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# Biolarvasides of *Cananga odorata* Flower and *Zyzygium polyanthum* Leaves Ethanol Extract on *Aedes aegypti* Larva

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**Abstract:** Dengue hemorrhagic fever (DHF) is one of the diseases caused by *Aedes Aegypti* mosquitoes. DHF is still a public health problem, including in Indonesia, due to the high mortality rate caused by this mosquito. The risk caused by the bite of the *Aedes sp* mosquito is quite dangerous, including acute fever, bleeding, shock, and even death, so that efforts are needed to control it. One of the natural ingredients that can use to eradicate *Aedes Aegypti* mosquitoes without causing negative impacts on the environment is the *Cananga* flower and *Zyzigium* Leaves. This study purposes of determining the optimum concentration of *Cananga* Flowers and *Zyzigium* Leaves Ethanol extract of which was having biolarvaside effect to *Aedes Aegypti* larvae. The method used a simple experiment with Posttest Only With Control Group Design. The data result of the research analyzed with Logit and Wilcoxon test. Achievement of those tests was $LC_{50}$ *Zyzigium* leaf extract on contact for 24 hours was 0.609-0.935, and $LC_{99}$ was between 1.064-1.973 while *Cananga* flower extract obtained $LC_{50}$ of 0.228-0.261 and the $LC_{99}$ was 0.446 to 0.558. Wilcoxon test for compared bioalarvaside effect results between *Cananga* Flower and *Zyzigium* Leaf received a significance value less than α (0.001<0.05), so it can ascertain that the *Cananga* Flower extract was stronger biolarvaside than *Zyzigium* Leaf extract.
**Keywords:** biolarvaside; ethanol extract; larvae

## INTRODUCTION

Aedes aegypti mosquitoes cause dengue Hemorrhagic Fever (DHF). Until now it is still a health problem for the Indonesia community, besides infection problems such as tuberculosis (Rifa'i, A. et al., 2019), or environmental pollutants such as fungi (Susanti, Y. et al., 2019) or bacteria such as *Staphylococcus aureus* (Mustika Sari, P. et al., 2019) and *Bacillus* (Fahani, A. et al., 2019). Indonesia has a high DHF mortality rate. Based on the report of the Indonesian Ministry of Health, it found that the period of dengue fever from January to February 2016 was 8487 people with 108 deaths. Dengue fever group of 5-14 years reached 43.44% and aged 15-44 years reached 33.25% (Indonesian Republic Ministry of Health, 2016).

The risk caused by the bite of the *Aedes sp* mosquito is quite dangerous, such as acute fever, bleeding, shock, and even death, so it needs control efforts. Efforts to control *Aedes sp* mosquitoes use chemical insecticides such as temephos. However, the use of chemical pesticides can harm the environment. These negative impacts include the death of natural enemies of disturbing organisms, the end of other

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beneficial organisms, disturb the quality and environmental balance because of the residual emergence of resistance in target animals (Istiana, 2012).

The natural material is one alternative that can use to eradicate *Aedes sp* larvae without causing negative impacts on the environment. Indonesia is a country rich in natural ingredients such as *Cananga odorata* (Lamk) *Hook*, *Syzygium polyanthum*, *Cinnamomum burmannii* (Huda, N. et al., 2019), *Eleutherine palmifollia* (L) *Merr* (Mahmudah, S. et al., 2019), *Hibiscus sabdariffa* L. (Sutiany, A. et al., 2019) *Ocimum basilicum* Linn (Pratama, HN. et al., 2020), *Citrus hystrix* D.C. (Kusumawardhani, N. et al., 2020). At present, research on the use of natural ingredients for the control of the *Aedes sp* mosquito vector has carried out intensively to reveal active substances in plants that can kill mosquito larvae, including *Cananga* odorata and Zyzygium (Indarti, 2014; Waskito, 2018; and Budi, et al., 2018). These natural ingredients are known to contain bioactive compounds, including polyphenols, essential oils, flavonoids, saponins, alkaloids, and tannins (Hariana, 2007). These materials easily found around us, relatively low prices, and safe for the environment.

The solvents to extract bioactive substances in a plant also needs to care. Solutions used to extract dynamic contents from the plant also depend very much on the polarity or solubility of them. Ethanol is a polar solvent that automatically dissolves active substances in plants that are also polar. The excess of ethanol compared to water is more selective. Molds and yeast are challenging to grow, less toxic, and neutral.

The results of the Nurhidayah study in 2017, which used water as a solvent to extract compounds in *Cananga* flower, were only able to kill *Aedes Aegypti* at 60% or 600,000 ppm concentrations. While another research in Dwiyanti et al., study in 2017, it knows that *Zyzigium* leaf water extract is only able to kill *Aedes Aegypti* larvae at a concentration of 32% or 320,000 ppm. The levels of these two plants are still high enough to kill *Aedes sp* larvae, so they not considered useful in killing *Aedes Aegypti* larvae, so alternative solvents other than water needed as ethanol which expected to kill larvae with lower concentrations. The base on the research above, we want to determine *Cananga* Flower dan *Zyzigium* Leaf in ethanol extract as biolarvaside to *Aedes Aegypti* larvae.

## MATERIALS AND METHODS

This research is experimental research and posttest only with control group design using five different concentrations; each treatment performed four times replication and every procedure of replication using 20 larvae. Materials are Ethanol extract from *Zyzygium polyanthum* leaves and *Cananga odorata* flower as biolarvaside to *Aedes Aegypti* instar III larvae. This research conducted in March-October 2018 at the Chemical Laboratory of Health Analyst Polytechnic Health Banjarmasin Indonesia.

The research samples used in this study were *Cananga* Flower (*Cananga* odorata Lamk.) And Salam Leaves (*Zyzygium polyanthum* W.) taken in the Bincau Area, Martapura Indonesia, and has been determined in the Laboratory of the Faculty of Mathematics and Natural Sciences, Lambung Mangkurat University.

The cleaned leaves and flowers are sliced into small pieces and then put into a clean, dry container and extract with Ethanol in maceration ways. Weighed the leaves and flowers each of 500 grams enter into a 2 L glass jar and add 96% ethanol solvent till cover the simplicia surface. Keep at room temperature for 2 x 24 hours while occasionally stirring to help accelerate the process of extraction of the active

substance in the simplicia. Filter to take the filtrate. The residue did the same way maceration. Accumulated each filtrate and evaporated with a temperature of <600C. The evaporated extract concentrated using a water bath until the viscous extract obtained. Put 100 mg, 250 mg, 500 mg, 750 mg, 1000 mg, and 1500 mg in 100 ml aqua dest from each ethanol extracts of flowers and leaves to a container. Entered 25 larvae *Aedes Aegypti* instar III on each glass test and then covered with gauze. The glass is placed in a room that meets the air condition conditions ie, 25-30$^{o}$C with humidity ranges from 81.5 to 89.5%. Observed and recorded the number of larvae that died after exposure to ethanol extract of *Cananga* flowers and *Zyzigium* leaf.

The data obtained were analyzed using *the Analysis of Variance* test to determine the differences between treatments and the Probit test to find out $LC_{50}$ and $LC_{99}$ of *Cananga* Flower and Zyzygium leaves ethanol extract.

## RESULTS AND DISCUSSION

### Characteristics of Examination Materials

The examination material used in this study was *Aedes Aegypti* larvae obtained by soaking filter paper containing *Aedes Aegypti* eggs into several small basins until collected *Aedes Aegypti* III / IV larvae with 4-5 mm size (about 5-6 days) with microscopic features: chest thorns start to clear and blackish-brown siphon. *Zyzygium polyanthum* W. and *Cananga odorata* Lamk. every 6 hours for 24 hours of observation

Table 1. Average of *Aedes Aegypti* Larvae Death After Exposure to Ekstrax Etanol

| Extract | Time exposure (hours) | Mortality in variabel concentration | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | C (+) | C (-) | 0,05% | 0,1% | 0,2% | 0,3% | 0,4% | 0,5% |
| *Zyzigium* | 6 | 0 | 80 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 12 | 0 | 80 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 18 | 0 | 80 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 24 | 0 | 80 | 0 | 0 | 0,5 | 1,75 | 2,25 | 2,75 |
| *Cananga* | 6 | 0 | 80 | 0 | 0 | 0 | 0 | 0 | 9 |
| | 12 | 0 | 80 | 0 | 0 | 0 | 7 | 14 | 48 |
| | 18 | 0 | 80 | 0 | 0 | 2 | 19 | 43 | 77 |
| | 24 | 0 | 80 | 0 | 14 | 39 | 56 | 76 | 80 |

Based on a table, it can see that the concentration of *Cananga* flower extract 0.05% has no effect at all on the death of *Aedes Aegypti* larvae, and only shows the mortality of larvae 10% after concentration was increased to 0.1 until 100% at 0.5. But *Zyzygium* ethanol extract has not shown an excellent ability to kill *Aedes Aegypti* larvae when compared to the strength of *Cananga* flower extract at the same treatment concentration. This result can see the highest level of *Zyzygium* extract, which is 0.5%, which is only able to kill as much as 13.75% of the population.

Table 2 .Comparing Percentage of Larva Deaths with Different Extract Concentrations After 24 Hour Contact

| Extract Consentration (%) | Average Mortality (%) from Zyzygium Extract | Average Mortality(%) from *Cananga* Extract |
|---|---|---|
| 0,05 | 0 | 0 |
| 0,1 | 0 | 10 |
| 0,2 | 2,5 | 40 |
| 0,3 | 8,75 | 66,25 |
| 0,4 | 11,25 | 93,75 |
| 0,5 | 13,75 | 100 |
| C (-) | 0 | 0 |
| C (+) | 100 | 100 |

Note :
C (-) : without any Extract
C (+) : treat with Temephos 0,01%

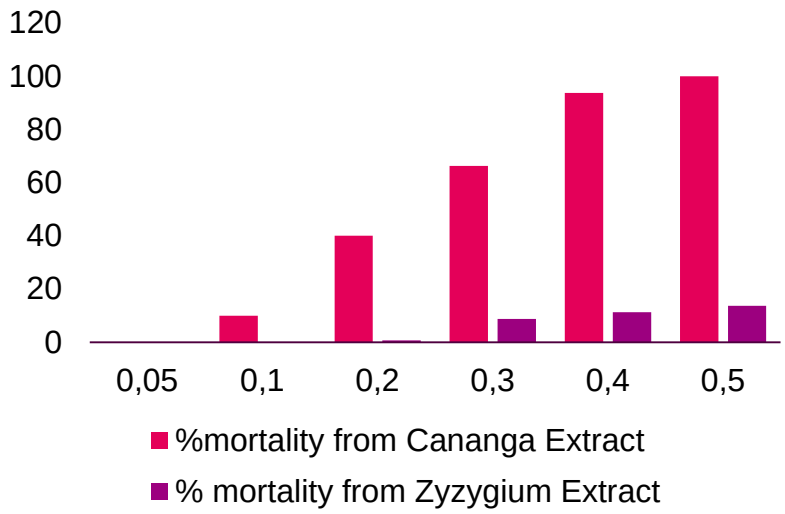


Figure 1. Graph Comparing The Percentage Mortality of Larvae with Different Extract Concentrations After 24 Hours Contact

**Data Analysis**

Data obtained is not standard, so the strength test of the extract against larvae mortality was tested using Wilcoxon. There was a significant effect between the biolarvaside of *Cananga* and *Zyzigium* Extract ( p = 0,001 <∝). To determine the extract strength against larvae mortality, it can state with a concentration that can kill larvae by 50% (= $LC_{50}$), and 99% ( = $LC_{99}$) uses probit test if data is standard and logit if not healthy. From the logit test results, the estimated LC50 for *Zyzigium* leaf extract on contact for 24 hours is 0.609 - 0.935, while LC99 is between 1.064 - 1.973 and for *Cananga* flower extract the $LC_{50}$ is 0.228 - 0.261 while the $LC_{99}$ is 0.446-0.558.

Table 3. Normality, Logit and Wilcoxon Statistical Results

| Uji | p | *Zyzygium* Extract | | *Cananga* Extract | |
|---|---|---|---|---|---|
| | | $LC_{50}$ | $LC_{99}$ | $LC_{50}$ | $LC_{99}$ |
| Normalitas | 0,000 - | 0,609 - | 1,064 - | 0,228- | 0,446 - |
| Logit | 0,011 | 0,935 | 1,973 | 0,261 | 0,558 |
| Wilcoxon | 0,001 | | | | |

In this study, two types of samples use, *Cananga* flowers, nd *Zyzygium* leaves using ethanol as a solvent. Choosing the use of ethanol as a solvent for active compounds in the sample with several considerations, among others, because it is more selective, and molds, yeast is more challenging to grow, less dangerous than other organic solvents (ether, chloroform, methanol), so that it expected could extract the active ingredients in the sample better than water. Previous research has been carried out on *Cananga* flowers by Nurhidayah (2017), and *Zyzigium* leaves by Dwiyanti, et al., (2017) using water as a solvent to extract its bioactive compounds but the results obtained have not shown the maximum effect in killing *Aedes Aegypti* larvae.

The ability of *Cananga* flower extract as biolarvaside compared to *Zyzigium* extract in this study was far better in all concentrations of treatments tested. The lowest concentration value of *Cananga* flower ethanol extract which has a biolarvaside effect is 0.1% with 10% larvae mortality, while in the ethanol extract of Zyzygium leaves, the lowest concentration that has a killing impact is a concentration of 0.5% with a mortality rate of 13, 75% which is that level has a 100% killing effect on *Cananga* flower ethanol extract.

Also, it can be seen in the negative control group (aqua dest) not showing the death of the test larvae, while in the positive control (Temefos 0.01%), 100% of the test larvae killed. This result indicates that the concentration of 0.5% *Zyzigium* leaf ethanol extract, which only killed 13.75% of the test population, was indeed ineffective when compared with positive control of 0.01% temefos.

Based on the probit test, it knows that $LC_{50}$ and $LC_{99}$ ethanol extract of *Cananga* flower is 0.244% and 0.506%, while *Zyzigium* leaves are 0.711% and 1.338%. Research on the effect of *Zyzigium* leaf ethanol extract granules on the ability to kill Anopheles aqonitus larvae has also been carried out by Heriansyah (2015) and obtained $LC_{50}$ of 2% and $LC_{99}$ of 7.7%. Research results in this study still show a high enough difference for bay leaf extract. This research is due to the use of different formulas, namely granules and extracts, so that the results obtained are also different. And also differences in the ability of water solubility of the two plant ethanol extracts. *Cananga* flowers are better as biolarvaside because the solubility of *Cananga* flower extracts in water is better than the solubility of ethanol extract of *Zyzigium* leaves. The ethanol extract of *Cananga* flowers after being suspended into water containing the larvae test was more soluble than the ethanol extract of *Zyzigium* leaves.

This theory is consistent with the hypothesis that the higher the solubility of a compound (solute) in a solvent, the easier the mixture or substance diffuses or touches with surrounding objects, including the test larvae contained in water as a medium or place where larvae located. While the ethanol extract of *Zyzigium* leaves after being suspended in water containing larvae test, does not show the ability to kill larvae optimally, this is due to the ethanol extract of *Zyzigium* leaves being slightly

soluble in water so that the number of active compounds (flavonoids, saponins, tannins, and essential oils) dissolved in water is also minimal, so it is not effective in killing larvae contained in water.

*Cananga* flower extract, which is more soluble in water, will also facilitate the process of diffusion of bioactive compounds contained in the extract to enter the body of larvae contained in water. Bioactive compounds in *Cananga* flowers such as flavonoids, saponins, and essential oils (Yahaya, 2019, and Phamkusolsil, 2012) also are polar so that they are soluble in ethanol (Suryani, 2015). According to the mechanism of flavonoid compounds in killing larvae is by coagulating proteins contained in the larva's body and as a respiratory poison that works by paralyzing the nerves in the respiratory system of insects. Saponin compounds work by inhibiting the absorption process and disrupt the digestive tract so that the larvae will starve and eventually die. Tannins work by influencing the failure of molting in larvae so that the larvae fail to become a pupa and eventually die (Hagerman, 2002). In this study, it has not explicitly indicated which active substances in *Cananga* flower ethanol extract have the most role in killing larvae in water. Hence, it still needs to be further studied by conducting tests identification of bioactive substances of *Cananga* flower from Banjarbaru ethanol extract and determine the concentration of each of these bioactive substances so that it can know with certainty which compounds play the most role in killing *Aedes Aegypti* larvae.

## CONCLUSION

The concentration of *Cananga odorata* flower ethanol extract was the most effective in killing *Aedes Aegypti* larvae at a level of 0.5% with a 100% mortality rate. In comparison, the concentration of *Zyzygium polyanthum* leaf ethanol extract at that concentration just had 10.75% mortality. The $LC_{50}$ and $LC_{99}$ ethanol extract of *Cananga odorata* flower are 0.228 - 0.261 and 0.446 to 0.558, while the ethanol extract of *Zyzygium polyanthum* leaf is 0.609 - 0.935 and 1.064 to 1.973.

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## CONFLICT OF INTEREST

There are no conflicts of interest in this study.

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