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# Bio-larvicidal Potential of Betel Leaves (*Piper betle* L) Ethanolic Extract in Addition of PEG 400 Diluent on *Aedes aegypti* Larvae

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**Abstract**

Dengue hemorrhagic fever (DHF) is a kind of vector transmitted disease, by *Aedes aegypti*. It is one of major public health problem around the world, including Indonesia, because it may lead to epidemics and death in a short time. The use of plant extracts as bio-larvicidal is thought to be a promising solution, and one of them is the betel leaves (*Piper betle* L). The addition of polyethylene glycol (PEG) as a diluent is thought may increase the dispersity of plant extract in the water which is larval medium of growth. Objectives: To determine the bio-larvicidal potential of 96% ethanolic extract of betel leaves (EEBL) in addition of PEG 400 diluent on the *Aedes aegypti* larval mortality. Material and Method: Betel leaves were extracted by maceration using 96% of ethanol. There are two kinds of EEBL concentration used, 0.2% dan 0.4%. PEG 400 was also added as diluent. The samples in this study were *Aedes aegypti* larvae at instar III-IV, with a total of 400 larvae. Evaluation was performed at 6, 12, 18, and 24 hours. The data obtained was then analyzed by Kruskall Wallis test and post-hoc Mann Whitney test. Result: In all of treatment groups, larval mortality was reached 100% at 24 hours. From the Kruskall Wallis test, p-value obtained was <0.05. From post-hoc Mann Whitney test, the p-value obtained in the comparation between treatment groups and positive control group was >0.05, and the p-value obtained in the comparation between treatment groups and negative control group was <0.05. Conclusion: EEBL in addition of PEG 400 diluent is potential as bio-larvicidal on *Aedes aegypti* larvae. It is also known that EEBL at concentration of 0.2% and 0.4% in addition of PEG 400 are as effective as temephos as larvicides on *Aedes aegypti* larvae.

**Keywords:** Betel leaves; PEG, Bio-larvicidal; Aedes aegypti.

**Abbreviations:** DHF: Dengue Hemorrhagic Fever; PEG: Polyethylene glycol; EEBL: 96% Ethanolic extract of Betel Leaves.

## INTRODUCTION

Dengue hemorrhagic fever (DHF) is one of vector transmitted disease, that mostly known around tropics area. DHF is transmitted by *Aedes aegypti* mosquito and it is still a major public health problem around the world, including in Indonesia. This disease may cause epidemic and lead to death in a short time (Karyanti and Hadinegoro, 2016). Data reported in the Indonesian Health Profile in 2021, the number of DHF cases has exceeded 100,000 cases, with 0.7% of them ending in death. The spread of DHF in Indonesia is mainly in Sumatra and Java Island, especially in urban areas with a high level of mobility and also in densely populated residential areas (Kementerian Kesehatan Republik Indonesia, 2022).

*Aedes aegypti* is the main vector of Dengue Hemorrhagic Fever (DHF) and Chikunguya. *Aedes aegypti* is a member of the order Diptera, Brassicaceae. The use of synthetic larvicide in controlling disease vectors, including *Aedes aegypti*, has several negative impacts which may lead to resistance and environmental pollution (Waskito and Cahyati, 2018). Alternative medicine to overcome those problems are being highly investigated. WHO also recommends proven safe and effective traditional medicinal plants, to be incorporated into the National Health System (WHO, 2018). One of Indonesia's native plants that are highly observed was betel leaves (*Piper betle* L). However, betel leaves are evergreen, that are commonly used as flavoring in chewing areca nut (betel nut chewing) (Patra, et al., 2022). Betel leaves contain several active compounds such as alkaloids, flavonoids, phenolics, saponins, tannins, and also essential oils (Maryanti, Manalu and Lesmana, 2022). These chemical compounds have bio-larvicides effect and not necessarily owned by other plants (Noshirma, 2019).

Water is the growth medium for larvae. But, when we applied plant extract on it, sometimes they are hard to dissolves well. One of polyether compounds that is often added in chemical formulation as a dispersant agent, is polyethylene glycol (PEG). The addition of PEG,

especially PEG 400 as a diluent is thought may increase the dispersity of plant extract in the water (Hutanu, et al., 2014). Previous study performed by Sembiring and Hasan (2020) stated that the minimum concentration of EEBL resulting larval mortality is 2.5%, but it's only reached 60% at first 6 hours of observation. It needs longer time to reached 100% of larval mortality. It is thought that by adding a dispersant agent, such as PEG 400, may increase the extract solubility in the water, so that active substance will be easily contact to the larva and caused death.

By considering those conditions, this study aimed to determine the bio-larvicidal potential of 96% ethanolic extract of betel leaves (EEBL) in addition of PEG 400 diluent on the *Aedes aegypti* larval mortality.

## MATERIALS AND METHODS

### Ethanolic extract of betel leaves (EEBL) Processing

Betel leaves collected were washed thoroughly, and then dried under the sun for about a week. When they were completely dry, it is continued by chopped them up using blender so that they turn into powder, and the simplicial were obtained. Those simplicial were then weighed and added by 96% of ethanol. This blend was stirred in a few minutes and then left to stand all day, repeated every day, for a week. This process resulting a macerate production, which was need to be concentrated by rotatory evaporator and water bath. Hereby, a thick extract was formed. This thick extract was prepared in two variation concentration, 0.2% and 0.4%. This step was performed in Pharmacology Laboratory, Universitas Muhammadiyah Surakarta.

**Figure 1**. Dried process of betel leaves under the sun.

**Figure 2.** Concentrating the macerate using rotatory evaporator.

### Sedimentation test

We prepared two container for two variation concentration of EEBL. The first container was for 0.2% of EEBL, and the other one was for 0.4% of EEBL. Each of the container were filled with EEBL which had been obtained from the extraction process, as much as 0.02 ml and 0.04 ml, respectively. At next, every container was 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 observed for 24 hours to see whether a precipitate formed. This step was also performed in Pharmacology Laboratory, Universitas Muhammadiyah Surakarta.

### Aedes aegypti larval preparation

Aedes aegypti's eggs that had been prepared on filter paper sheets were used the filter paper sheets were placed to a container which has been filled by distilled water before. Prepare fish feed and blender a little. Mix the fish feed that has been blended slightly, to the container prepared before. Wait for 2 to 3 days, so that the eggs will drip. This step was performed in Parasitology Laboratory, Universitas Muhammadiyah Surakarta.

### Larvicidal test

For this study, we adopt the guidelines for Laboratory and Field testing of Mosquito larvicides by WHO (2005). There were 4 study groups designed. They are the negative control group, the positive control group, the treatment group with 0.2% of EEBL, and the treatment group with 0.4% of EEBL. A 3% of PEG 400 was used as negative control, while 1% of temephos was used as positive control. Repetition was done for 4 times, so that we prepared 4 containers for each study group. Total number or larva involved in this study was 400, with 25 larvae prepared in each of container. In negative control group, each of container were added with 3 ml of PEG 400 and distilled water until the volume reached 100 ml, while in positive control group, each of container were added with temephos and distilled water until the volume reached 100 ml. In the treatment group with 0.2% of EEBL were added with 0.2 ml of EEBL, 3 ml of PEG 400 and distilled water until the volume reached 100 ml.

And in the treatment group with 0.4% of EEBL were added with 0.4 ml of EEBL, 3 ml of PEG 400 and distilled water until the volume reached 100 ml. Larval mortality was observed every 6 hours, for 24 hours. The larvae are considered to be dead when they sink or appear unresponsive when touched using a stick. All of this process was performed in Parasitology Laboratory, Universitas Muhammadiyah Surakarta.

### Data analysis

The number of larval-death in each study group and in each observation-period were recorded, and analyzed using Kruskall Wallis test and post-hoc Mann Whitney test.

## RESULTS AND DISCUSSION

### Sedimentation test

From both containers prepared for this test, it was obtained that no precipitate formed, directly nor in 24 hours of observation. It means that the addition of PEG 400 may increase the dispersion of EEBL in water, so that active substance kept soluble in it.

### Larvicidal test

In larvicidal test, we count the number of larval mortalities in each study group and in each repetition performed. The data obtained presented in the following table.

**Table 1.** Larval mortalities.

| Study group | Larval mortalities (mean ± standard deviation) | | | | Percentage of larval mortalities in 24 hours |
|---|---|---|---|---|---|
| | 6 hours | 12 hours | 18 hours | 24 hours | |
| Negative control group | 0±0 | 0±0 | 0±0 | 0±0 | 0% |
| Positive control group | 25±0 | 25±0 | 25±0 | 25±0 | 100% |
| 0.2% of EEBL | 25±0 | 25±0 | 25±0 | 25±0 | 100% |
| 0.4% of EEBL | 25±0 | 25±0 | 25±0 | 25±0 | 100% |

### Discussion

In first study group, the negative control group, the percentage of larval mortality was 0%. There was no single larva were found to be dead in this study group. Here, we could say that PEG 400 has no larvicidal effect, especially on *Aedes aegypti* larva. Zhong, et al. (2013) stated that PEG 400 is a kind of hydrophilic polymer which is inexpensive and non-toxic. PEG 400 also has high solubility in water and any other solvent, such as alcohol and acetone. This addition of PEG, or we call it by PEGylation, aimed to increase the solubility of plant extract in water, so that the active substance will be evenly distributed, and may increasing its exposure to the larva. But, in other side, it's non-toxic effect will not affect the larval death, so that the larval mortality in this study was purely caused by active substance of the plant extract, not by PEG. Therefore, the usage of PEG as negative control in this study was appropriate.

The second study group, that is the positive control group, which using 1% of temephos as larvicidal agent, it was found that the percentage of larval mortality was 100%. All of *Aedes aegypti* larva in this group were considered to be dead since first-6 hour of observation. Temephos was neural toxic for larva. There is acetylcholine accumulation in larval tissue because cholinesterase inhibition by temephos. This results in hyperexcitation, tremor, and convulsion of larva, resulting fatigue and lead to death. Those mechanism was proven by the determination of temephos as part of *Aedes aegypti* eradication program in Indonesia (Pambudi, et al., 2018). Hence, the determination of temephos as positive control in this study was appropriate.

In the treatment groups, 0.2% and 0.4% of EEBL, the percentage of larval mortality was 100%. The same condition to the positive control group. It means that EEBL was proven to contain active substances that are have larvicidal effect on *Aedes aegypti* larva. Various literatures state that betel leaves contain a number of secondary metabolites that are larvicidal, such as alkaloids, flavonoids, saponins, tannins, essential oils, and many more. Most of them are mainly act by interference the central nervous system via cutaneous or respiratory absorption. In this type of intoxication, there is acetylcholinesterase (AChE) inhibition which may lead to death. And as we know, this mechanism was similar to temephos. Some other mechanisms of action were involving GABA system, leading to seizures and inhibition of mitochondrial activity (de Souza Wuillda, et al., 2019; Sembiring and Hasan, 2020; and Kumara, et al., 2021). Another active substance that is thought to have a strong larvicidal effect from Betel leaves is caryophyllene. This substance causes a damage on cellular function because it binds strongly to the NS3 protease in the nucleus (Prabhu, et al., 2022).

The data in table 1 was then statistically analyzed. To see the normality of data distribution, the Saphiro-Wilk Test was used, and the p-value obtained was $<0.05$. Meanwhile, to see the homogeneity of data distribution, the Homogeneity of Variance Test was used, and the p-value obtained was $<0.05$. Based on these two results, it can be said that the requirements for using the ANOVA test were not met, so an alternative test with Krusskal-

Wallis Test was used, and the p-value obtained was <0.05. Here, we conclude that there is at least one group of data that is significantly different, so the analysis continued with Post Hoc Mann Whitney Test. Comparison of data in the negative control group and the treatment groups, both 0.2% and 0.4% of EEBL, obtained the p-value of <0.05. It means that the data in both of study group was significantly different. Furthermore, it can be interpreted that EEBL with a concentration of 0.2% and 0.4% with addition of PEG diluent, was a potential bio-larvicides to *Aedes aegypti* larva. Different condition appears when compared to study performed by Rosyadi and Swastika (2020), where EEBL was also used but without the addition of PEG, it was seen that there was a difference in the larval mortality within 24 hours. In Rosyadi and Swastika's study (2020), in EEBL with a concentration of 0.2% and 0.4%, larval mortality at 24 hours was 44% and 65%, respectively. Whereas in our study, with the same concentration and observation time, but with the addition of PEG, larval mortality reached 100%. This result reinforces the previous findings that EEBL with a concentration of 0.2% and 0.4% with addition of PEG diluent, was a potential bio-larvicides to *Aedes aegypti* larva. The addition of PEG 400 as a diluent effectively increases the solubility of EEBL in the water, so that it's active substances evenly distributed and exposed to the larva. We also compared our data in the positive control group and both of treatment groups, where the resulting p-value was >0.05. It means that the data in all of study group was similar. In other words, we could say that EEBL in concentration of 0.2% and 0.4% with addition of PEG have similar effectivity as larvicide on *Aedes aegypti* larva.

## CONCLUSIONS

Ethanolic extract of Betel Leaves (*Piper betle* L) in addition of PEG 400 diluent is potential as bio-larvicidal on *Aedes aegypti* larvae. It is also known that Ethanolic extract of Betel Leaves at concentration of 0.2% and 0.4% in addition of PEG 400 are as effective as temephos as larvicides on Aedes aegypti larvae

***Authors' Contributions*:** Listiana Masyita Dewi designed the study. Hilda Zaniba Ariffah carried out the laboratory work and analyzed the data. Listiana Masyita Dewi and Hilda Zaniba Ariffah wrote the manuscript. All authors read and approved the final version of the manuscript.

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