research conducted by Prakoso *et al*. (2017) revealed that flavonoid compounds act as respiratory poisons or respiratory inhibitors that cause inhibition of the airway of the *Aedes aegypti* mosquito. This compound works by entering the respiratory tract and making the mosquito's respiratory muscles weak and withered. This causes the mosquito to be unable to breathe and results in death.

In conclusion, although *Eucheuma cottonii* extract shows the potential as a natural larvicide against *Aedes aegypti* larvae, its effectiveness still has some limitations that need to be considered. The variability of LC_{50} and LT_{50} values from various studies suggests that the content of active compounds in these extracts may be influenced by the extraction method, type of solvent, and environmental and biological conditions of the larvae. In addition, the possibility of resistance due to the use of too high concentrations is also a challenge in long-term utilization. Therefore, further research is needed to explore the optimal formulation, a more detailed mechanism of action, and an

integrated approach to the use of *Eucheuma cottonii* as a larvicide, so that its use is not only practical but also sustainable and environmentally friendly in dengue vector control.

CONCLUSION

The conclusion obtained from this study is that the ethanol extract of *Eucheuma cottonii* has low effect on the mortality of instar III larvae of *Aedes aegypti* mosquitoes, the LC_{50} value is 42.35 ppm after 72 hours, and the LT_{50} value 52.77 hours (>24 hours) at a concentration of 200 ppm; its effectiveness still has some limitations that need to be considered.

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AUTHOR CONTRIBUTIONS

ES & NTN: collecting research data, drafting the article, final manuscript revision; DFM: Creating research concept, final manuscript revision; ER: creating research concepts, drafting articles, revising manuscripts.

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