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# Larvicidal Activity of Bay Leaf (*Syzygium polyanthum*) Ethanolic Extract in Addition of PEG Diluent on *Aedes aegypti* Larvae

## (Aktivitas Larvasida Ekstrak Ethanol Daun Salam (*Syzygium polyanthum*) Dengan Penambahan Pengencer PEG Terhadap Larva Nyamuk *Aedes aegypti*)

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## ABSTRACT

**Background**: Dengue Fever (DF) is a viral infection disease transmitted by *Aedes aegypti*. It is one of Indonesian endemic diseases that reported to occur throughout the year. To break the transmission chain of DF, the use of larvicides is preferred, especially using natural ingredients, such as bay leaves (*Syzygium polyanthum*). Polyethylene glycol (PEG) as a dispersant may prevent the clumping of material test so that it can be distributed evenly in water which is the medium of growth for larvae. **Objectives**: To determine the effectiveness of ethanolic extract of bay leaves (EEBL) in addition of PEG diluent on the mortality of *Aedes aegypti* larvae. **Material and Method**: Bay leaves as the main material were extracted using 96% of ethanol, and were applied in two variation concentration, 0.75% and 1%, while the diluent added was PEG. The samples used in this study were *Aedes aegypti* larvae at stages III-IV, with a total of 25 individuals for each treatment group. Evaluation was performed every 6 hours, for 24 hours, then the results were recorded and analyzed using the Kruskall Wallis test and the Mann Whitney test. **Result**: In both of variation concentration used, at 24 hours of observation it was obtained the mortality of *Aedes aegypti* larvae was 100%. The p-value obtained for the Kruskall Wallis test was <0.05. From Mann Whitney test, when each of treatment group was compared to the positive control, abate®, the p-value obtained is >0.05, while when they were compared to the negative control, PEG, the p-value obtained is <0.05. **Conclusion**: 96% ethanolic extract of bay leaves in addition of PEG diluent is effective as *Aedes aegypti* larvicides. It is also known that EEBL at concentration of 0.75% and 1% in addition of PEG are as effective as abate® as *Aedes aegypti* larvicides.

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## INTRODUCTION

Dengue Hemorrhagic Fever (DHF) is an endemic disease caused by Dengue virus throughout the tropics and some of the subtropics area all around the world (Syamsir & Daramusseng, 2018). An increase in DHF patients often occurs from year to year which is closely related to seasonal or climatic changes (Diah et al., 2021). The World Health Organization (WHO) estimates that around 2.5-3 billion people are currently living in dengue fever transmission zones (Wang et al., 2020). World Health Organization, (2023) also estimate that 70% of the patients mainly come from Asia. Based on Indonesian Health Profile in 2021, the number of DHF patients in Indonesia reached 103,509 people, with 725 of them ending in death. The morbidity rate (IR) per 100,000 population is 38.15, with a case fatality rate (CFR) of 0.7. These cases are spread throughout Indonesia, especially in parts of Sumatra, the entire island of Java, parts of Sulawesi, Bali and Nusa Tenggara. Provinces with a high incidence of DHF are known to be centers of activity for residents with high mobility and a dense population, and some are tourist areas (Kementerian Kesehatan Republik Indonesia, 2022).

There is no vaccine found effectively provides protection against Dengue virus. Therefore, to break the transmission chain in DHF spreading is very dependent on controlling *Aedes aegypti*, the vector of the disease. This vector control can be carried out physically, chemically, biologically, or genetically, and in its any stage of live. One of the most preferred methods is by using chemical control using larvicides (Huljani & Ahsanunnisa, 2019). However, chemical larvicides have some lacks, such as increasing of resistances and the risk of contamination of larvicides residues in water. Alternative resources are urgently needed. Natural larvicides have been shown to make a significant contribution as a new alternative in efforts to reduce it (Riyadi et al., 2018).

Bay leaves (*Syzygium polyanthum* or also knowns as *Eugenia polyantha* are widely known and often used as spices, to enhance the food taste because it has a distinctive aroma (Harismah & Chusniatun, 2016). In other way, bay leaves were chosen as an alternative larvicides, because they contain flavonoids, tannins, alkaloids, and saponins which are toxic for larvae (Susiwati et al., 2018). Besides, bay leaves are thought to be utilized because they contain eugenol, a kind of essential oil which is not owned by all types of plants (Fatimah et al., 2022). A number of previous studies have explained several uses of bay leaves. Research by Dewi & Arlita, (2021) shows that the ethyl acetate fraction from bay leaves has an antibacterial effect against *Staphylococcus aureus* bacteria. Another study performed by Oktiansyah et al., (2022) stated that bay leaves extract was effective as a repellent against *Culex quinquefasciatus* mosquitoes. A similar thing was also conveyed by Chaiphongpachara et al., (2020) who stated that bay leaves are one of the plants that contain essential oils which are effectively used as larvicides.

In the application of natural larvicides, it is often constrained by the lack of solubility of the active substance in water which is the growth medium for the larvae. This may affect the effectiveness of larvicides on larval mortality. Therefore, it is necessary to add a dispersant substance. Polyethylene glycol (PEG) is a polyether compound that is often added in the formulation of a material, including in medicine. The addition of PEG, especially PEG 400, is known to be a good dispersant, so it may increase the solubility of a substance or drug, including larvicides, in water (Hutanu, 2014). Based on the previous studies stated above, this research was conducted to determine the effectiveness of ethanolic extract of bay leaves in addition of PEG 400 diluent on the mortality of Aedes aegypti larvae.

## MATERIAL AND METHODS

### Materials

The main material used as larvicide is bay leaves (*Syzygium polyanthum*), obtained from farmers in Klaten area, Central Java, and have been determined by Balai Besar Penelitian dan Pengembangan Tanaman Obat dan Obat Tradisional, Tawangmangu (B2P2TOOT) with letter number KM.04.02/2/2027/2022. In the extraction process also uses 96% of ethanol, manufactured by PT. Molindo Raya Industrial. The addition of 3% of PEG 400, manufactured by PT. Subur Kimia Jaya,was used as a dispersant agent and was also used as a negative control. As a positive control, abate®, manufactured by PT. BASF Indonesia, was chosen. The samples studied were *Aedes aegypti* larvae at stage III-IV obtained from the Parasitology Laboratory collection at the Faculty of Medicine, Universitas Muhammadiyah Surakarta.

### Methods

#### Extraction Process

The first stage of this study was the extracting process of bay leaves (*Syzygium polyanthum*) by maceration method using 96% ethanol which was carried out at the Pharmacology Laboratory, Universitas Muhammadiyah Surakarta. Initially, bay leaves are dried under the sun for about a week, then crushed using a blender to make them into powder. The powder is then weighed and added by 96% of ethanol. The mixture is stirred for a few minutes, then left to stand all day. This process was repeated for 7 days with daily stirring. The macerate obtained was then concentrated using a rotatory evaporator and water bath, so that a thick extract was formed. The concentration of 96% ethanolic extract of bay leaves (EEBL) to be used is 0.75% and 1%. (Sutrisna, 2016)

#### Solubility Test

The second stage is the solubility test. We used 2 variations of EEBL concentration, they are 0.75% and 1%, so that we prepared 2 beaker glasses for this stage. They were filled with EEBL which had been obtained from the previous process, as much as 0.075 mL and 0.1 ml, respectively. Each of them was

then added with 0.3 ml of PEG 400 and then added with distilled water until the volume reached 10 ml. The mixture was stirred until homogeneous and then sonicated for about 15 minutes. Solubility results were observed visually. (Budiati et al., 2023)

### Larvicide Test

The next stage is the larvicide test which was carried out at the Parasitology Laboratory, Universitas Muhammadiyah Surakarta. This process follows the guidelines for Laboratory and Field testing of Mosquito larvicides by World Health Organization, (2005). We divided this study into 4 study groups, the positive control group, the negative control group, the treatment group with 0.75% of EEBL and the treatment group with 1% of EEBL. Each study group was repeated 4 times. At first, we prepared 16 beaker glasses, each of them was filled with 50 ml distilled water and 25 larvae. The first 4 beaker glasses used for the positive control group were added with abate® and distilled water until the volume reached 100 ml. The second 4 beaker glasses used for the negative control group were added with 3 ml of PEG and distilled water until the volume reached 100 ml. The third 4 beaker glasses used for the treatment group with 0.75% of EEBL were added with 0.75 ml of EEBL, 3 ml of PEG and distilled water until the volume reached 100 ml. The last 4 beaker glasses used for the treatment group with 1% of EEBL were added with 1 ml of EEBL, 3 ml of PEG and distilled water until the volume reached 100 ml. The larvae in each beaker glasses were then observed at 6, 12, 18 and 24 hours and the number of larvae that died was recorded. The percentage of the larva mortalities in 24 hours was calculated by this following formula.

$$\text{Percentage of the larva mortalities in 24 hours} = \frac{\text{The number of larvae that die in 24 hours}}{\text{The number of larvae prepared in each beaker glass}} \times 100\%$$

The data obtained was then tabulated and processed statistically using Saphiro-wilk test, Homogeneity of Variance Test, continued by Kruskall Wallis Test and Mann Whitney Test.

## RESULTS AND DISCUSSION

### Extraction Process

Extract yield is a comparison between simplicia and extract obtained. In this study, from 1000 grams of simplicia used, 72.5 grams of extract was obtained. It means, the yield produced in this study was 7.25%.

### Solubility Test

At each concentration of EEBL added with PEG 400 and distilled water, it was obtained that the mixture remained homogeneous without forming precipitate. Thus, it can be said that the addition of PEG able to maintain the dispersion level of the extract in water media.

## Larvicidal Test

The data from the larvicidal test are recorded in table 1 as presented below, while the the larvicide test process is shown in Figure 1

Table 1. Number of Larval Mortalities

| Study group | Repetition | Number of larval mortalities at each of observation time | | | | Average of the larva mortalities in 24 hours | Percentage of the larva mortalities in 24 hours |
|---|---|---|---|---|---|---|---|
| | | 6 | 12 | 18 | 24 | | |
| Positive control group (abate®) | I | 25 | 0 | 0 | 0 | 25±0 | 100% |
| | II | 25 | 0 | 0 | 0 | | |
| | III | 25 | 0 | 0 | 0 | | |
| | IV | 25 | 0 | 0 | 0 | | |
| Negative control group (3% of PEG) | I | 0 | 0 | 0 | 0 | 0±0 | 0% |
| | II | 0 | 0 | 0 | 0 | | |
| | III | 0 | 0 | 0 | 0 | | |
| | IV | 0 | 0 | 0 | 0 | | |
| Treatment group of 0.75% EEBL | I | 25 | 0 | 0 | 0 | 25±0 | 100% |
| | II | 20 | 5 | 0 | 0 | | |
| | III | 20 | 5 | 0 | 0 | | |
| | IV | 20 | 5 | 0 | 0 | | |
| Treatment group of 1% EEBL | I | 25 | 0 | 0 | 0 | 25±0 | 100% |
| | II | 25 | 0 | 0 | 0 | | |
| | III | 25 | 0 | 0 | 0 | | |
| | IV | 25 | 0 | 0 | 0 | | |

Note :

EEBL : 96% ethanolic extract of bay leaves

Figure 1. Containers used for each group in the larvicide test

Figure 2. Process of observing the condition of Aedes aegypti larvae

From the data above, it can be seen that in the positive control group, the mortality of larvae at each replication reached 100%. Abate® contains temephos as the active substance, which works by inhibiting the cholinesterase enzyme in the larvae, causing disturbances in nerve activity due to accumulation of acetylcholine in the tissues (Suparyati, 2020). Thus, it can be said that the usage of abate® as a positive control is appropriate. In the negative control group, there was no larval death in each replication. In other word, the larval mortality in negative control group was 0%. This shows that PEG does not contain larvicidal active substance. The PEG itself did not affect the larval mortality, so that the addition of PEG as a diluent in this study and as a negative control was also appropriate.

In both of EEBL treatment groups it was found that the larval mortality at 24 hours of observation reached 100%. It means that EEBL at a concentration of 0.75% and 1% has larvicidal activity against *Aedes aegypti* larvae. As it is known that bay leaves as main material in EEBL contains several kinds of active substances that are larvicidal, such as flavonoids, tannins, alkaloids, saponins, and eugenol. Flavonoids enter the larvae's body through the siphon and then cause damage to the larva's respiratory system. This is shown by changing the position of the larvae which aligns its body to the water surface to make it easier in oxygen uptake. Continued respiratory disturbance will result in larval death (Cania & Setyaningrum, 2013). Tannin compounds cause the death of *Aedes aegypti* larvae by inhibiting the absorption of nutrients. This metabolic disorder is due to decreased activity of the protease enzyme in converting amino acids (Kumara et al., 2021). Alkaloids act on several neurotransmitter receptors and affect the sodium channel of the larval nerve cell membrane thereby preventing the transmission of nerve impulses in *Aedes aegypti* larvae which trigger uncontrolled muscle movements, paralysis, seizures, and lead to death (de Souza Wuillda et al., 2019). Saponins, besides being able to reduce the activity of protease enzymes that affect food absorption, are also able to inhibit the acetylcholinesterase enzyme which causes the accumulation of acetylcholine. This causes chaos in the impulse delivery system in the larval body and may cause the muscles keep contracting until fatigue, and finally death occurs (Ervina, 2014). Eugenol, which is one of the essential oil contents in bay leaves, also acts on the

nervous system, thus strengthening the effect of muscle contractions on the larvae (Cahyani & Asngad, 2020). With these ingredients, bay leave extract is believed to have the ability to act as a larvicide. A study with similar results has been carried out by Tri & Ilham, (2020) where the best effectiveness of bay leaves extract against *Aedes aegypti* larvae was obtained at a concentration of 213 ppm, as well as study conducted by Nurlailah & Thuraidah, (2020) which showed LC50 in the bay leaves larvicide test against *Aedes aegypti* larvae was 0.609-0.935, although this result was lower than *Cananga odorata* flower extract. Similar study by (Rohmayani et al., 2022) also states that bay leaves extract is effective as a larvicide, even though in her study the subjects used were *Culex quinquefasciatus* larvae.

The results from normality test using the Shapiro-Wilk test and the homogeneity test with the Homogeneity of Variance test showed that the p-value for each group and for each repetition was <0.05. In other words, it can be stated that the data obtained is abnormally and in-homogeneously distributed. Thus, the statistical analysis performed next is a non-parametric statistical test, that was Kruskall Wallis test. The Kruskall Wallis test is used to see whether there are groups of data that have significant differences. The p-value obtained from this test is <0.05. Therefore, it can be interpreted that there is at least one group of data that is significantly different. And to see which groups that have these differences, then proceed with the Mann Whitney post hoc test. This post hoc test result shown in table 2.

**Table 2. The Mann Whitney post hoc test result**

| | Negative control group | Positive control group | 0.75% of EEBL group | 1% of EEBL group |
|---|---|---|---|---|
| Negative control group | | 0.008* | 0.008* | 0.008* |
| Positive control group | 0.008* | | 1.000 | 1.000 |
| 0.75% of EEBL group | 0.008* | 1.000 | | 1.000 |
| 1% of EEBL group | 0.008* | 1.000 | 1.000 | |

In Mann Whitney test, each of EEBL treatment group was compared to the negative control group, and a p-value of <0.05 was obtained, which means that there was a significantly difference in the data between groups. In other words, EEBL with a concentration of 0.75% and 1% proved to be effective as a larvicidal against *Aedes aegypti* larvae. Furthermore, the EEBL treatment group was compared to the positive control group, and a p-value of >0.05 was obtained, which means that there was no significantly

difference in the data between groups. In other words, EEBL with a concentration of 0.75% and 1% has the same effectiveness as abate® as a larvicidal against *Aedes aegypti* larvae.

This finding is in line with study conducted by Lumowa & Nova, (2015) and Setyaningsih & Swastika, (2016) who also explored the larvicidal effect of EEBL on *Aedes aegypti* larvae. The two studies also stated that the EEBL was effective as a larvicide on *Aedes aegypti* larvae. However, in both of them, there was no 100% larval mortality in the treatment group, where the highest EEBL concentration used in Lumowa & Nova, (2015) study was 0.75% and in Setyaningsih & Swastika, (2016) study was 10%. This was answered through this study, where the concentration of EEBL used was 0.75% and 1% with the addition of PEG, it was found that it may increase the effectiveness of EEBL as a larvicidal against *Aedes aegypti* larvae. Addition of PEG increases the dispersion of the extract in water which is the living medium for *Aedes aegypti* larvae, thereby increasing exposure to the active substance to the larvae, resulting in increased larval mortality.

## CONCLUSION

96% ethanolic extract of bay leaves (EEBL) in addition of PEG diluent is effective as *Aedes aegypti* larvicides. It is also known that EEBL at concentration of 0.75% and 1% in addition of PEG are as effective as abate® as *Aedes aegypti* larvicides. Because the concentration variations used in this study are quite limited and have a short range, the LC50 and LC90 cannot be evaluated. Therefore, it is necessary to carry out similar study with a further concentration range and variation so that data on LC50 or LC 90 can be obtained. Additional examinations such as GC-MS (Gas Chromatography–Mass Spectrometry) tests or tube tests can also be carried out to detect active ingredients contained in the extract and its amount.

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## CONFLICT OF INTEREST

The author declares that there is no conflict of interest

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