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Effectiveness Extract Larvacides of Kecombrang Flower (*Etlingera elatior*) on Death of *Aedes aegypti* Mosquito Larvae

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ABSTRACT

The prevention and control of dengue hemorrhagic *fever can be done by using natural larvicides whose content is safer, such as kecombrang plant (Etlingera elatior).* ***Purpose:*** *to analyze the effectiveness of extract larvacides of kecombrang flower with various concentrations on the mortality of Aedes aegypti larvae.* ***Methods:*** *this research used a pure experimental method (true experimental) with a post test with only one group design. The independent variable was kecombrang flower extract (Etlingera elatior) with concentrations of 0% (control), 2%, 4%, 6%, and 8% with 5 replications and an observation time of 6 hours. The dependent variable was the death of Aedes aegypti larvae. Data analysis consisted of descriptive and inferential analysis using the One-Way Anova test followed by the Post Hoc LSD (Least Significant Difference) test. Results and Discussion: the results of the research showed that there was a significant difference in mean mortality between various concentrations extract of kecombrang flower (p=0.000) and the most effective concentration was 6%.* ***Results:*** *There was a significant difference in mean mortality between various concentrations extract of kecombrang flower (p=0.000) and the most effective concentration was 6%. The result of probit test showed that the LC50 value was 6.577 and LC90 was 12.113 for 6 hours.* ***Implications:*** *The results of this study indicated that extract of kecombrang flower can help government efforts in controlling dengue fever and can be used by the community.* ***Conclusion:*** *kecombrang flower extract (Etlingera elatior) was effective in being used as a vegetable larvicide.*

Keyword: *Dengue Hemorrhagic Fever (DHF), Kecombrang (Etlingera Elatior) Flower, Aedes Aegypti Larvae.*

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INTRODUCTION

Dengue hemorrhagic fever (DHF) is a disease caused by the dengue virus carried by mosquitoes of the *Aedes* genus (*Aedes aegypti and Aedesalbopictus*). Generally, this virus can appear in tropical areas rather than subtropical in the development and growth of mosquitoes. The signs of DHF are prolonged pain in the solar plexus and bleeding in the mouth, nose, gums, and red spots on the skin surface (Kemenkes RI, 2017). To date, dengu*e* is still a serious health problem and the of death in several countries in Asia and Latin America. Based on a report from the World Health Organisation (WHO) in 2020, the global incidence of DHF has increased dramatically in recent decades. Half of the world's population is at risk of DHF with an estimated 100-400 million people infected each year. The number of dengue cases reported to WHO has increased 8-fold over the last 20 years, from 505,430 cases in 2,000 years to more than 2.4 million in 2019, while the number of deaths reported to WHO has increased 8-fold over the past 20 years. In 2019, while the number of deaths reported between 2000 and 2015 increased from 960 to 4,032 cases (WHO, 2020). In 2020 to 2022, there was a slight decrease in cases of dengue due to the COVID-19 pandemic and lower reporting figures. However, in 2023, there was a spike in dengue cases globally (WHO, 2024a). The highest number of dengue cases was recorded in 2023, affecting over 80 countries in all regions of WHO. WHO recorded more than 6.5 million cases of dengue fever and more than 7,300 deaths related to dengue fever in 2023 (WHO, 2024b).

Based on data from the Ministry of Health of the Republic of Indonesia (Kemenkes RI) 2021, dengue cases are found in 472 districts or cities in 34 provinces, and deaths due to dengue cases occur in 219 districts or cities. It was informed that in 2021 there were 73,518 cases of DHF and a mortality of 705 cases(Kemenkes RI, 2022). Tasikmalaya is one of the cities in West Java Province that is a DHF endemic area. The number of DHF cases in Tasikmalaya City in 2021 was 909 cases, a decrease compared to the previous year which amounted to 1,409 cases. The Incidence Rate (IR) of DHF in 2021 was 133.2 per 100 population, which is the third highest IR value in West Java, while the CFR rate was 2.31% (Dinkes Jabar, 2022).

As a disease caused by the *Aedes aegypti* mosquito vector, it is necessary to take steps to control dengue vectors. Vector control is a measure to reduce risk factors for disease transmission by vectors by making vector breeding habitat disappear, reducing the number and age of vectors, suppressing contact between humans and vectors, and cutting the chain of transmission of dengue disease (Kemenkes RI, 2017). Vegetable larvicides are derived from nature such as plants or herbs. (Yuliana et al., 2021) The advantages of plant-based larvicides are that they do not cause adverse effects on the environment, animals, and humans and do not cause resistance. the environment, animals, and humans and does not cause resistance (Ishak, 2018) The use of plants as plant-based larvicides has been investigated for safe use. Some plants are known to contain compounds that can control *Aedes aegypti* mosquitoes (Nirma et al., 2017).

Etlingera elatior or kecombrang plant is a plant from Indonesia that is used to enhance the flavor of traditional and as a source of nutrients such as protein, amino acids, fatty acids, and other mineral compounds (Levitta Juti, Sri Adi, Tiana Milanda, Mutakim, Irma Melyani, 2019)

Kecombrang is a plant that is widely consumed by people as a mixture of cooking ingredients or spices. In addition, it turns out that kecombrang is a versatile plant, with all parts of the leaves, flowers, and fruit that can be used as natural preservatives, medicine, and others. This plant is usually used fresh, can be eaten directly as fresh vegetables, and can also be boiled to drink the water (Syam, 2017).

In daily life, kecombrang is used for hygiene purposes, such as soap, shampoo, and deodorant. In the medical aspect, kecombrang can be used to treat ear infections, diarrhea, typhoid fever, and loss of appetite. Stems and leaves of the plant kecombrang stems and leaves are used to treat tuberculosis and cough, while the rhizome is used to treat fever and coral stones (Silalahi et al., 2019).

Kecombrang (*Etlingera elatior*) is one of the plants with larvicidal activity. *Etlingera elatior* is a plant species that belongs to the genus Etlingera. The flowers of kecombrang (*Etlingera elatior*) contain several compounds, some of which are flavonoids, terpenoids, saponins, tannins, alkaloids, and essential oils (Rahmawati et al., 2020).

Kecombrang has secondary metabolite compounds such as saponins, tannins, quercetin, lainglycosides, anthocyanins, kaemprefol, quercitrin, ergosterol 5,8- peroxide, diarylheptanoids, labdane diterpenoids, cytostenone, catechin, kaempfrol 3-glucoronide, decanal, dodecanal, 1-tetradecane, detoxycurcumin, dodecyl esters, sterols, ß-cinene, ß-farnesene and various terpenes (Silalahi et al., 2019). Phytochemical test results in the study Koraag *et al.*, (2016), investigated that the kecombrang flower contained flavonoid compounds, polyphenols, saponins, and essential oils with a content of 17% in it. Based on the above explanation, a study was conducted on the effectiveness of kecombrang flower extract (*Etlingera elatior*) on the mortality of *Aedes aegypti* mosquito larvae (Koraag et al., 2016).

METHODS

This research used a pure experimental method (true experimental) with post test only control group design. The concentration used is divided into 5 groups, namely control concentration (0%), 2%, 4%, 6%, and 8% with 5 repetitions based on the frederer formula.

$$(t-1)\,(r-1) >$$

Where, t = number of treatments (concentrations), and r = number of repetitions. The number of samples used is based on the standard WHO (2005) which is 25 *Aedes aegypti* larvae with 5 repetitions (WHO, 2005). Based on the calculation results of the formula, 625 larval samples were obtained. In this research, each group was replicated with 5 treatments and 5 repetitions.

The equipment used in this study is rice paper, trays, blenders, gloves, digital scales, knives, filters, measuring cups, glass jars, stirrers, filter paper, funnels, plastic wrap, label paper, cup test effectiveness test equipment, 25 250 ml measuring cups, 1 1000 ml measuring cup, thermometer, 3 pipettes, ph meter, clock dipper / stirring rod, clock, flashlight, label paper, cup test, and measuring

cup. The materials used were kecombrang (Etlingera elatior) flowers, 70% ethanol, distilled water, and Aedes aegypti larvae from the Laboratory of the Center for Research and Development of Disease Vectors and Reservoirs (B2P2VRP) Salatiga.

Making simplisia of kecombrang flower extract (Etlingera elatior) starts with providing the necessary tools and materials. First, kecombrang (Etlingera elatior) flowers are washed thoroughly to remove dirt, then cut into small pieces and stored on a tray. Next, it is dried naturally by aerating at room temperature which is protected from sunlight. After waiting for approximately 5 days to dry, kecombrang is then mashed into powder. Produced approximately 550 gr simplisia kecombrang flowers. Powder of kecombrang flower (Etlingera elatior) was then macerated for 3x24 hours using 70% ethanol solution in a glass jar tightly closed using plastic wrap. The results of the solution of kecombrang flower (Etlingera elatior) that has been macerated within 3x24 hours, then evaporated using a rotary evaporator to remove the alcohol content at temperatures ranging from 40-50 oC, then produced 100% concentration of kecombrang flower extract (Etlingera elatior) as much as 150 ml.

Larvicide Testing of Kecombrang (*Etlingera elatior*) Flower Extract on the Mortality of *Aedes aegypti* :

a. Calculation of the concentration of kecombrang flower extract (Etlingera elatior)
b. Measure room temperature and humidity using a clock thermometer before testing the larvicide.
c. Make control by providing 100 ml of distilled water in a 250 ml measuring cup.
d. Making a mixture of plant-based larvicides and distilled water with the required concentrations.
e. Prepare Aedes aegypti larvae placed in 25 test cups.
f. Larvicide Test
   The larvicide test was conducted by taking 25 Aedes aegypti instar III larvae using a dipper, then the larvae were put into a measuring cup that had been filled with kecombrang flower extract (Etlingera elatior) with various concentrations to be used.
g. Measuring the pH of the water and the temperature of the larval testing water using a pH meter
h. Waiting and observing the development and death of Aedes aegypti instar III larvae for 6 hours. Observations were made at the 1st, 2nd, 3rd, 4th, 5th and 6th hours.
i. Counting the dead larvae according to the observation time.
j. Determining whether the larvae were alive or dead by shining a flashlight on them and providing stimulation in the form of movement, then seeing that the larvae remained still or moved to determine their death.
k. Counting the number and mortality of Aedes aegypti larvae for 6 hours.
l. Re-measure the water temperature, pH, and humidity of the room.
m. Recording the observation results in the research sheet

The data analysis used in this research was the One-way Anova test and the Post Hoc LSD (Least Significant Difference) follow-up test. As for the decision to take the hypothesis (Ho) is accepted or rejected based on the magnitude of the significant value. Probit analysis was used to determine the Lethal Concentration (LC) to determine the toxicity that causes death in Aedes aegypti instar III larvae. Probit analysis to determine the estimated dose of larvicide that can cause death in Aedes aegypti larvae using 50% (LC50) and 90% (LC90).

RESULTS

3.1 Results of Descriptive Analysis

Table 1. Deaths of *Aedes aegypti* Larvae

Concentration	Deaths of *Aedes aegypti* larvae at hour-						Percentage of Larvae Death
	1	2	3	4	5	6	
Control (0%)	0	0	0	0	0	0	0%
2%	0	0	6	19	34	33	26.4%
4%	0	0	6	19	34	52	41.6%
6%	0	7	22	38	49	66	52.8%
8%	0	13	47	57	71	97	76%

The results of observations in table 1, in total each concentration used involved 125 larvae. The total of each concentration used involved 125 larvae, from the results of the number at each concentration It is known that the highest mortality occurred at a concentration of 8% with a percentage of 76% mortality, and the lowest mortality in the control concentration (0%) with no mortality.

Table 2. Descriptive Analysis

Concentration	Percentage of Larvae Death	Standard Deviation
Control (0%)	0%	0.000
2%	26.4%	0.548
4%	41.6%	1.673
6%	52.8%	1.304
8%	76%	1.673

Table 2, showed the results of the percentage of larval mortality and standard deviation of each concentration. The standard deviation of each concentration of kecombrang flower extract. The highest percentage of mortality was at a concentration of 8% as much as 76%. The highest standard deviation was found in the 8% concentration with a standard deviation of 1.673 and the lowest in the control concentration (0%). The standard deviation of 1.673 and the lowest is at the control

concentration (0%) with a value of 0.000.

3.2 Result of Probit Analysis

Table 3. Probit Analysis

	Probability	Estimate	Lower bound	Upper bound
Probit	0.500	6.577	5.359	7.413
	0.900	12.113	9.948	21.394

Table 3, showed that the probit analysis of LC50 larvicide of kecombrang (*Etlingera elatior*) flower extract was 6.577 with an interval of 5.359 to 7.413, which means that the larvicidal extract of *Etlingera elatior* can kill 50% of the larvae. (*Etlingera elatior*) can kill 50% of *Aedes aegypti* larvae. *Aedes aegypti* mosquitoes at a concentration of 6.577%. While LC90 larvicide of kecombrang flower extract (*Etlingera elatior*) is 12.113%. With an interval of 9.948 to 21.394, meaning that the larvicidal (*Etlingera elatior*) flower extract can kill 90% of the *Aedes aegypti* mosquito larvae at a concentration of 12.113%.

DISCUSSION

1. Larvicidal Effectiveness of Kecombrang Flower Extract (*Etlingera elatior*) on Mortality of *Aedes aegypti* Mosquito Larvae

This study was conducted to determine the effect of larvicidal extract (*Etlingera elatior*) on the mortality of *Aedes aegypti* larvae as a vegetable larvicide. Based on the results of One-Way Anova test showed a *p-value* of 0.000 ($p \leq 0.05$), so it can be concluded that the extract of (*Etlingera elatior*) affects the death of *Aedes aegypti* larvae.

The death of *Aedes aegypti* mosquito larvae occurs because kecombrang flower extract (*Etlingera elatior*) has a chemical content that can make larvae die. Kecombrang flowers contain compounds such as saponins, flavonoids, tannins, steroids, quinones, triterpenoids, and polyphenolics that function as vegetable insecticides (Sirojudin et al., 2022). Kecombrang flowers contain alkaloid compounds, polyphenols, flavonoids, terpenoids, saponins, steroids, and essential oils. Polyphenol compounds are acidic which means they can release H + ions from their hydroxyl groups (MULIA et al., 2020).

The content of compounds in kecombrang flowers are flavonoids, saponins, terpenoids, and tannins (Lachumy et al., 2010). *Aedes aegypti* larvae are said to be dead if the larvae have not moved when given a response. Alkaloid compounds have content that can damage cell walls and damage the nervous system by inhibiting the activity of the enzyme acetylcholinesterase in the larval body(R. Putri et al., 2017).

The terpenoid compound can be said to be a contact poison because its content can enter the insect cuticle and also plays a role in inhibiting the entry of nutrients to the larvae (D. M. Putri et al., 2018). Essential oils are produced from various parts of the plant. Essential oils are widely used in

cosmetics, medicines, food, and aromatherapy which makes mosquitoes can't stand the smell (Nurhaen et al., 2016). Essential oils contain insecticides that act as repellents, attractants, and fumigants that inhibit mosquito appetite (Hartati, 2012).

Flavonoids can enter through the respiratory system in larvae which can cause damage until it ends in death in larvae (Koraag et al., 2016) Saponins are stomach toxins that enter through the digestive tract. Furthermore, damage to the larval digestive tract occurs which has an impact on larval feeding (Nadila et al., 2017) Tannins can disrupt the digestive system of insects. Tannins bind to proteins necessary for growth in the digestive tract, causing disruption of the digestive process and larval growth, and causing larval death (Muta'ali & Purwani, 2015).

The poison from kecombrang flower extract enters the body of *Aedes aegypti* larvae acting as a contact poison and stomach poison. Contact toxins from essential oil compounds are insecticides that enter the insect's body through the skin's surface. Insects will die when in contact with the insecticide. Then alkaloid compounds, saponins, and tannins act as stomach poisons in the digestive organs of insects and are absorbed by the walls of the digestive tract. Then, body fluids carry the insecticide into the insect's nerves causing damage to internal organs and eventually causing death to the larvae (Sartika, 2018).

Based on phytochemical screening by Rahmawati et al., (2020), the extract contains the highest yield of alkaloid compounds, namely 4.62% which is 4.62% then terpenoids 3.08%, flavonoids 2.11%, saponins 1.92%, essential oil 1.67% and tannin 0.31%(Rahmawati et al., 2020). The extract of kecombrang flowers contains secondary metabolite compounds, namely alkaloids, steroids or triterpenoids, saponins, flavonoids, and tannins. So it can be concluded that kecombrang flower extract (*Etlingera elatior*) contains active compounds that can be used as a vegetable larvicide. can be used as a vegetable larvicide (Syahrani, 2021).

The results of this study are supported by previous research conducted by Koraag et al. (2016), the results showed that concentrations of kecombrang extract 0.5%; 0.75%; 1%; 1.25%; 1.5% and 1.75%, able to kill *Aedes aegypti* mosquito larvae for 24 hours(Koraag et al., 2016). The results of this study show that there are differences in the effectiveness of larvicidal administration of kecombrang flower extract on the death of *Aedes aegypti* larvae with various concentrations that can be used as an alternative to vegetable larvicides so that it can be used to replace chemical larvicides in efforts to prevent *dengue* disease.

2. Best Concentration of Kecombrang Flower Extract (*Etlingera elatior*)

Based on the results of the One-Way Anova inferential analysis test, mortality of *Aedes aegypti* larvae began at hour 2 at concentrations of 6% and 8%. *Aedes aegypti* larvae started from the 2nd hour at concentrations of 6% and 8%. As for the 2% and 4% concentrations, larval mortality began at the 3rd hour. Judging from the results of the study at a concentration of 8% there was 76% mortality, 6% concentration had 52.8% mortality, 4% concentration had 41.6% mortality, and 2% concentration had 26.4% mortality. Deaths and 2% concentration there were 26.4% deaths, while in the control group, there was no death of *Aedes aegypt*i larvae in all treatments.

The value of LC can measure the toxicity of the extract content used. Acute toxicity is said to

be highly toxic if the value is in the range of <1%, toxic 1-10%, moderately toxic 10-50%, slightly toxic 50-99%, and non-toxic 100%. Based on the LC results of this study, it can be seen that kecombrang flower extract is toxic, which means it is effective in killing *Aedes aegypti* larvae (Humpage A, Falconer I, Bernard C, Froscio S, 2011).

Based on the results of the explanation above, it can be seen that the most effective kecombrang flower extract is at a concentration of 6%. This concentration is included in the toxic category, which means it can kill mosquito larvae. *Aedes aegypti* mosquito larvae. This extract can be used as an alternative plant-based larvicide alternative to chemical larvicides because it is safe for humans and more environmentally friendly.

CONCLUSIONS

Based on the results of the research that has been carried out, it can be concluded as follows:

1. There was a difference in the effectiveness of the larvicidal application of kecombrang flower extract at 0% concentration (control), 2% concentration, 4% concentration, 6% concentration, and 8% concentration on the death of *Aedes aegypti* larvae.
2. The concentration of kecombrang flower extract was most effective at 6% concentration.
3. The concentration of kecombrang flower extract that can kill 50% (LC50) is 6.577% and the concentration that can kill as much as 90% (LC90) is 12.113%.

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