

EFFECTIVENESS OF KECOMBRANG FLOWER (*ETLINGERA ELATIOR*) AS A LARVICIDE FOR *Aedes Aegypti* INSTAR III MOSQUITOES

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ABSTRACT

Dengue hemorrhagic fever (DHF) is a disease caused by dengue virus infection where the vector is the female *Aedes aegypti* mosquito. DHF causes high mortality and morbidity because a vaccine for DHF has not yet been found. DHF therapy is still supportive and symptomatic, causing this disease to remain a health problem for the community. The purpose of this study was to determine the effectiveness of kecombrang flower (*Etlíngera elatíor*) as *Aedes aegypti* instar III larvicide. This research was conducted at the *Institute of Tropical Disease* (ITD) Universitas Airlangga in July 2023. The research design used was true experimental design with *post-test only group control design*. Divided into 7 groups, namely negative control, 0.1%, 0.6%, 1.1%, 1.6%, 2.1% and 2.6%. The number of samples used in the study was 560 larvae. Each group contained 20 larvae with 50ml of solution containing kecombrang flower extract. Repetition was done 4 times at each concentration. The test used was *Kruskal-Wallis* ($p < 0.05$), *Post-Hoc Mann Whitney U test* ($p < 0.05$). The results showed the highest average larval mortality at concentrations of 2.1% and 2.6% at 100% and the lowest average at a concentration of 0.1% at 28.75%. In the *Kruskal-Wallis test*, each treatment group showed significant differences ($p < 0.05$). The *Mann Whitney U post hoc* test showed significant differences between the two treatment groups.

Keywords: *Aedes aegypti*, Kecombrang flower (*Etlíngera elatíor*), Larvicide

ABSTRAK

Demam berdarah dengue (DBD) merupakan penyakit yang disebabkan oleh infeksi virus dengue dimana vektornya adalah nyamuk *Aedes aegypti* betina. DBD menyebabkan angka kematian dan kesakitan yang tinggi karena vaksin DBD belum ditemukan. Terapi DBD yang masih bersifat suportif dan simtomatik menyebabkan penyakit ini masih menjadi masalah kesehatan masyarakat. Tujuan penelitian ini adalah untuk mengetahui efektivitas bunga kecombrang (*Etlíngera elatíor*) sebagai larvasida nyamuk *Aedes aegypti* instar III. Penelitian ini dilakukan di *Institute of Tropical Disease* (ITD) Universitas Airlangga pada bulan Juli 2023. Desain penelitian yang digunakan adalah true eksperimen design dengan *post-test only group control design*. Dibagi menjadi 7 kelompok yaitu kontrol negatif, 0,1%, 0,6%, 1,1%, 1,6%, 2,1% dan 2,6%. Jumlah sampel yang digunakan dalam penelitian adalah 560 larva. Setiap kelompok berisi 20 larva dengan 50 ml larutan yang mengandung ekstrak bunga kecombrang. Pengulangan dilakukan sebanyak 4 kali pada masing-masing konsentrasi. Uji yang digunakan adalah uji *Kruskal-Wallis* ($p < 0,05$), uji *Post-Hoc Mann Whitney U* ($p < 0,05$). Hasil penelitian menunjukkan rata-rata kematian larva tertinggi pada konsentrasi 2,1% dan 2,6% sebesar 100% dan rata-rata terendah pada konsentrasi 0,1% sebesar 28,75%. Pada uji *Kruskal-Wallis* setiap kelompok perlakuan menunjukkan perbedaan yang signifikan ($p < 0,05$). Uji *post hoc Mann Whitney U* menunjukkan perbedaan yang signifikan antara kedua kelompok perlakuan.

Keywords: *Aedes aegypti*, bunga kecombrang, *Etlíngera elatíor*, larvasida

INTRODUCTIONS

Dengue Hemorrhagic Fever (DHF) causes high mortality and morbidity due to the absence of a DHF vaccine and the primarily supportive and symptomatic nature of its treatment. This disease is transmitted by the *Dengue* virus through the bites of *Aedes aegypti* and *Aedes albopictus* mosquitoes^{1,2}.

In 2019, Southeast Asia recorded 658,301 cases of DHF, with Indonesia being one of the five countries in the region with the highest DHF cases³. In January to September 2022, there were 87,501 DHF cases and 816 deaths in Indonesia⁴. East Java reported 8,894 cases and 110 deaths⁵, with Surabaya ranking third, following West Java and Bali, in the number of DHF cases in 2018 from January to October⁶.

Mosquito population control is crucial to combat DHF cases. Temephos 1% is a commonly used chemical larvicide. However, its long-term use can lead to mosquito resistance, and it can also contaminate drinking water⁷.

The use of natural substances as an alternative is being considered due to their environmentally friendly nature. Local plants, such as *Etilingera elatior*, also known as “Kecombrang flowers,” can serve as larvicides. Kecombrang flowers contain useful larvicidal components, such as alkaloids, flavonoids, tannins and saponins⁸.

Alkaloid in kecombrang flowers are toxic to insect nerves, while flavonoid inhibit the insect's respiratory system. Tannin disrupt the metabolism of the insect's digestive system by inhibiting protease enzymes. Saponins lead to an inflammatory process in the insect's gastrointestinal mucosa, causing fluid extravasation and significant loss of body fluid^{8,9}.

Based on above explanation, the researchers are interested in studying the effectiveness of kecombrang flowers (*Etilingera elatior*) as a larvicide for *Aedes aegypti* instar III. The concentrations used in this study are 0.1%, 1.1%, 1.6%, 2.1% and 2.6%. The extraction method used in this study is maceration with 96% ethanol as the solvent.

The goal of this research is to determine the effectiveness of kecombrang flowers (*Etilingera elatior*) extracts as a larvicide for *Aedes aegypti* instar III. This study aims to measure the effectiveness of kecombrang leaves on the mortality of third instar *Aedes aegypti*.

MATERIAL AND METHOD

Materials

The equipment used in this research includes a micropipette, blender, rotatory evaporator, cloth, vacuum, spatula rod, digital scale, sieve, Erlenmeyer flask, measuring glass and reaction tube. The materials used consist of kecombrang flowers (*Etilingera elatior*), distilled water (aquadest), tween 20, 96% ethanol and *Aedes aegypti* instar III.

Methods

This study is a true-experimental research with a post-test only group control design. The research was conducted at the Entomology Laboratory of the Institute of Tropical Disease (ITD), Universitas Airlangga, Surabaya. The study consist of seven groups, including one negative control (aquadest 50ml) and six treatment groups with concentration 0.1%, 0.6%, 1.1%, 1.6%, 2.1% and 2.6%.

Larva samples were obtained from rearing at Entomology Laboratory ITD, Universitas Airlangga. The larvicide test used third instar *Aedes aegypti* larvae that were not fed for 24 hours. The samples used were *Aedes aegypti* larvae that had reached instar III. Each treatment group had 20 larvae with four repetitions as calculated using the Freeder formula.

Data collection was carried out after 24 hours of larval exposure to the extract. The extract is considered effective if the statistical test result yield $p < 0,05$. Data analysis was conducted in staged, starting with a normality test using the Saphiro-Wilk test to determine whether the data was normally distributed. Subsequently, a homogeneity test was performed using the Lavene test to assess data homogeneity. If the result of both normality and homogeneity test had $p > 0,05$, a One-Way ANOVA test would be conducted. However, in this study, the data did not follow a normal distribution and were not homogeneous, so a non-parametric Kruskal-Wallis test was used. If the Kruskal-Wallis test yield $p < 0,05$, a post hoc Mann Whitney-U test would be conducted to identify groups with significant differences.

Preparations of Kecombrang Flower (*Etilingera elatior*) Extract

This study used 5.5 kg of kecombrang flowers were obtained from Bangli, Bali. After that, the kecombrang flower will be checked and adjusted to the morphological characteristics of the kecombrang flower. The flowers were washed with running water until clean and the drained. The flower petals were cut into smaller pieces and dried in a shaded area. Once dried, the flowers were ground into a fine powder using a blender and sieved to obtain kecombrang simplisia powder.

The extraction process involve macerating the simplisia powder with 96% ethanol solvent for 24 hours to ensure complete immersion, with stirring every 8 hours. The macerate was poured and filtered, and the residue was macerated again with the solvent for a total of three repetitions to extract all secondary metabolites. All the solvent macerates obtained were the evaporated using a rotatory evaporator, resulting in a concentrated extract 41.5g as semi-solid.

Preparation of Concentration Series

The kecombrang flower extract was made into a 2.6% stock solution, which was diluted to create required concentration series. The stock solution was prepared using the formula $\% = (g/ml) \times 100\%$. To dissolve the extract, 16,9g was mixed with 60ml of aquadest. The concentration series of 0.1%, 0.6%, 1.1%, 1.6%, 2.1% and 2.6% were created using the dilution formula $V1 \times M1 = V2 \times M2$. In the larvicidal activity test, there were 7 treatment groups with diluted kecombrang flower extract.

RESULT

Result of the preliminary test for the research can be observed in **Table 1**.

The mortality rate for all samples was observed at a concentration of 1.5%, while in the negative control group, no larval deaths were recorded.

Table 1 Preliminary test of the effectiveness of kecombrang flower extract on the mortality of *Aedes aegypti* instar III mosquito larvae

Concentration of Kecombrang Flower Extract	Total Number of Tested Larva	Mortality
Kontrol negatif	20	0
0,5%	20	19
1,5%	20	20
1,75%	20	20
3%	20	20
5%	20	20
10%	20	20

Continuing from this data, probit analysis was performed to determine the LC50 and LC95

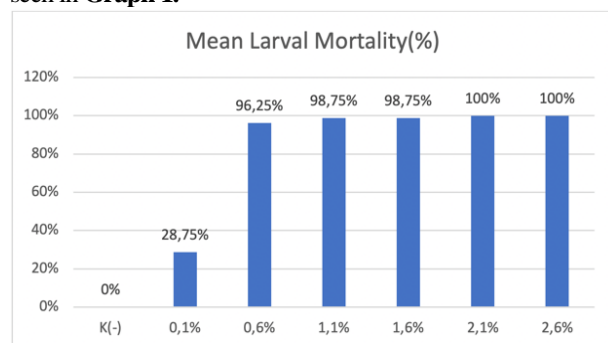
values to determine extract concentration to be used in the actual test. The LC50 and LC95 values can be seen in **Table 2**.

Table 2 Preliminary probit analysis test

	Estimate	95% Confidence Limits for Dosis	
		Lower Bound	Upper Bound
LC50	0.334	0.181	0.511
LC95	0.795	0.588	1.439

from the data in **Table 2**, it can be conducted that to achieve as 50% larval mortality rate, an estimated dose of 0.034% is required, with dose range that can be used from 0.181% to 0.551%. In contrast, to achieve a 95% larval mortality rate, an estimated dose of 0.795% is needed, with a dose range that can be used from 0.588% to 1.439%.

The average mortality data for larvae in actual test can be seen in **Graph 1**.



Graph 1 Average mortality of *Aedes aegypti* instar III larvae

Based on **Graph 1**, the percentage of larval mortality increases linearly with the concentration provided. The highest average mortality rate was obtained at concentrations of 2.1% and 2.6%, while the lowest was observed at a concentration of 0.1%. Subsequently, a statistical test was conducted to determine the effectiveness of the kecombrang flower extract.

Normality and homogeneity in this study yielded result of $p < 0,05$, leading to the use of the non-parametric Kruskal-Wallis test. The Kruskal-Wallis test resulted in a $p < 0,001$ ($p < 0,05$), indicating that the kecombrang flower extract is effective as a larvicide for *Aedes aegypti* instar III. To identify which groups have significant differences, a post hoc Mann Whitney-U test was performed. A significant difference ($p < 0,05$) was found when comparing a concentration of 0.1% with concentrations of 0.6%, 1.1%, 1.6%, 2.1% and 2.6%.

DISCUSSION

The reasearch result indicate that larval mortality is directly proportional to the concentration of the given extract. this is because a greater number of secondary metabolites contained within the extract, such as flavonoids, alkaloids, tannins, and saponin enter the larvae's bodies. The varying result can be attributed to the mosquito's threshold for detoxifying substance that enter its body¹⁰.

Flavonoid compounds can cause paralysis in the insect's respiratory muscle system. Alkaloids work by inhibiting acetylcholinesterase enzymes, which can result in nerve damage and the degradation of insect cell walls. Tannins inhibit protease enzymes, and larval digestive activity decreases, causing insect to obtain less food. Saponins trigger an inflammatory process in the insect's gastrointestinal mucosa, leading to decreased appetite. Furthermore, saponins cause fluid extravasation in the larva's body, resulting in significant^{11,12}.

The result of this research show better effectiveness compared to the study conducted by Palgunadi¹¹. In Palginadi's study, a mortality rate of 99,2% was obtained at a concentrations of 15%. This difference can be attributed to various factors such as extraction method, solvent use, and the geographical location of sample collection. The maceration method was chosen due to its simplicity and the use of basic equipment, preventing damage to thermolabile substances like flavonoids, alkaloids, and tannins. The use of 96% ethanols as the solvent was based on its high polarity, ensuring optimal compound extraction. This is because the active compounds in plant have nearly the same polarity as 96% ethanol¹³. Geographical location affects air humidity, soil quality, rainfall, and plant age, all of which influence the secondary metabolites contained in kecombrang flowers¹⁴.

CONCLUSIONS

The concentrations of kecombrang flower (*Etingera elatior*) extract at 0.1%, 0.6%, 1.1%, 1.6%, 2.1%, and 2.6% are

effective for use as larvacides for *Aedes aegypti* instar III mosquitoes.

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