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Utilization of Bintaro (*Cerbera manghas* L.) leaves extract as an *Aedes aegypti* L. mosquito killer: How is its effectiveness?

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Abstract. *Aedes aegypti* L. Mosquito is the only vector of Dengue Hemorrhagic Fever (DHF). The chemical-based control agents have a negative effect if used for a long time. Therefore, this research focused on finding the natural agent that may act as an adulticide, like Bintaro (*Cerbera manghas* L.). Bintaro leaves contain active compounds such as cerberin (one of the alkaloids), flavonoids, tannins, steroids, and saponins that have a mortality effect on mosquitoes. The study was to determine the effectiveness of Bintaro leaves extract as an adulticide against *Aedes aegypti* mosquitoes. This experimental research uses the posttest-only control group design and uses *Ae. Aegypti* mosquito aged 3-5 days as the sample. There were seven different concentrations of Bintaro leaves extract as a treatment, namely 300 ppm, 350 ppm, 400 ppm, 450 ppm, 500 ppm, 550 ppm, and 600 ppm. The method used in this research is using a 250 ml Duran bottle. The results of the study showed that the Bintaro leaves extract at a concentration of 600 ppm was more effective against the mortality of Ae. Aegypti, but not significantly different from the 400 ppm, 450 ppm, 500 ppm, and 550 ppm treatments. And the LC50 probit regression analysis was 455.602 ppm, and the LC90 value was 1735.956 ppm.

1 Introduction

Dengue fever (DHF) is one of the infectious diseases in humans caused by the dengue virus [1]. The disease is transmitted from one person to another through the injection of *Aedes aegypti* mosquitoes. The *Aedes aegypti* mosquito is the only major vector in the spread of dengue fever [2]. A special characteristic marking the *Aedes aegypti* mosquito is the presence of a band mark or silvery white stripes on the abdomen of the mosquito [3]. The distribution of *Aedes aegypti* mosquitoes in Indonesia is quite massive, directly proportional to the data on dengue fever cases that occur [4]. The high number of dengue fever cases in Indonesia has led to various ways of handling the vectors of this disease. Generally, dengue vector management is not environmentally friendly, resulting in long-term resistance effects, such as the use of chemical insecticides [5]. Adulticide has almost the same meaning as an

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insecticide, but the limitation of this adulticide is that the object used as a target must be an adult or 2-5 days old [6]. This poses a challenge for researchers to empower bioactive compounds in plants as an alternative effort to eradicate dengue fever vectors [7].

Plants are one of the natural materials that can be used to develop alternative adulticides because they store many bioactive compounds such as saponins, flavonoids, alkaloids, and tannins [8]. Bintaro plants are one of the potential plants that contain these bioactive compounds. Bintaro (*Cerbera manghas* L.) Is one type of plant of the Apocynaceae family. Bintaro plants are often found in yards, parks, gardens, and roadsides [9]. Bintaro has a positive response to alkaloids, flavonoids, steroids, saponins, and tannins [10–13]. Each of these bioactive compounds has a different role and has been tested to be used as a bio-adulticide. Leaves have greater varieties and amounts of phytochemicals compared to fruit, seeds, stems and roots [14]. In addition, the ethanolic extract of *Cerbera manghas* leaves contains carbohydrates (reducing sugars), alkaloids, tannins, steroids, flavonoids and glycosides [15–17].

The indicator used in measuring how toxic the compound or extract used in the adulticide process is by measuring its Lethal Concentration [18]. Lethal concentration is the concentration or dose value that gives the effect of death according to the predetermined LC value [19]. Therefore, this research aims to analyze the effectiveness of Bintaro leaves extract (*Cerbera manghas* L.) On the mortality of *Aedes aegypti* mosquitoes as a natural adulticide. In addition, this study also aims to analyze the LC50 and LC90 values of Bintaro leaves extract used as a natural adulticide.

2 Method

2.1 Study area

The research used a quantitative approach with experimental research. The study uses a post-test-only control group design that involves an experimental group and a control group. The object of research is adult-stage *Aedes aegypti* mosquitoes that are bred independently. This study uses independent variables in the form of Bintaro (*Cerbera manghas* L.) Leaves extract concentrations which are divided into seven concentrations, namely 300 ppm, 350 ppm, 400 ppm, 450 ppm, 500 ppm, 550 ppm, and 600 ppm with a total of three replicates. The control group used is 96% ethanol. The dependent variable is the number of mortalities of *Aedes aegypti* mosquitoes resulting from each concentration used as test material.

2.2 Preparation of *Aedes aegypti* mosquitoes

Aedes aegypti mosquito eggs using a homemade ovitrap made from an old bottle with filter paper on the inside, placed in a place with abundant mosquito intensity and indications of dengue fever cases. If there are *Aedes aegypti* mosquito eggs attached to the filter paper, the water in the bottle can be emptied, and the eggs are allowed to remain attached to the filter paper until later breeding. *Aedes aegypti* mosquito eggs that have been obtained can be put into a basin that contains water for 2-3 days until they become larvae. *Aedes aegypti* larvae can be characterized by their resting position which forms a 45-degree angle, as well as the presence of large enough siphons [20].

The collected *Aedes aegypti* larvae were placed in a rearing box that was lined with a thin net. The larvae are waited and checked periodically until they reach the pupal phase. When they become mosquitoes, put sugar water in a 100 ml Erlenmeyer and put a cotton swab on the mouth of the Erlenmeyer. This is done to feed the mosquitoes in the rearing box. Mosquitoes in the rearing box must be identified before being used as test material. The species of mosquito used as test material must be confirmed to be *Aedes aegypti*. Identification is carried out using an insect microscope so that the mosquito morphology can be seen clearly.

2.3 Adulticide test

The adulticide test was conducted to determine whether natural bioactive compounds of Bintaro leaves can cause mortality to *Aedes aegypti* mosquitoes. A total of 15-20 mosquitoes were put into a 250 ml duran bottle that had been cleaned. Bintaro leaves extract was dripped and leveled into the bottle according to the determination of concentration and the number of replicates. Observation of mosquito activity was carried out every 6 hours for 24 hours. This extract test indicates good results if the number of *Aedes aegypti* mosquitoes that experience mortality is large. Adulticide data were taken by direct observation technique, each mortality obtained in each concentration and replication. After the data was collected, it was analyzed statistically by following a series of One-way ANOVA test steps.

2.4 Probit regression test LC_{50} and LC_{90}

The LC values used as a measure of mortality in this study are LC_{50} and LC_{90}. The LC analysis method used is probit analysis which provides observations on the effects of which extract concentrations can cause 50% and 90% mortality of species. The data used for the probit regression test are those that have concentration categories, total samples, and the number of mortalities to perform the probit regression test.

3 Results and Discussion

The percentage of mortality of *Aedes aegypti* mosquitoes tested using Bintaro leaves extract (*Cerbera manghas* L.), obtained mortality mortality data, and the of mosquitoes at each concentration with a predetermined time indicator can be seen at Table 1.

Table 1. Mortality data of *Aedes aegypti* mosquitoes after being treated with Bintaro leaves extract as a reference for calculating LC_{50} and LC_{90}.

Treatment (ppm)	Repetition	Percentage of dead mosquitoes			
		6 hours	**12 hours**	**18 hours**	**24 hours**
300	1	15%	20%	25%	35%
	2	15%	25%	35%	45%
	3	10%	15%	30%	40%
Average		**13%**	**20%**	**30%**	**40%**
350	1	10%	15%	25%	35%
	2	10%	15%	35%	40%
	3	10%	25%	35%	45%
Average		**10%**	**18%**	**32%**	**40%**
400	1	10%	20%	35%	45%
	2	15%	25%	30%	40%
	3	5%	15%	35%	50%
Average		**10%**	**20%**	**33%**	**45%**
450	1	10%	20%	30%	40%
	2	5%	20%	30%	55%
	3	10%	30%	45%	50%
Average		**8%**	**23%**	**35%**	**48%**

Treatment (ppm)	Repetition	Percentage of dead mosquitoes			
		6 hours	**12 hours**	**18 hours**	**24 hours**
500	1	15%	20%	45%	65%
	2	10%	30%	55%	60%
	3	15%	25%	30%	45%
Average		**13%**	**25%**	**43%**	**57%**
550	1	5%	25%	40%	50%
	2	10%	25%	45%	65%
	3	15%	35%	50%	55%
Average		**10%**	**28%**	**45%**	**57%**
600	1	15%	25%	45%	60%
	2	5%	25%	40%	55%
	3	15%	35%	45%	65%
Average		**12%**	**28%**	**43%**	**60%**
Control	1	10%	10%	10%	20%
	2	0%	0%	0%	5%
	3	0%	10%	15%	15%
Average		**3%**	**7%**	**8%**	**13%**

The increasing of concentration used provides different mortality percentage data. As in Table 1, the results obtained tend to increase. This is evidenced by the percentage of mortality of *Aedes aegypti* mosquitoes at concentrations of 300 ppm, 350 ppm, 400 ppm, 450 ppm, 500 ppm, 550 ppm, and 600 ppm which are 40%, 40%, 45%, 48%, 57%, 57%, and 60% respectively, so it can be said that the Bintaro leaves extract used as a treatment group has an adulticide effect on *Aedes aegypti* mosquitoes. In line with Wulandari's research [21] which states that Bintaro leaves contain bioactive compounds that can provide mortality effects on insects.

Table 2. One-Way ANOVA test data of research results for mosquito mortality.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	4683.333	7	669.048	13.963	.000
Within Groups	766.667	16	47.917		
Total	5450.000	23			

The difference in mortality effects obtained due to the provision of different concentrations of Bintaro leaves extract is also reinforced by the results of the One-Way ANOVA test in Table 2. The One-Way ANOVA test results show that there are significant differences in the mortality of *Aedes aegypti* mosquitoes based on the different concentrations of extracts given. The toxic effect of bioactive compounds contained in Bintaro leaves (*Cerbera manghas* L.) is also influenced by the level of concentration given. The higher the concentration, the stronger the toxic effect.

Table 3. Post-Hoc test result.

Treatment (ppm)	Average	LSD Notation		
Control	13.33	a		
300	40.00		b	
350	40.00		b	
400	45.00		b	c
450	48.33		b	c
500	56.67			c
550	56.67			c
600	60.00			c

A post-hoc test was conducted to find which data provided significant differences in mortality data from the effect of giving different concentrations of extracts. Further information on the results of the extract test can also be seen in Table 3 in the form of results from the post-hoc test. The results obtained explain if the most effective concentration to provide mortality in *Aedes aegypti* mosquitoes is at a concentration of 600 ppm, but not significantly different from the concentrations of 550 ppm, 500 ppm, 450 ppm, and 400 ppm. Different results were obtained in mosquitoes given the 96% ethanol control treatment. without the provision of Bintaro leaves extract (*Cerbera manghas* L.), the mortality value of this control group was only 13%. Bintaro leaves extract has a significant effect on mortality in *Aedes aegypti* mosquitoes.

Table 4. Analysis Result of Regretion Probit LC_{50}

Probit Regretion Analyisis	**Estimate (%)**
LC_{50}	455,602

Table 5. Analysis Result of Regretion Probit LC_{90}

Probit Regretion Analyisis	**Estimate (%)**
LC_{90}	1735,956

Based on the results of the LC50 and LC90 probit regression tests in Table 4 and Table 5, the LC50 value is 455.602 ppm, and the LC90 value is 1735.956 ppm. Explaining if Bintaro leaves extract can cause 50% mortality of the total mosquito object at a concentration of 455.602 ppm, and Bintaro leaves extract can cause 90% mortality of the total mosquito object at a concentration of 1735.956 ppm. This proves that the lower the LC50 and LC90 values, the higher the toxicity level. The results of the LC test provide information on the Bintaro leaves extract can be toxic and cause the mortality of *Aedes aegypti* mosquitoes.

The effectiveness of Bintaro leaves extract (*Cerbera manghas* L.) on the mortality of *Aedes aegypti* mosquitoes is based on bioactive compounds contained in Bintaro leaves. Bioactive compounds in Bintaro leaves that play a role in providing mortality of *Aedes aegypti* mosquitoes are alkaloids, flavonoids, saponins, tannins, and steroids [12–16–17–22]. Alkaloids have been found to work as mosquito adulticides by causing death and affecting the development of mosquito larvae. The alkaloids harmaline, harmine, harmalol, and harman extracted from *Peganum harmala* seeds show considerable toxicity to *Aedes albopictus* larvae [23]. DL-methionine, another alkaloid, shows larvicidal efficacy against *Aedes aegypti* larvae when applied to natural water sources [24]. Alkaloids extracted from Lupine seeds, such as lupanine and 13-OH-lupanine, are also effective against the larvae of *Culex pipiens*, *Musca domestica*, and *Xenopsylla cheopis* [25]. In addition, Amaryllidaceae alkaloids and their derivatives, such as cyclopaldic acid and 1,2-O, O'-diacetyllycorine, show adulticidal activity against *Aedes albopictus* [26]. Alkaloids in Bintaro leaves have the effect of inhibiting calcium ion channels in *Aedes aegypti* mosquitoes. The effect exerted on calcium ions is due to the cerberin compound contained in the alkaloid compound. In addition, alkaloid compounds can cause damage to the digestive tract in the stomach of the *Aedes aegypti* mosquito [27]. These alkaloids and their derivatives can cause mortality, reduce larval and pupal duration, and inhibit mosquito larval development, making them potential candidates for the development of bioinsecticides for mosquito control programs [28].

Flavonoids, such as daidzein, have been found to work as insecticides for adult mosquitoes by inhibiting the activity of specific enzymes involved in mosquito development and detoxification processes [29–30]. These enzymes include glutathione S-transferase Noppera-BO (Nobo) and cytochrome P450 monooxygenase, which are important for insect hormone biosynthesis and insecticide detoxification, respectively [31–32]. The inhibitory activity of flavonoids against these enzymes has been observed at the atomic and enzymatic levels, providing insight into how they work. Flavonoids have also been shown to exhibit larvicidal activity, suppressing mosquito larval development more efficiently than other flavonoid-type inhibitors. The use of flavonoid extracts from plants, such as *Ageratum conyzoides* and *Sphaeranthus indicus*, has been proposed as an environmentally friendly alternative to synthetic insecticides for mosquito control. These findings indicate that flavonoids in Bintaro also have the potential to be used as selective and safe reagents for mosquito control.

Additionally, saponins work as insecticides for adult mosquitoes by interacting with cholesterol, causing interference in the synthesis of ecdysteroids, which are important for insect growth and development [33]. They also have toxic effects on harmful insects, including anti-feeding properties, disruption of the molting process, growth regulation, and mortality [34]. This activity is also facilitated by steroid compounds found in Bintaro leaves. Steroid compounds can interfere with the molting process owned by *Aedes aegypti* [35–36]. Saponins also can act as protease inhibitors or cytotoxic agents against certain insects [37]. However, the use of saponins as insecticides has several limitations. They have strong toxicity to mammals due to their cytotoxic and hemolytic activities [38]. The hydrophilic nature of saponins limits their penetration through the cuticle of lipophilic insects [39]. Moreover, the structural complexity of saponins makes it difficult to identify and synthesize the active molecules. Next, tannin compounds has demonstrated antifeedant activity and inhibition of larval growth in insect pests, including cotton leafworm and rice weevil [40]. In addition, tannins extracted from *Magonia pubescens* have shown larvicidal activity against the *Aedes aegypti* mosquito [41]. The exact mechanism of action of tannins as insecticides is not clearly understood, but it is suggested that the alkaline midgut pH, surfactant, and peritrophic membranes in insects may help tolerate moderate tannin concentrations in the diet [40]. Further research is needed to fully understand the physiological and toxicological impacts of tannins on insect herbivory and their potential as natural alternatives to synthetic insecticides. However, taken together, the findings of this study suggest that Bintaro leaf extract holds promise as an adulticide for Aedes aegypti mosquitoes, attributed to the activity of secondary metabolite compounds.

4 Conclusion

The conclusion that can be drawn from the research on the effectiveness of natural adulticides from Bintaro leaves extract on the mortality of *Aedes aegypti* mosquitoes is that the effect of Bintaro leaves extract (*Cerbera manghas* L.) on the mortality of *Aedes aegypti* mosquitoes can be found. The best treatment that can cause the highest mortality is at a concentration of 600 ppm, but not significantly different from the results given by concentrations of 400 ppm, 450 ppm, 500 ppm, and 550 ppm. The LC50 value of Bintaro leaves extract against *Aedes aegypti* mosquito mortality is at a concentration of 455.602 ppm. The LC90 value of Bintaro leaves extract on the mortality of *Aedes aegypti* mosquitoes is at a concentration of 1735.956 ppm. Bintaro leaves extract can be declared toxic because it has an LC50 value < 1000 ppm.

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