Potential Active Material of Stem Extract *Apium graveolens L.* As Biolarvacides *Aedes aegypti*

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Abstract. *Aedes aegypti* is the main vector of transmission of dengue hemorrhagic fever (DHF). The death rate from dengue fever in Indonesia is still quite high. As biolarvicides, celery stems (Apium graveolens L.) are known to contain alkaloids, flavonoids, tannins, and saponins. This study aimed to identify the biolarvicide effect of celery (Apium graveolens L.) stem extract on the death of Aedes aegypti mosquito larvae by examining the lethal concentration (LC_{50}) value. This research uses an experimental laboratory design with a post-test only control group. 6 groups made up the treatment group: the Aquades negative control group, the Abate 0.01% positive control group, P1 (5,000 ppm celery stem extract), P2 (10,000 ppm celery stem extract), P3 (20,000 ppm celery stem extract), and P4 (30,000 ppm celery stem extract). Each treatment used 10 instar III larvae, and each treatment was repeated two times. According the study's findings, at a concentration of 30,000 ppm (P4), the percentage of larval mortality reached 100%, resulting in the highest number of deaths of larval. The lowest larval mortality occurred at a concentration of 5,000 ppm (P1) with a larval mortality percentage of 55%. The LC_{50} value of celery stem extract (*Apium graveolens* L.) was 4,518 ppm, which was in the concentration range of 258–7,531 ppm. This means that the concentration of 4,518 ppm of celery stem extract (*Apium graveolens* L.) is toxic because it causes 50% mortality of Ae. aegypti instar III. The Kruskal-Wallis test results showed that the p value (Asymp. Sig.) was 0.099 (0.099 > 0.05), which means that there was no difference in mortality of Ae. aegypti in different treatments at P1, P2, P3, and P4.

Keywords: Potential · Celery Stem Extract (Apium graveolens l.) · Biolarvacides · Aedes aegypti larvae

1 Introduction

DHF (dengue hemorrhagic fever), filariasis, malaria, chikungunya, and encephalitis are some of the diseases that can be transmitted by mosquitoes [1]. In developing countries like Indonesia today, diseases are transmitted by mosquitoes and are cases that deserve special attention. One of them is dengue fever, it is transmitted by the *Aedes aegypti*

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mosquito's bite [2]. Dengue fever is characterized by an abrupt rise in temperature and bleeding symptoms, and which often results in shock and death [3].

The mortality rate due to dengue fever in Indonesia is still quite high (>5%) in the provinces of Aceh, South Sumatra, West Kalimantan, Central Kalimantan, East Kalimantan, Southeast Sulawesi, Lampung, and West Nusa Tenggara [4].

Based on data from the West Nusa Tenggara (NTB) Provincial Health Office, in 2016, dengue cases reached 1,939 people spread across 10 regencies/cities throughout NTB. The city of Mataram has the highest number of dengue cases and death rates in the West Nusa Tenggara area, compared to other areas. A total of 589 cases and 7 people died in all sub-districts is something that needs to be taken into account [5]. This dengue virus may result in an increase in hematocrit, purpura, conjunctival hemorrhage, epistaxis, melena, hepatomegaly, shock, and Heart rate dropped to 20 mmHg or less, systolic pressure dropped to 80 mmHg or less, platelet count fell to 100,000/mm3 in thrombocytopenia from days 3 to 7, and hemoconcentration increased [6].

Several efforts to prevent mosquito vectors have been carried out by health workers from the health office chemically by giving abate powder (temephos). Synthetic larvicides are well known to be practical, effective, affordable, and simple to use. However, its continuous use can lead to increased resistance in mosquito larvae and can also have negative impacts such as water pollution due to residues left in water reservoirs and can have a negative impact on health [7].

One alternative that can be done is the use of botanical insecticides to reduce environmental pollution problems. Insecticides contain ingredients that are rapidly degraded in nature and have a small impact on the environment, so they are not harmful. Therefore, vegetable insecticides can be used as an alternative to synthetic insecticides that contain chemicals that can harm the environment [8]. One of the plant insecticides with potential as biolarvicides is celery stem (*Apium graveolens* L.).

Celery is known to contain alkaloids, flavonoids, tannins, and saponins that can work as biolarvicides. Alkaloids and saponins are stomach poisons for larvae [9]. Flavonoids work to interfere with the respiratory system of larvae, and tannins affect the failure of moulting in larvae so that they die before developing into pupae [10]. Several studies have shown that the use of celery insecticides can be used to control mosquito larvae. Based on this, this research was conducted with the aim of knowing the biolarvicide activity of celery (*Apium graveolens* L.) stem extract on *Ae. aegypti* mortality by looking at the Lethal Concentration (LC_{50}) value. The results of this study are expected to produce biolarvicides to break the life cycle of *Ae. aegypti* so that the population can be inhibited, and the number of DHF sufferers can be reduced.

2 Research Method

2.1 Design of Research

This research uses an experimental laboratory design with a post-test only control group. This research was conducted at the Chemistry and Biology Laboratory of Polytechnic Medica Farma Husada Mataram. *Ae. aegypti* larvae received direct treatment by placing

them in a solution of celery (*Apium graveolens L.*) stem extract at various concentrations. The larvae of *Ae. aegypti* instar III served as the study's subjects with siphon characteristics already black.

2.2 Tools, Materials, and Research Samples

Celery stem (*Apium graveolens L.*), *Ae. aegypti* larvae instar III, temephos (Abate 0.01%), mineral water, ethanol 96%, aquades, blender, glass jar, erlenmeyer, beaker, rotary evaporator, volumetric flask, spatula, analytical balance, aluminum foil, plastic tub, glass plastic, dropper, volume pipette, gauze, filter paper, and hand counter top.

2.3 Procedures

2.3.1 Drying Simplicia

Simplicia drying is done by the drying method with an oven. The drying process begins with a sorting process, then the product is washed and dried according to the drying method until the moisture content reaches 10%. Dry stems have the characteristic of being easily crushed when squeezed and a significant discoloration occurs. Oven drying was carried out at 50 °C for 6 h.

2.3.2 Preparation of Celery Stem Extract (Apium Graveolens L.) (Maceration Method)

The dried celery stem was blended until they became powder (simplicia) and ready to be extracted. The simplicia was extracted by the maceration method using 96% ethanol as solvent and allowed to stand for 24 h, then filtered using filter paper to obtain the filtrate and residue. The resulting filtrate was then concentrated using a rotary evaporator to produce a concentrated extract. The concentrated extract is then put in a cup to remove the remaining solvent.

2.3.3 Biolarvicide Test of Celery Stem Extract (Apium Graveolens L.)

120 tail *Aedes aegypti* larvae instar III were utilized in this investigation. The treatment group consisted of six groups, including the Aquades negative control group, the Abate 0.01% positive control group, the P1 treatment group, namely the administration of 5,000 ppm celery stem extract, the P2 treatment group (the 10000 ppm concentration of celery stem extract), the treatment group treatment P3 (giving celery stem extract with a concentration of 20,000 ppm), and treatment group P4 (giving celery stem extract with a concentration of 30,000 ppm). Each concentration extract, totaling 50 ml, was put into a plastic cup. Each treatment group was repeated 2 times, with the number of larvae in each treatment being 10 larvae. Then they left for 24 h to see the effect of mortality due to exposure to the extract. Larvae of *Ae. aegypti* that died were counted using a hand counter.

2.4 Data Analysis

Determining the percentage (%) of larval mortality treated with ethanol extract of celery stem (Apium graveolens L.) with various concentrations determined employing the formula:

$$\%\ larval\ mortality = \frac{dead\ larvae\ number}{\text{number of test larvae}} \times 100\%$$

Meanwhile, to determine the value of toxicity and effectiveness measured by the lethal concentration (LC50) of ethanol extract of celery stem (Apium graveolens L.), namely by means of data on dead larvae, concentration, and number of larvae entered and analyzed by the SPSS 23.0 program with analysis probit. There were differences in biolarvicides from various concentrations of ethanol extract of celery stem (Apium graveolens L.) using the Kruskal-Wallis statistical test.

3 Research and Discussion Results

The biolarvicide testing on *Ae. aegypti* in this study was carried out by the dipping method because the mosquito larvae are aquatic or live in water [11]. The *Aedes aegypti* larvae of instar III were used in this study because at this stage, the larvae need food to develop so that the celery juice can be drunk by the larvae. At the pupa stage, it does not need food anymore. The percentage of larval mortality due to exposure to ethanol extract of celery stem (*Apium graveolens L.*) to larvae of *Ae. aegypti* instar III is shown in Table 1.

Based on Table 1, the most significant number of larval deaths occurred in P4 (celery stem extract concentration of 30,000 ppm), with the percentage of larval mortality reaching 100%. The lowest larval mortality occurred in P1 (a celery stem extract concentration of 5,000 ppm) with a larval mortality percentage of 55%. The higher the concentration of celery stem extract (*Apium graveolens* L.) given, the higher the percentage of larval mortality, and the higher the concentration of the treatment used, the higher the toxic substances it contains. This is in line with the assertion made by Ustiawaty J and Zacharia

Table 1. Mortality of *Ae. aegypti* larvae after being tested with celery stem extract (*Apium graveolens L.*) in various concentrations.

Treatment	Number of Test Larva	Average Number of Larvae mortality	Larvae Mortality Percentage (%)
K- (aquades)	10	0	0
K+ (Abate)	10	10	100
P1 (5.000 ppm)	10	5,5	55
P2 (10.000 ppm)	10	8	80
P3 (20.000 ppm)	10	9	90
P4 (30.000 ppm)	10	10	100

Table 2. LC_{50} value of celery stem (*Apium graveolens* L.) extract against *Ae. aegypti* instar III.

Extract	value	Estimate (ppm)	IB (ppm)
Celery stem extract (Apium graveolens L.)	LC50	4.518	258

E that the mortality rate of Ae. aegypti instar III increases with extract concentration [11]. Celery stem (*Apium graveolens* L.) extract was effective in killing *Ae. aegypti* instar III. This is in accordance with the statement of the Pesticide Commission (1995) that an extract's efficacy is in terms of the concentration in killing test larvae if the concentration is able to kill as many as 10–95% of the larvae tested. Increased toxicity absorbed by *Ae. aegypti* as a test animal exceeds its tolerance limit, causing damage to cells and tissues of the larvae [12].

The percentage of mortality in the P4 treatment (celery stem extract concentration of 30,000 ppm) was the same as the positive control, reaching 100% after the larvae of *Ae. aegypti* contact for 24 h. This indicates that the mortality of *Ae. aegypti* in the test solution was probably caused by the presence of toxic compounds capable of killing *Ae. aegypti*. In the positive control of 100% mortality, abate which is a positive control, is anti-cholinesterase, which works by binding to the cholinesterase enzyme, causing continuous muscle contractions that cause the death of the larvae [13]. While in the negative control, there was no larval motality. This shows that aquadest is not able to kill larvae because *Aedes aegypti* larvae can live in clean water.

According to the data in Table 2, celery stem extract (*Apium graveolens* L.) has an LC_{50} value of 4,518 ppm, which lies in the concentration range of 258–7,531 ppm. This means that the concentration of 4,518 ppm of celery stem extract (*Apium graveolens* L.) caused 50% mortality of *Ae. aegypti* instar III. Based on the classification of Wagner (1993) in Rochmat A et al. (2016), the level of toxicity of a plant is assessed based on the mortality rate of the test material (very toxic if the LC_{50} value is 30 mg/L, toxic if 31 mg/L < LC50 1,000 mg/L, and non-toxic if > 1,000 mg/L) [14]. Based on this classification, celery stem extract (*Apium graveolens* L.), at a concentration of 4518 ppm was considered toxic so that it was able to kill 50% of *Ae. aegypti* instar III. According to Kartikasari D. and Novitasari M., the lower the LC_{50} value, the better, because a small concentration can kill the population, so the effectiveness of using raw materials is achieved [15].

Celery stem extract (*Apium graveolens* L.) enters the bodies of insects and has a larvicidal effect. According to Kristanti et al., chemical compounds in plants that have the potential as biolarvicides are cyanide, flavonoid, saponin, tannin, alkaloid, steroid, and essential oil group [16]. Compounds that have biolarvicidal activity are also contained in celery plants. Celery stems are known to contain alkaloids, flavonoids, tannins, and saponins that can work as biolarvicides. Alkaloids and saponins are stomach poisons in larvae [9]. Saponins that function as insect feeding inhibitors (antifectants) [10]. Additionally, saponins are known to lessen the surface tension of the digestive tract's mucous membranes in larvae, causing the walls to corrode and obstructing the larvae's ability to digest [9]. Saponins also interferes with the development and molting of the larvae so that the larvae will not be able to develop to the next stage [17]. Alkaloids, especially

Table 3. The results of the Kruskal-Wallis test.

Test Statistics[a,b]	
	Number of Larvae Deaths
Chi-Square	9.255
df	5
Asymp. Sig.	.099

a. Kruskal Wallis Test.
b. Grouping Variable: Concentration.

the three main hormones found in insects, namely brain hormones, edixone hormones, and growth hormones, have the ability to limit the growth of insects (larvae). Metamorphosis failure may result from a lack of these hormones' development. Alkaloids can damage the larval nervous system by inhibiting the action of the acetylcholinesterase enzyme, which will interfere with excitatory transmission, causing a decrease in muscle coordination and causing death. It can also degrade cell membranes to enter and damage cells [18]. Tannins can affect molting failure in larvae so that they die before developing into pupae [10]. Flavonoids can enter through the cuticle of Ae. aegypti, then damage the cell membrane of *Ae. aegypti* [19]. Flavonoids cause body cavity permeability in *Ae. aegypti* so that hemolymph cannot be completely distributed [20]. By entering the larva's body through the respiratory system, flavonoids work as a respiratory poison that will cause the nerves to wither, the respiratory system is disrupted causing the larvae to be unable to breathe and then die [21] (Table 3).

According to the Kruskal-Wallis test results, the p value (Asymp. Sig.) was 0.099 ($0.099 > 0.05$), which means that there was no difference in mortality of *Ae. aegypti* in different treatments. This means that the concentration of P1 (5,000 ppm), which is the lowest concentration, has been effective in eliminating 55% of Ae. aegypti. P2 (a celery stem extract at a concentration of 10,000 ppm) caused an 80% larval mortality rate, P3 (a celery stem extract at a concentration of 20,000 ppm) caused a 90% larval mortality rate, and P4 (a celery stem extract at a concentration of 30,000 ppm) caused a 100% larval mortality rate.

According to observations, celery stem extract (*Apium graveolens* L.) can increase the mortality of Ae. aegypti if the higher the concentration used in therapy. This can be seen at various different concentrations, indicating the large number of motality of *Ae. aegypti* in each treatment with different concentrations. The higher the concentration of celery stem extract (*Apium graveolens* L.) given, the higher the mortality of *Aedes aegypti* larvae. According to the Pesticide Commission (1995), the concentration of celery stem extract (*Apium graveolens* L.) stated that the concentration of celery stem extract (*Apium graveolens* L.) was successful in killing the test larvae if it was able to eliminate 10–95% of the test larvae [12]. Increased toxicity absorbed by *Ae. aegypti* as a test animal exceeding its tolerance limit will result in damage to cells and tissues of the larvae.

4 Conclusion

Celery stem extract (*Apium graveolens* L.) was effective in killing *Ae. aegypti*. Celery stem extract (*Apium graveolens* L.) was able to kill larvae up to 50% Lethal Concentration (LC_{50}) at a concentration of 4,518 ppm.

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