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Bintaro Leaves (*Cerbera manghas*): Toxicity to *Aedes aegypti* Instar III Larvas

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Abstract

DHF cases tend to increase from year to year in Indonesia. So the government makes efforts to control cases of Dengue Fever (DHF) which are usually done chemically and harm the environment and health. There is a need for safer, more effective, and environmentally friendly controls, such as using natural ingredients as natural larvicides. Among the natural ingredients having potential as a larvicide is Bintaro leaves (Cerbera manghas). The purpose of this study was to determine the toxicity of Bintaro leaf extract against third instar larvae of Aedes aegypti mosquitoes. This experimental study used 7 treatments of Bintaro leaf extract concentration (5 %, 15 %, 25 %, 35 %, 45 %, 65 %, 75 %), abate as a positive control, and distilled water as a negative control. Each treatment used ten instar III Aedes aegypti larvae with four repetitions. The data obtained were then analyzed using probit analysis to determine the toxicity of Bintaro leaf extract to Aedes aegypti larvae by calculating the LC50 and LC90 values. The results showed that the most effective concentration was 75 % because it could kill 100% of the test larvae. The LC50 value of 5,097 % and the LC90 value of 25,300 % indicate that the level of toxicity is very toxic. The probit regression analysis shows a linearity line equation y = 1.15 + 1.43x with a correlation (R2) of 0.512 which indicates that the correlation is strong enough. It is related to the content of flavonoids, tannins, saponins, triterpenoids which are toxic to the abdomen, nervous system, and respiratory system of larvae. From the research results, Bintaro extract with a concentration of 75 % can be used as a natural larvicide candidate. Furthermore, further research to see the toxicity to the environment can be done.are toxic to the abdomen, nervous system, and respiratory system of larvae. From the research results, Bintaro extract with a concentration of 75 % can be used as a natural larvicide candidate. Furthermore, further research to see the toxicity to the environment can be done.

Introduction

Dengue fever is often a concerning problem, especially in the health sector for the community. DHF is a disease caused by the dengue virus (Halstead, 2012). Dengue virus is a type of flavivirus virus consisting of 4 serotypes, namely DEN-1, DEN-2, DEN-3, and DEN-4 (Costa et al., 2012). There is still no vaccine for it (World Health Organisation, 2014). Patients with DHF are characterized by symptoms of fever for 2-7 days, accompanied by a decrease in platelets, headache and muscle aches (Itrat et al., 2011) as well as abdominal pain, vomiting, diarrhea, weakness, and joint pain (Halsey et al., 2012). In general, the spread of dengue disease is carried by mosquito vectors of the Aedes genus, namely Aedes aegypti and Aedes albopictus. The main vector of dengue fever is the Aedes aegypti mosquito (Hamid et al., 2017). It has a biting preference indoors, which is different from Aedes albopictus having a bitting preference outside the home

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or plantation (McBride et al., 2015). The more vectors or populations of the Aedes aegypti mosquito, the higher the number of dengue fever sufferers (Rahayu et al., 2019).

Dengue fever in Indonesia from year to year tends to be high, especially in the provinces of Java. They have high dengue cases, especially in East Java. East Java occupied the highest dengue cases in 2011-2014, with 2,657 cases compared to other Java provinces (Sholihah et al., 2020). The increase in the number of DHF sufferers in East Java in 2017 was 7,866 cases and increased again in 2018 there were 9,425 cases (BPS, 2018) in addition to the number of cases in East Java, the high mortality rate also occupied the highest number in 2017 as many as 105 patients (Kemenkes, 2018). Malang Regency is one of the regions in East Java that has high dengue cases compared to others. DHF cases in Malang Regency in 2017 were 451 cases, an increase of 682 cases in 2018 (BPS, 2018). Efforts made by the government through the health department are the fogging program (Yee et al., 2017), larvae eradication using abate (Suriami et al., 2020), cypermethrin (A. P. de Araujo et al., 2019) as a vector control program.

The use of larvicides and chemical insecticides in the long term will cause negative impacts on the environment. It can cause environmental pollution, residues in the environment (Gutierrez et al., 2014) and can cause resistance in mosquitoes (Senthil & Sengottayan, 2020) . Then it will eventually lead to failure in a control vector program. The use of chemical insecticides using pyrethroids (Demok et al., 2019), malathion (Morales et al., 2019), temephos (Grisales et al., 2013), deltamethrin (Dusfour et al., 2015) is no longer effective because it causes resistance. It is necessary to find other alternatives to prevent these negative impacts by making larvicides from natural materials safer, more effective, and environmentally friendly. Larvicides from natural ingredients or natural larvicides can be obtained from mahogany plants (Shaalan et al., 2010), Jatropha, brotowali, grapefruit (Gutierrez et al., 2014), Maja leaves, curry leaves, srigading leaves, belustru roots (Patil et al., 2010), basil (Maurya et al., 2012), and sweet orange (Warikoo et al., 2012) which has been done by previous researchers. However, the toxicity test of bintaro leaves (Cerbera manghas) has never been carried out on the instar III Aedes aegypti larvae, so this research is needed.

The use of plants as natural larvicides has encouraged research that has potential as sources of larvicides, one of which is the Bintaro plant (Cerbera mangos). The bintaro plant can be used for the seeds, skin, fruit, and leaves because these parts contain active compounds, but in this study, only the leaves from the bintaro plant were used because they have higher levels of active compounds (Susilo et al., 2020). The active compounds contained in bintaro leaves are steroids (triterpenoids), saponins, alkaloids, flavonoids, tannins (Kristiana et al., 2015). Some of these substances are toxic and function as a lethal effect on larvae (Susilo et al., 2020), inhibiting mosquito development and reducing appetite (Kinney et al., 2014). The presence of active compounds in the bintaro plants can encourage its use as a potential natural larvicide for research on the toxicity test of bintaro leaf extract against the instar III larvae of the Aedes aegypti mosquito. This research will be used as the basis for further research to explore the active ingredients in the Bintaro plant (Cerbera mangos) as a candidate for larvicides.

Method

This research is an Experimental Research Group study using Post Test Only Group Design. It was conducted at the Chemistry Laboratory of the University of Muhammadiyah Malang and the Laboratory of Materia Medica Batu. Aedes aegypti larvae were collected through landing collections from residents' homes in Malang Regency. This research took time from August to November 2020.

Samples were obtained through the larval landing collection stage. Landing collection of larvae is done by taking the larvae in the bathroom tub using a larval filter and placing them in a tray. The larvae obtained were then sorted into instar III Aedes aegypti larvae. Then the larvae are acclimatized for 24 hours to let them physiologically adapt to the environment.

The next stage is the identification of larvae. Identification of larvae is done easily by looking directly at the characteristics of Aedes aegypti larvae which at rest condition, the

larvae are perpendicular to the water surface. Next, the larvae were taken from the tray using a dropper, dripped with slight alcohol, placed on a glass slide, and given a clear polish. Furthermore, identify under a microscope by looking at the morphological characteristics of Aedes aegypti larvae based on the identification key. Namely, Aedes aegypti larvae consist of the head, thorax, abdomen, abdomen tip, ventral brush, tuft, and comb. Then particular characteristics distinguish it from other larvae, namely having a jagged comb.

Then, the stage of making extracts is by taking the leaves of the Bintaro plant and choosing the ones that are still good, then washing the leaves with running water. The washing process is then continued by chopping process so that the leaves dry quickly and weigh the leaves as much as 6 kg, then dry the leaves for ± one week at room temperature so that the compounds contained in the leaves are not damaged. After drying, then mashed with a blender until it becomes powder, put into a glass beaker, and added with 3 liters of 96% ethanol. Then it is stirred and closed tightly for 24 hours for maceration. After 24 hours, it will be filtered with filter paper to obtain the filtrate or juice, then vacuumed. The filtrate obtained will be evaporated using a rotary evaporator, and the extract is placed in a glass cup, then the finished product is inserted into the extract bottle. After that, a phytochemical test was carried out. The result is the bintaro leaf extract has active compounds of flavonoids, tannins, saponins, and triterpenoids.

The toxicity test stage involved the treatment group and the control group. The control group consisted of negative control, given distilled water, and a positive control given abate. The treatment group consisted of 7 levels of Bintaro leaf extract, namely 5%, 15%, 25%, 35%, 45%, 65%, 75%. The total number of larvae used was 360 larvae. In this study, the leaf extract of Bintaro (Cerbera mangos) was used to make stock solution diluted with concentrations of 5%, 15%, 25%, 35%, 45%, 65%, and 75%. The 5% concentration was obtained by diluting 2.5 ml of stock solution of Bintaro leaf extract with 47.5 ml of distilled water, and so on. Toxicity test of bintaro leaf extract was carried out with ten larvae for each treatment unit/each plastic cup, which was then transferred to a plastic cup containing the diluted extract. The treatment in the sample group duration was 24, 48, and 72 hours. Then make observations and count the number of dead larvae or larval mortality at that time.

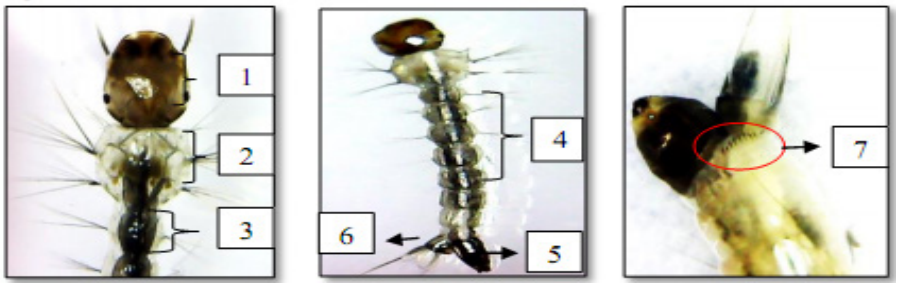
Data analysis in this study using the application program Ms. Excel and SPSS. The data obtained were entered in Ms. Excel to determine the percentage of larval mortality, then proceed with probit analysis used to determine toxicity. One of the toxicity test methods is LC50 and LC90. The values of LC50 and LC90 were obtained from the data on the mortality of the test animals. Test animal mortality data is a reference number for calculating the lethal concentration value. After knowing the lethal concentration value, it is adjusted to the level of toxicity of a substance.

Results and Discussions

The initial stage in this study was to identify Aedes aegypti larvae collected from larval collection activities.

Picture 1. Shows the results of the identification of Aedes aegypti larvae with a magnification of 600x.

Based on Figure 1, the identification of Aedes aegypti larvae using a Portable LCD



Description: 1. Caput, 2. Thorax, 3. Abdomen, 4. Ventral brush, 5. Tuft, 6. End of abdomen, 7. Serrated comb that shows the special characteristics of Aedes aegypti larvae.

Digital Microscope (G-600 China 3.6 MP) found that the Aedes aegypti larvae have several parts, including head, thorax, abdomen, ventral brush, tuft, comb. The particular feature that distinguishes Aedes aegypti larvae from other larvae is that they have a serrated comb.

The activity test stages of Bintaro leaf extract were collected from the results of the number of dead larvae from each concentration.

Table 1. The results of the percentage mortality of Aedes aegypti larvae at 72 hours.

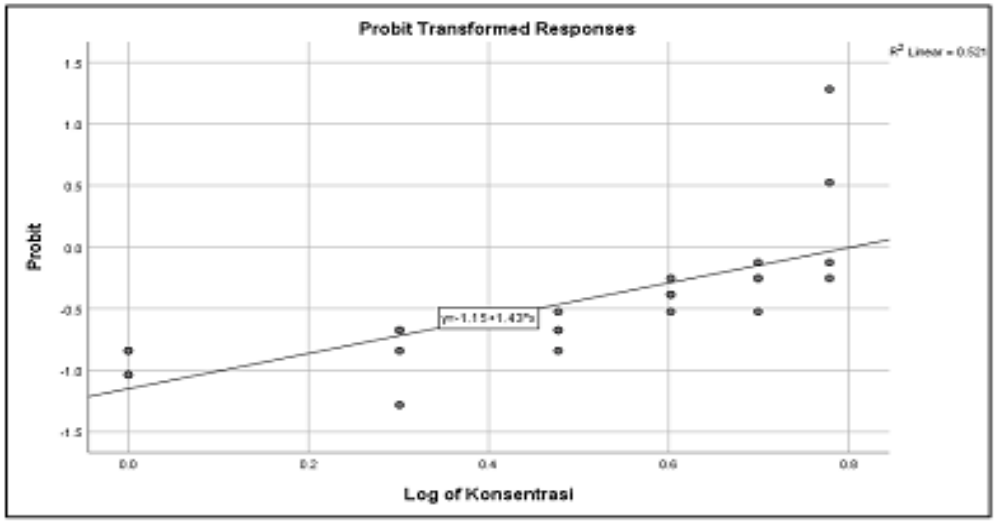
Concentration (%)	Average Mortality (%)
5 %	30 %
15 %	35 %
25 %	50 %
35 %	70 %
45 %	77,5 %
65 %	82,5 %
75 %	100 %

Source : Primary Data, 2020

Based on Table 1 shows that the percentage of mortality from treatment with Bintaro leaf extract concentration at 72 hours, it is known that the highest larval mortality is at a concentration of 75% with a percentage (100%), while the lowest larval mortality occurs at a concentration of 5% percentage (30%). Furthermore, The LC50 value at 72 hours has a value of 5.097% and the LC90 value 25.300%, which means that the administration of bintaro leaf extract on larval mortality can cause 50% and 90% of test animal deaths. It shows that bintaro leaf extract has been applied to tested animals can kill Aedes aegypti larvae.

According to Ullah et al., (2016) the level of toxicity consists of several categories, including (1) extremely toxic having a value <1, (2) very toxic having a value of 1-50, (3) moderately toxic having a value of 51-500, (4) slightly toxic has a value of 501-5000, (5) practically non-toxic has a value of 5000-15,000, and (6) less dangerous has a value of > 15,000. The results of probit analysis of LC50 and LC90 were 5.097 % and 25.300%, indicating that the level of toxicity is very toxic because the values of LC50 and LC90 are in the range of 1-50.

Image 1. Graph of Linear Probit Regression and Linearity Equation



Source : Primary Data, 2020

According to Banjarnahor et al., (2021), there is a correlation between probit concentration (x) and larval mortality (y) in probit regression analysis which will be interpreted based on the level of relationship, which has several categories, including (1) very strong having a value of 0 .80 – 1.00, (2) strong has a value of 0.60-0.79, (3) quite strong has a value of 0.40-0.59, (4) low has a value of 0.20-0.39, (5) very low has a value of 0.00-0.19. Based on Figure 1 shows that the graph of the probit regression analysis obtained a linearity equation, namely y = 1.15 + 1.43x with correlation (R2) has a value of 0.521, which indicates that the correlation between concentration probit with larval mortality is

quite strong because it has a value between 0.40-0.59 (Banjarnahor et al., 2021).

A phytochemical test on bintaro leaf extract was applied to find out the active compounds contained in bintaro leaves. Table 3 shows the phytochemical test result on Bintaro leaf extract. It contained flavonoids, tannins, saponins, and triterpenoids which were positive.

Table 2. Phytochemical Test Results

Active Ingredients	Parameters	Result
Flavonoids	Orange, brick red, pink, dark red	+
Tannins	Dark brown, dark blue	+
Saponins	Permanent foam	+
Triterpenoids	Orange, brownish orange	+

Source : Primary Data, 2020

The study results for the effect of bintaro leaf extract (Cerbera manghas) obtained from the activity test of bintaro leaf extract was an increase in the mortality of Aedes aegypti larvae instar III along with the increase in the concentration of bintaro leaf extract. It is proven that the larval mortality percentages at concentrations of 5%, 15%, 25%, 35%, 45%, 65%, 75% respectively were 30%, 35%, 50%, 70%, 77.5%, 82.5%, 100% (table 1) so that bintaro leaf extract is proven to have a larvicidal effect on Aedes aegypti larvae instar III refers to World Health Organization, (2005) which states that the concentration is considered to affect if it causes death 10-95%.

The toxicity test of Bintaro leaf extract on larvae was based on the LC50 and LC90 values. Based on the results of probit analysis, the LC50 value has a value of 5.097 % and the LC90 25.300 %, which means that the administration of bintaro leaf extract on larval mortality can cause 50% and 90% of test animal deaths. The smaller the LC50 and LC90 values, the higher the level of toxicity, the higher the number of larvae death. Based on the analysis results, natural larvicides based on bintaro leaves (Cerbera mangos) have a very toxic level of toxicity. According to Ullah et al., (2016), the level of toxicity shows it is a very toxic category because the LC50 and LC90 values have values between 1-50 mg/kg (%). In addition, the smaller the value of LC50 and LC90, the higher the level of toxicity of a compound. Conversely, the greater the value of LC50 and LC90, the lower the level of toxicity of a compound.

The phytochemical test showed bintaro leaf extract contains flavonoid compounds, tannins, saponins, and triterpenoids. It may be related to larval death. A concentration of 75% can kill 100% of larvae. Because the higher the concentration, the higher the content of active compounds that are toxic in Bintaro leaves. It is marked by the increasing number of dead larvae (Wahyuni & Yulianto, 2018). The larvae getting high concentrations, the content of these compounds will work quickly in suppressing the activity of the respiratory and nervous systems and break down cells quicker in the walls of the digestive tract of larvae so that the larvae will decrease their appetite (Steinwascher, 2018). The larvae's growth will be hampered even larvae will also die more quickly. In contrast to the larvae that received a low concentration, the workings of the active compound content were also slower. Even did not experience poisoning if it was low, or it could be said that it had no significant effect on larval mortality (I. . Araujo et al., 2018).

Bintaro leaf extract can be used as a bio larvicide because it has a toxic effect on Aedes aegypti larvae. As Kristiana et al., (2015) mentioned, the contents in bintaro leaves include flavonoids, tannins, saponins, steroids, and triterpenoids. Some of these compounds have toxic properties that can kill larvae (Susilo et al., 2019). It is per that flavonoid compounds work as inhibitors or inhibit the respiratory system or as respiratory toxins. Flavonoids have a way of working. Namely by entering the larva's body through the respiratory system that will cause withering of the nerves and damage to the respiratory system then cause the larvae to be unable to breathe and die (Kristiana et al., 2015), so the eggs do not hatch into larvae (Cahyati et al., 2019). In addition, flavonoids cause damage when

these compounds enter through the siphon, causing the larvae to change their position so that they are parallel to the water surface to get more oxygen intake (Subagiyo et al., 2017). According to Sutiningsih et al., (2017) saponins are bioactive compounds as toxic substances/ stomach poisons that enter through the mouth because the larvae usually take food from their place of life. In addition, saponins can damage cell membranes and disrupt the endocuticular protein layer causing toxic compounds to enter the larval body (Steinwascher, 2018), inhibiting metamorphosis or inhibiting the development of eggs to larvae (Cahyati et al., 2017). According to Kinney et al., (2014), tannin can reduce appetite which results in disruption of growth in larvae. Meanwhile, triterpenoids have anti feedant properties, which can cause the larvae to die (Carlos et al., 2015).

Based on the research conducted, it shows that the greater the concentration, the more effective it is to kill larvae so that bintaro leaf extract has the potential as a natural larvicide to kill Aedes aegypti larvae.

Conclusions

The research shows that bintaro leaf extract can be used as a larvicidal candidate for Aedes aegypti. The most effective concentration is 75% because it kills 100% of larvae and has an LC50 value of 5.097% and an LC90 25.300%. It is classified as very toxic. Further research to see the toxicity to the environment can be carried out.

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