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Effect of Kecombrang (*Etlingera elatior*) as Larvacide to Control *Aedes aegypti* (Diptera: Culicidae)

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ABSTRACT

Kecombrang (*Etlingera elatior*) as an Indonesian forest plant (generally called kincung, kantan, honje, vanity, tamarind acid, sambuang, scale beetle, siantan, and kecicang), flowering terna, fruit, and its seeds of Zingebereaceae family which are used as vegetable ingredients spices and control of plant pests. *Etlingera elatior* consist of saponins plays role as an insecticide and larvicide. Saponins can reduce the surface tension of the mucous membrane of the larval digestive tract, so that the wall of the tract become corrosive. Flavonoids as inhibiting agent for the eating of insects and fungus those which attack them, impacting the looting genes in the nitrogen nodule bacteria. This research objective is to find the LC_{50} and LC_{90} of kecombrang as larvicidal of *Aedes aegypti* and to survey the insect associated with this plant species. The studies showed LC_{50} values of 0.053% of kecombrang extract is effective to the larvae of *Ae. Aegypti*. The survey conducted from June-July 2019 on two plots of kecombarang plantation in the forest edge of Mount Leuser National Park in Pamah Village, Semilir District, Langkat Regency to invent the types of insects by using 3 tools, namely *sticky yellow trap*, *pit fall trap* and *malaise trap*. According to observation results, there are 12 families of Diptera were found on *E. elatior* plantation.

Keywords: *forest plant, insect control, bio-insecticide*

1. INTRODUCTION

Kecombrang (*Etlingera elatior*) is a type of shrub with a height of 1-3 m, trunked, erect, flaky, forming rhizomes and green. The leaves are single, lancelets, the tip and base are pointed but flat, the leaves have a length of 19-29 cm and width of 5-24 cm, the leaves are pinnate and green. Kecombrang flower is a tubular compound with a stem length of 40-80 cm. Stamen length is ± 7.5 cm and yellow. Its pistil is small and white with crown flower, hairy and rarely pink. Kecombrang seeds are square or oval in white or pink. The fruit is small and brown. The roots are fibrous and dark yellow [1] as shown in Figure 1.

Figure 1 Kecombrang flower (*Etlingera elatior*)

Ethnobotany is a study of interactions between local communities and their natural environment, especially regarding the use of plants in daily life [2]. The use of plants in daily life can include plants as food, environmentally friendly vegetable pesticides and insect control. In Southeast Asia, *Culex quinquefasciatus, Captotermes curvignathus, Aedes aegypti*, were the presence of insects which controlled by kecombrang [2-4]. Besides, kecombrang used can control some dengue fever is an acute disease caused by dengue virus, which is transmitted by the *Ae. aegypti* mosquito. This disease is found in the tropics and subtropics [3].

The incidence of DHF in Indonesia in 2004 was still quite high, from 497 cities throughout Indonesia there were still DHF cases in 374 cities in various provinces with a total number of 65,431 cases and 595 fatalities. The incidence of DHF in North of Sumatra Province in 2004 is categorized as quite high with 5713 cases and 24 fatalities [4]. DHF prevention programs are more reliant on vector control, namely mosquitoes (adults) *Ae. aegypti*. Vector control is the efforts to eradicate dengue which is carried out to break the chain of transmission. The main eradication of dengue fever is eradication of mosquito nests or control with 3M Plus not by fogging [5].

One form of DHF treatment with vector control is to use synthetic insecticides as larvicides. There are two broad categories of insecticides that are often used as household insecticides, namely insecticides functioning to kill insects and to repel insects (Repellent) [6]. One of the plants that can be used as natural insecticides is kecombrang *(E. elatior*). The active ingredient contained in kecombrang

consists of saponins, flavonoids, polyphenols and essential oils [7-9]. According to [10], saponins plays important role as insectides and larvicides. The saponin can also reduce the surface tension of the mucous membrane of the larvae digestive tract, so that the wall of the tracts becomes corrosive [11]. Saponins that is found in food and is consumed by insects can reduce the activity of digestive enzymes. In addition, the absorption of food containing flavonoids could be a plant defense to inhibit the eating of insects due to its toxic property [12]. Whereas, [13] shows that pink rind of kecombrang fruit with a concentration of 50% has a higher antibacterial activity than the fruit seeds, both against *Bacillus cereus* and *Eschericia coli*.

1.1. Materials and Methods

This research was conducted from June to July 2019, there were some steps done, those were collecting leaves and flowers of kecombrang, then collecting *Ae. aegypti* III instar larvae collected in laboratory in Faculty of Agriculture, University of North Sumatra. This research used a completely randomized design (CRD) as the research design. The population used in this study was Mosquito eggs (in dry form using filter paper media that were obtained from the P2B2 Medan Research Institute). The determination sample number was based on [3] using 2 controls and 4 treatments with 19 instar III larvae in each treatment group, included in a test glass containing 100-190 mL of water for each treatment with 6 replicates, so that a number of 480 larvae were needed. The criteria of this study were active *Ae. aegypti* larvae that had reached Instar III, whereas the non-criteria on of this study was free larvae. The independent variable of this study was the various methanol fractions of kecombrang stem extract (*E. elatior*), then the dependent variable of this study was the death of *Ae. aegypti* larvae.

1.1.1. Insects sampling

Samples of kecombrang leaves and flowers were taken randomLy in the forest edge of Mount Leuser National Park in Pamah Village, Semilir District, Langkat Regency to invent the types of insects detected using 3 tools, namely sticky yellow trap, pit fall trap, and malaise trap in kecombrang plantation (described in Figure 2). Collected insect brought to the laboratory of Pests in Faculty of Agriculture, Universitas Sumatera Utara and identified using key identification of [14-16].

1.1.2. Kecombrang leaves and flowers extraction

Kecombrang leaves and flowers samples were taken using a knife, a machete and sack to store plant samples. The weight of the kecombrang leaves sample obtained was

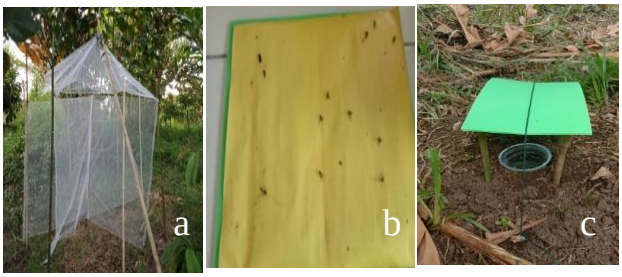

Figure 2 Tools used: (a) *Malaise trap*, (b) *sticky yellow trap*, (c) *pit fall trap* for caught insects in Kecombrang

2400 g and the weight of the kecombrang flower sample obtained was 829 g.

Kecombrang leaves and flowers extraction was done by maceration method. Kecombrang leaves and flowers samples were dried under UV light, then crushed to a powder. It was obtained 622 g and 240 dry weight of leaves and flowers sample (simplicia). Then maceration was conducted utilized 75% ethanol solvent. Simplicia was put into a pumpkin then 75% ethanol was added then allowed to stand for 5 d, matched 2 times for 19 min (speed 90 rpm). After 5 d, the slurry was added with 75% ethanol then shaken to obtain filtrates. The filtrates were then evaporated using a rotary evaporator at a temperature of 60 °C – 70 °C to gain a thick and thick leaves extract of 105.75 g and a flower of 45 g.

1.1.3. Testing of extracts against Aedes aegypti larvae

Larvicide test was carried out to determine the ability of kecombrang leaves and flowers extract to kill *Ae. aegypti* larvae. Two tests were conducted, namely a preliminary test and a final test/follow-up test. In the preliminary test, each kecombrang leaves and flowers extract were divided into 6 treatment groups with the concentrations of 0.5%; 0.75%; 1%; 1.24%; 1.5% and 1.75% respectively, with 4 repetitions determined by the Federer formula [17].

$$(n-1)\,(r-1) \geq 24$$

In which, n = number of treatments (concentration); r = number of repetitions.

Each group contained 24 larvae with 4 treatments. The number of larvae is set at 24 tails according to WHO provision [3, 18, 24]. The control used is positive control in the form of *B. thuringiensis* at 1 ppm and negative control in the form of water. Each control was repeated 4 times. In the final/follow-up test, the concentration range of the leaf and flower extracts used was determined based on the LC50 and LC95 values of the preliminary test results. In this study, kecombrang leaves and flowers extracts were not replaced during the experiment. If there is larval mortality in the control of >10% then the study must be repeated, whereas if death <10% then it must be corrected using Abbot's formula [19].

$$\textbf{\textit{Mortality}}\ (\%) = \frac{x - y}{x} \times 100$$

Where, x = percentage of larvae that survived in the control group; y = percentage of larvae who survived in the treatment group.

The larvae used in this study was *Ae. aegypti* larva instar III with criteria move active (healthy) larvae. Larvae were obtained from Experimental Animal Laboratory P2B2 Research and Development Centre in Medan. The tools and materials used were test tray, bowl or container, dropper, larvae filter and glass beaker. Deep larvae tray was moved using pipette to beaker glass. A total of 24 larvae were put into each test container containing 190 mL water, kecombrang leaves and flowers extract in each test container due to the concentration group. Observation was done in 1 hr, 3 hr, 6 hr, 9 hr, and 23 hr. During the test, no feeding was given to larvae. The number of dead larvae was counted at every hr of observation. Death indicator of larvae were not moving or not responding to any stimulation.

1.1.4. Data analysis

To determine LC_{50} and LC_{95} from the extract leaves and flowers, it was used probit analysis. Then determination of the difference in killing power concentration of kecombrang leaves and flower extract used ANOVA. To gain the killing power difference of leaf and flower extract concentration towards *Ae. aegypti* larvae, it was utilized independent t test. Data processing was done with SPSS 19.00.

1.2. Our Contribution

This paper presents some improvements based on [20–23] were researched used bioactive extracts of toxic substances that enter the body of larvae will inhibit the growth of larvae. While [12] states kecombrang flavanoid compounds will inhibit eating of insects and are toxic. Then, this finding of the research is the LC_{50} and LC_{95} are the basis of knowledge for formulating larvicides from kecombrang flower leaves.

1.3. Paper Structure

The rest of the paper is organized as follows. Section 1 introduces the preliminaries used in this paper, which include ethnobotany *E. elatior* (kecombrang). The use of plants in daily life can include plants as food, environmentally friendly vegetable pesticides and insect control. Section 2 presents kecombrang sampling, extraction, measured of mortality *Ae. aegypty* and checking framework based on the LC_{50} and LC_{95}. In this section also discussed the insect associated on the plantation of the kecombrang.

Section 3 the learning framework. Then, the framework is extended to the methanol fraction of kecombrang stem extract (*Etlingera elatior*) has an effect as larvaside on *Ae. aegypti* larva instar III. *E. elatior* species was used as a control for the *Ae. aegypti* mosquito causing Dengue Hemorrhagic Fever (DHF) and as a potential environmentally, friendly plant pesticide in Indonesia.

2. RESULTS AND DISCUSSION

2.1. Extract of Kecombrang as Larvacide

Then probit analysis results from the test of kecombrang leaves and flowers extract on *A. aegypti* larvae revealed the number of larvae deaths by 50% (LC_{50}) and 95% (LC_{95}). For the preliminary test, the test concentrations for leaves and flowers were the same, namely 0.5%, 0.75%, 1.0%, 1.24%, 1.5% and 1.75%, respectively. The basis for selecting the concentration of the test was based on the results of research [19], which conducted a test of kecombrang stem extract on *A. aegypti* larvae with hexane solvent. It obtained LC50 in the value of 1%. These results were not different significantly with a research finding from [23], which conducted a test of kecombrang stem extract with a methanol solvent with 0.98% LC_{50} value. Based on these results, an initial concentration was taken for the preliminary test with the category of below 1% and above 1%. This procedure was applied to two samples, namely leaves and flowers. Preliminary test results in the form of kecombrang leaves and flowers extract on the death of *Ae. aegypti* larvae can be seen in Table 1 below.

Table 1 LC_{50} and LC_{95} values of mortal *Ae. aegypti* larvae (Preliminary Test)

	Kecombrang leaves		**Kecombrang flowers**	
	LC_{50}	**LC_{95}**	**LC_{50}**	**LC_{95}**
Estimate (%)	1.112	1.723	0.862	1.396
Lower bound (%)	0.624	1.502	0.624	1.287
Upper bound (%)	1.287	2.438	1.124	1.765

Based on the results of probit analysis in the preliminary test on leaves sample in Table 1, LC_{50} value was 1.112% and LC_{95} was 1.723%. While flowers extract obtained LC_{50} value of 0.862% and LC95 of 1.396%. Leaves sample gained the LC_{50} value above 1%, while LC_{50} of the flower sample was below 1%. The concentration value ranges were taken below the LC_{50} value to above the LC_{50} value, so based on the results, concentration ranges were made for further tests or the actual test for leaves extract, those were 0.75%; 1.0%; 1.24%; 1.5%; 1.75%; and 2.0% respectively. While kecombrang flower extracts had a range of further test, the efficacy of kecombrang leaves and flowers extract concentration [9, 24] were 0.05%; 0.1%; 0.24%; 0.5%;

0.5%; 0.75% and 1.0% respectively. The concentration range of flowers extract administration was lower than the concentration range of leaves extract. This was due to LC_{50} values of Kecombrang leaves and flowers extract. Observation was conducted in a number of times, namely 1st, 3rd, 6th, 9th and 23th hr. Kecombrang leaves extract could kill 95% of larvae, starting at concentrations of 1,723% to 2,438%, while flowers extract started at concentrations of 0.624% to 1,287%. LC_{50} and LC_{95} values of flowers extract were lower than kecombrang leaves extract. The mean of *Ae. aegypti* larvae mortality increased with time.

The giving of positive control, which was *Bacillus thuringiensis* (1 ppm), showed that all larvae were 100% death at the 3rd hr. Whereas in the treatment of kecombrang leaves extract, the larvae did not die 100% until the 23th hr. In contrast to the treatment of leaves extract, the administration of kecombrang flowers extract treatment revealed the death of 100% larvae at the 23th hr of 0.24%; at the 9th hr of 0.50%; at the 3rd hr of 0.75% and at the 1st hr of 1%. The administration of negative control in the form of water, larvae death did not occur until the 23th hr of observation.

As the consequence, the hypothesis test was 23th hr. One Way Anova is a comparative hypothesis test for numerical variables normally distributed to more than two groups. From this hypothesis test, it is known that the p value is 0,000. The p-value <0.05 means that there is a significant difference in the number of larvae dying during observation between the two concentrations.

2.2. Insects Collected on the Kecombrang

The insects collected on Kecombrang plantation consists of 7 orders and 37 families with 917 insect populations. Order of Diptera with 12 Families, consist of Anthomylidae, Asilidae, Bibionidae, Calliphoridae, Cecidomyiidae, Culicidae, Lonchaeidae, Muscidae, Otitidae, Scathophagidae, Tabanidae, and Tephritidae were dominant recorded in Kecombrang plantation from June to July 2018 used by sticky yellow trap, pit fall trap and malaise trap. Those 12 families collected were dominant assumed availability of food sources in kecombrang plantation [22].

Based on the composition of the insects identified from the use of three tools, it was found that the use of sticky yellow trap was more effective compared to the use of pit fall trap and Malaise trap tools. This research data is supported by [1], which has similar calculation with data collection of the research, consist of 13 families recorded in Bogor. The status of insects, consist of herbivore [6 orders; 15 families]; predators consist of 3 orders and 7 families, while parasitoid comes from 2 orders (Hymenoptera and Diptera) and 4 families, then pollinators is only founded from order Lepidoptera, namely the family Noctuide and Satyridae. Meanwhile scavenger recorded 2 orders, 4 families, such as Hymenoptera: Braconidae, Evaniidae, Ichneumonidae and Diptera: Calliphoridae.

Based on the results of the leaves extract test, it revealed that the kecombrang leaves and flowers extract had biolarvacide ability. Kecombrang leaves extract showed an increase in mortality of *Ae. aegypti* larvae along with the increasing concentration of the extract, as well as the kecombrang flowers extract. Kecombrang flowers extract had the ability to kill *Ae. aegypti* larvae at lower/smaller concentrations compared to kecombrang leaves extract. LC50 and LC95 values of kecombrang flowers extract were much lower compared to kecombrang leaves extract. This shows that kecombrang flowers extract is more effective in killing *Ae. aegypti* larvae than kecombrang leaves extract. The lower the LC_{50} value of a plant extract, the better it functions, due to the plant toxicity.

According to [22], LC_{50} value can be used to determine the level of toxicity of a plant extract. A plant extract can be considered very toxic if it has an LC_{50} value below 29 ppm (0.003%), and is considered toxic if an LC_{50} value is of 29-1000 ppm (0.003%-0.1%) [23]. Based on this research, kecombrang flowers extract can be categorized as toxic. Previous research also uses kecombrang flowers extract [24, 25], which is conducted to see the effectiveness of kecombrang flowers extract against *Aedes* spp. The spraying of kecombrang flowers extract into the body of adult *Aedes* spp. mosquitoes (*Ae. aegypti* and *A. albopictus*) reveals that kecombrang flower extract has the ability to kill 50% of the population of *Aedes* spp. at concentrations of ≥4.5% [23]. This means that kecombrang flowers extract is also effective in killing adult *Aedes* spp.

Based on the results of the study [25], it was found that kecombrang flowers extract contained quite complex active compounds such as flavonoids, terpenoids, saponins, tannins, alkaloids and anthraquinone [24], while kecombrang leaves extract contained quite plenty of active flavonoid compounds [26]. The active compound like tannin can be useful as antibacterial, antiviral and antiparasitic. The presence of tannin compounds in kecombrang flowers extract can act as poison for larvae,
Based on the results of the analysis, it is known that the average of *Ae. aegypti* larvae mortality due to the administration of kecombrang flowers extract is greater than the administration of kecombrang leaves extract. This means that kecombrang flowers extract is more effective in killing *Ae. aegypti* larvae than kecombrang leaves extract. Several supporting studies [27] on the effectiveness of kecombrang (*E. elatior*) leaves extract as antioviposition of *Ae. aegypti* mosquitoes shows that kecombrang leaves extract with concentrations above 24% was effective as antioviposition of mosquitoes, which can reduce the number of mosquito egg [27].

Research used to [6] reveals that the repellent activity of kecombrang leaves essential oil gains result in the repelence index (IR) or the resistance of kecombrang leaves essential oil by 94.38% (>90%) [28]. This result is also supported by research [19], which says that kecombrang essential oil is effective in killing termites (*Coptotermes curvignathus* sp) with an LC_{50} value of 3.072%. Research by [26] uses fragrant pandanus leaves extract (*Pandanus amaryllifolius* Roxb.) as *Ae. aegypti* biolarvaside with an LC_{50} value of 2388.46 ppm. According to [29-30], seed extract of

Lansium domesticum Corr has an LC50 of 9367.5 ppm, while [31] say that *Aloe vera* leaves extract has an LC_{50} ranging from 2.041%-0.130%, which has killing ability towards *Ae. aegypti*. Then [32] identified that ceremai leaves extract (*Phyllantus acidus*) had an LC_{50} of 0.505% with ethanol extract, whereas fragrant vetiver (*Vetiveria zizanoides*) had an LC50 of 1373.6 ppm [33-34].

All of these studies have LC_{50} values below LC_{50} values of 0.053% of kecombrang flowers in this study, so it can be said that kecombrang flowers extract is more effective than some of the other plant extracts. When compared with the positive control (*B. thuringiensis*), the results obtained from both kecombrang leaves and flowers extract showed less effective outcome to be compared to positive controls, but kecombrang flowers extract can still be developed as an alternative to larvacide. Kecombrang flowers extract has a low LC_{50} value and is categorized as toxic and even very toxic. Further research is needed to identify and isolate active compounds contained in kecombrang flowers, so that they can be retested on *Ae. aegypti* larvae.

3. CONCLUSION

From the research, it can be concluded that the methanol fraction of kecombrang stem extract (*Etlingera elatior*) has an effect as larvaside on *Ae. aegypti* larva instar III. Therefore, natural insecticides were used, one of which was Kecombrang (*E. elatior*), which contains saponins and flavonoids that have a larvicidal effect. The 12 families of Dipterans have been found on Kecombrang plantation.

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