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Mortality Effect of Bintaro (*Cerbera manghas* L.) Seed Extract Against *Aedes aegypti* (Diptera: Culicidae) Mosquitoes

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Abstract. Bintaro *(Cerbera manghas* L.*)* seeds contain phytochemical compounds like cerberin, flavonoid, steroid, tannin, and saponin. These compounds may act as a natural eradication of *Aedes aegypti* L. in the adult stage or are called natural adulticides. This study aimed to determine the effectiveness of Bintaro seed extract on the mortality of Ae. aegypti mosquitoes. This experimental research uses the post-test-only control group design. There were seven Bintaro seed extract concentrations as treatment, namely 300ppm, 350ppm, 400ppm, 450ppm, 500ppm, 550ppm, and 600ppm. The control treatment used ethanol (96%). The research sample was 480 mosquitoes aged 3-5 days (for three repetitions). Each treatment uses 20 mosquitoes in a Duran bottle 250 mL. The data were processed using SPSS type 22 with normality and homogeneity tests, then analyzed using the *Kruskal-Wallis* test to determine differences in effective concentrations on mosquito mortality. The results showed that Bintaro seed extract at a concentration of 600ppm was the most effective on the mortality of Ae. aegypti mosquitoes but not significantly different from the other concentrations. While the results of the LC50 probit regression analysis were 453.071 ppm, and the LC90 value was 1737.760 ppm.

1 Introduction

Dengue fever in Indonesia is still a problem in the community, as evidenced by the incidence of dengue fever which has increased every year [1]. Based on data from the Ministry of Health in 2019, the increase in dengue fever cases in the vulnerable years 2010-2019. Recorded that the highest incidence rate of dengue occurred in 2010, 2016, and 2019. The cause of high dengue cases is due to increased mobility and population density, community behavior, climate, and environmental conditions [2]. The condition of the home environment that is clean and there are many puddles of clean water can cause the spread of dengue, because the mosquitoes that cause dengue are more adaptable and prefer to lay their eggs in clean water compared to dirty water [3]. Other factors that cause high dengue cases include vector behavior, disease vector density, and disease vector control [4].

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The main vector of dengue fever is the *Aedes aegypti* mosquito, which has a preference to bite inside the house, while the second vector of dengue fever is the *Aedes albopictus* mosquito, which has a preference to bite outside the house or plantation [5]. *Aedes aegypti* mosquitoes prefer dark places, for example under tables, under chairs, behind long-hanging clothes, and in dark bathrooms [6]. The breeding ground for *Aedes aegypti* mosquitoes is in the form of water reservoirs, for example, water collected in containers [7]. The *Aedes aegypti* mosquito has a perfect metamorphosis, starting from the egg, larva, pupa, and adult mosquito. The *Aedes aegypti* mosquito has a very active time when sucking human blood, namely during the day [8], and at that time the *Aedes aegypti* mosquito also spreads the dengue virus.

Dengue virus spread by *Aedes aegypti* mosquitoes requires an incubation period of 4 - 7 days, this virus belongs to the group B Arthropod virus (Arbovirus) which is identified in four types of serotypes, namely: DEN-1, DEN-2, DEN-3, and DEN-4 [9]. The spread of the dengue virus begins after the mosquito sucks the viremic blood, the mosquito salivates from its proboscis, the virus enters the vector's stomach and multiplies, then the dengue virus is found in the salivary glands [10] and the vector is ready to continue the chain of transmission by being transferred from mosquitoes to humans [11]. The dengue virus can cause dengue fever. Dengue fever is commonly found in tropical and subtropical regions of the world, [12]. Dengue fever has characteristics, namely sudden high fever accompanied by bleeding manifestations, and tends to cause injury and death [13].

Many efforts have been made by the government through the Ministry of Health to break the chain of the spread of dengue fever caused by the dengue virus. One of them is the socialization of the mosquito nest eradication program with 3M (*menutup, menguras, mengubur* – closing, draining, and burying) in water reservoirs, either artificial or natural water reservoirs. This is intended for the community to independently control dengue vectors [14]. Other efforts made by the government in controlling dengue vectors include fogging using insecticides and eradicating mosquitoes using abate. The fact field that is done by the community in breaking the chain of the spread of dengue is the use of chemical insecticides such as mosquito sprays, electricity, ointment, and burns. One of the contents contained in these insecticides is malathion and themephos which are insecticides from the organophosphate class [15], and cypermethrin, palmetrin, transflutrin and D-alletrin which are insecticides from the pyrethroid group [16].

The continuous use of chemicals to control adult-stage *Aedes aegypti* mosquitoes has various negative impacts. Among them, it can cause resistance in mosquitoes, endanger environmental health because it can be a pollutant, and spread free radicals [17]. According to [18], the use of chemical insecticides such as organochlorines, carbamates, pyrethroids, and organophosphates such as malathion, if used for a long period will cause resistance, so other alternative solutions are needed. More effective alternative solutions are needed to break the chain of the spread of *Aedes aegypti* mosquitoes, such as the utilization of natural extracts for adulticides. Adulticide from natural extracts can be obtained from *Piper betle* [19], *Ayzygium aromaticum* [20], *Spondias mombin* [21], *Zanthoxylum limonella* [22], *Melaleuca cajuputi* [23] which have been done by previous researchers. However, in this study employs Bintaro (*Cerbera manghas* L.) seeds as a natural extract for adulticidal purposes due to the presence of various chemical compounds within the seeds that exhibit potential adulticidal activity against Aedes aegypti mosquitoes. Furthermore, investigations focusing on the adulticidal properties of natural extracts derived from Bintaro seeds remain limited in the existing scientific literature.

Bintaro seeds contain active compounds that are toxic such as flavonoids, steroids, saponins, alkaloids, and tannins [24]. Flavonoid compounds act as antimicrobials and attack the nerves of vital insect organs [25]. Steroid compounds act as a protective agent by inhibiting the molting process in insects [26]. Tannin compounds act as antifeedants [27], which damage protein absorption and block the digestive process, causing a decrease in the work system of the digestive organs. Saponin compounds act as stomach poisons, contact poisons, and respiratory poisons in insects that cause cell damage, and disrupt the respiration process so that it can cause death to *Aedes aegypti* mosquitoes [28]. The alkaloid compound contained in Bintaro seeds is cerberin, which is a bitter-tasting and toxic substance that can inhibit calcium ion channels in the heart muscle of mosquitoes which causes death [29]. Flavonoid compounds act as antimicrobials and attack the nerves of vital insect organs [30]. So, the aim of this research is to investigate the influence of Bintaro (Cerbera manghas L.) seed extract on the mortality of Aedes aegypti mosquitoes, with the objective of determining the potential of the extract as an effective adulticidal agent in controlling the population of Aedes aegypti mosquitoes.

2 Method

The approach used in this study was quantitative. The data then generated in the form of the percentage of mortality of adult-stage *Aedes aegypti* mosquitoes were analyzed statistically. The type of research used is experiment research to find the effectiveness of Bintaro fruit seed extract (*Cerbera manghas* L.) on the mortality of adult-stage *Aedes aegypti* mosquitoes. The posttest-only control group design was used in the study. The treated group is referred to as the experimental group, while the untreated group is the control group. The adulticide test method is using with a 250 mL Duran bottle with seven treatments namely Bintaro seed extract concentrations of 300ppm, 350ppm, 400ppm, 450ppm, 500ppm, 550ppm, 600ppm, and one control treatment (96% ethanol). The research sample was *Aedes aegypti* mosquitoes aged 3-5 days with as many as 480 which were used for three replicates. The research stages consist of collecting Bintaro seeds and making extracts, looking for *Aedes aegypti* mosquito larvae, identifying larvae including *Aedes aegypti*, treating larvae until they become mosquitoes by feeding poultry pellets and sugar water, after mosquitoes aged 3-5 days, adulticide tests were carried out using Duran bottles and Bintaro seed extracts with seven concentration treatments and one control treatment. Each treatment was filled with 20 mosquitoes in each bottle. Data from the study were processed using SPSS type 22 with normality and homogeneity tests, then analyzed using the Kruskal-Wallis test to determine differences in effective concentrations on mosquito mortality.

The adulticide test procedure using a Duran bottle is described as follows. (1) The Duran bottle used for the adulticide experiment was washed using warm water and soap once, and then the bottle was washed using running water three times. (2) the Duran bottle is put into the oven at 50 degrees Celsius for ± 20 minutes until the bottle is dry, then the bottle is smeared with extract according to the concentration of 0.1 ml. (3) the Duran bottle that has been smeared with the extract is leveled by turning the bottle so that the extract is perfectly flat. (4) After that, the Duran bottle as a whole is covered with aluminum foil, and a small gap is left as a place to see during observation. (5) The top side of the Duran bottle is closed using aluminum foil and given a small hole for breathing for mosquitoes. (6) The Duran bottle that has been given the extract and closed using aluminum foil is placed in a container that does not have light for 24 hours to ensure that the extract in the bottle is dry. (7) After 24 hours the bottle can be used for an adulticide test by inserting 20 mosquitoes in each bottle. (8) Observations were made every six hours in 24 hours to note the mortality of mosquitoes.

3 Results and Discussion

The outcomes of the investigation regarding the efficacy of Bintaro seed extract on Aedes aegypti mosquito mortality are presented in Table 1. The results indicate that the highest mortality rate among Aedes aegypti mosquitoes occurred in the groups treated with Bintaro seed extract concentrations of 450ppm (U3), 550ppm (U3), and 600ppm (U3), where a total of 14 mosquitoes exhibited mortality, corresponding to a percentage value of 70%. The lowest mortality rate among the treated mosquitoes occurred at a concentration of 300ppm (U1), where a total of 4 mosquitoes experienced mortality, corresponding to a percentage value of 20%. The control group (U1) exhibited the lowest mortality rate among all tests conducted, with 0 mosquitoes showing mortality, representing a percentage value of 0%.

Table 1. The mortality data of *Aedes aegypti* mosquitoes.

Treatment (ppm)	Repetition	Number of dead mosquitoes				Total	Percentage (%)	Mean
		6 hours	12 hours	18 hours	24 hours			
300	1	2	0	2	0	4	20	28.33
	2	0	2	1	2	5	25	
	3	1	2	1	4	8	40	
350	1	2	3	1	3	9	45	31.67
	2	3	1	1	0	5	25	
	3	1	3	0	1	5	25	
400	1	2	2	1	2	7	35	35
	2	1	1	1	5	8	40	
	3	2	1	1	2	6	30	
450	1	3	1	1	3	8	40	53.33
	2	2	2	2	4	10	50	
	3	2	4	0	8	14	70	
500	1	4	1	4	2	11	55	55
	2	0	0	4	5	9	45	
	3	4	3	4	2	13	65	
550	1	3	4	0	6	13	65	55
	2	0	2	2	2	6	30	
	3	3	7	0	4	14	70	
600	1	4	4	0	4	12	60	63.33
	2	4	5	0	3	12	60	
	3	5	3	4	2	14	70	
Control	1	0	0	0	0	0	0	3.33
	2	0	1	0	0	1	5	
	3	0	0	0	1	1	5	

The presence of various active compounds contained in Bintaro seeds can cause death in *Aedes aegypti* mosquitoes. This is following Table 1 which states that mosquito mortality at concentrations of 450ppm, 550ppm and 600ppm can kill mosquitoes by 70%. This is because the higher the concentration given, the higher the toxic concentration of Bintaro seed extract (*Cerbera manghas* L.) which can cause a large number of mosquitoes to die [31]. In contrast to the control concentration which can only kill mosquitoes as much as 0%, this is because mosquitoes that get low concentrations, the workings of the active compounds are slow so it can be said that low concentrations do not have an even effect on mosquito death [32].

Each increment in concentration utilized yields varying mortality percentage data, resulting in a tendency towards increased outcomes. This trend is evidenced by the mortality percentages of Aedes aegypti mosquitoes at concentrations of 300ppm, 350ppm, 400ppm, 450ppm, 500ppm, 550ppm, and 600ppm, which are recorded as 28.33%, 31.67%, 35%, 53.33%, 55%, 55%, and 63.33%, respectively. Hence, it can be inferred that the Bintaro seed extract employed as a treatment group exhibits an adulticidal effect on the *Aedes aegypti* mosquito. This finding aligns with the research conducted by [31], which posits that Bintaro contain bioactive compounds capable of inducing mortality in insects. *Cerbera manghas* contains various bioactive compounds including saponins, terpenoids, alkaloids, phenolic acids, flavonoids, cardiac glycosides, steroids, iridoids, lignans, and other compounds [31–33]. The main phytochemical compounds in *Cerbera manghas* are cardiac glycosides, followed by terpenoids and phenolic acids [34]. This plant also exhibits antioxidant, anticancer, anti-inflammatory, DNA damage protection, and antimicrobial activities [35]. In addition, a derivative of *Cerbera manghas* aldehyde called cyclopentenopyridine has been found to have activity against plant viruses, insects, and plant pathogenic bacteria [36]. *Cerbera manghas* leaf extract also shows antibacterial activity against plant and animal pathogens, as well as antioxidant activity [35–37–38]. *Cerbera manghas* seeds contain cerberin, a cardiac glycoside toxin that blocks calcium ion channels in heart muscle [37–39].

Furthermore, the normality test results (Shapiro-Wilk test) based on Table 2 informs that the mortality results of *Aedes aegypti* mosquitoes at a concentration of 300ppm [W(3) = 0.923, p = 0.463], a concentration of 350ppm [W(3) = 0.750, p = 0.000], a concentration of 400ppm [W(3) = 1. 000, p = 1.000], 450ppm concentration [W(3) = 0.964, p = 0.637], 500ppm concentration [W(3) = 1.000, p = 1.000], 5500ppm concentration [W(3) = 0.842, p = 0.220], 600ppm concentration [W(3) = 0.750, p = 0.000], Control group [W(3) = 0.750, p = 0.000]. The data on normality is considered abnormal due to two concentrations having a Sig value of less than 0.05 (<0.05), namely the 350ppm concentration and the 600ppm concentration.

Table 2. Normality test result.

Treatment (ppm)	Shapiro-Wilk		
	Statistic	**df**	**Sig.**
300	.923	3	.463
350	.750	3	.000
400	1.000	3	1.000
450	.964	3	.637
500	1.000	3	1.000
550	.842	3	.220
600	.750	3	.000
Control	.750	3	.000

The results of the homogeneity test with the Levene test in Table 3 inform that the variance of the data on the mortality results of *Aedes aegypti* mosquitoes is not homogeneous because the Sig value is less than 0.05 [F (7, 16) = 2.854, p = 0.039]. Therefore, the data cannot be continued using the one-way ANOVA parametric test considering that 2 conditions must be met, namely; data must be normal and data must be homogeneous. Both conditions are not met, so the data can be continued with a non-parametric test to replace one-way ANOVA, namely the Kruskal-Wallis non-parametric test.

Table 3. Homogeneity test result.

Levene Statistic	df1	df2	Sig.
2.854	7	16	.039

The results of the Kruskal-Wallis test based on Table 4. inform that the Assymp Sig value is 0.018. This value is less than 0.05 (<0.05), indicating that there is a difference in the average mortality of *Aedes aegypti* mosquitoes in the effect of each extract concentration. Furthermore, there is a further test after the Kruskal-Wallis test which is useful for knowing the concentration that has a difference in the mortality of *Aedes aegypti* mosquitoes and can be seen in Table 5. Based on Table 5, it is indicated that the concentration of Bintaro seeds effective for killing Aedes aegypti mosquitoes is 600ppm. However, this concentration does not exhibit a significant difference from the concentrations of 500ppm, 550ppm, 450ppm, 400ppm, 350ppm, and 300ppm.

Table 4. Kruskal-Wallis test result.

Test Statistics[a,b]	
Chi-Square	16.868
Df	7
Asymp. Sig.	.018

Table 5. The notation of Kruskal-Wallis further test result.

Treatment (ppm)	Mean	Notation	
Control	3.33	a	
300ppm	28.33	a	b
350ppm	31.67	a	b
400ppm	35	a	b
450ppm	53.33	a	b
500ppm	55	a	b
550ppm	55	a	b
600ppm	63.33		b

Based on the results of the Kruskal-Wallis test used to determine the presence of differences in the concentration of Bintaro seed extract on mosquito mortality, can be seen in Table 4 and Table 5. The results stated that the concentration of 600ppm was effective in killing *Aedes aegypti* mosquitoes but was not significantly different from the concentrations of 550ppm, 500ppm, 450ppm, 400ppm, 350ppm, and 300ppm. This occurs because the determination of the concentration range used is not high enough, namely only a range of 50ppm at each concentration, and temperature conditions in the unfortunate area in February-March when mosquito rearing is less stable.

Then, Table 6 shows that the probit regression test obtained an estimate of the LC50 of Bintaro seed extract at a concentration of 453.071ppm. This means that giving Bintaro seed extract with a concentration of 453.071ppm will cause 50% mortality of *Aedes aegypti* mosquitoes. Furthermore, the results of the probit regression test obtained an estimate or estimate of the LC90 of Bintaro seed extract at a concentration of 1737.760ppm. The estimated LC90 has a value of 1737.760ppm which means that giving Bintaro seed extract with a concentration of 1737.760ppm can have an impact of 90% mortality of *Aedes aegypti* mosquitoes.

Table 6. The results of probit regression analysis.

Probit Regression Analysis	Estimate (ppm)
Probit LC50	453.071
Probit LC90	1737.760

Based on the results of the LC_{50} and LC_{90} probit regression tests in Table 6, the LC_{50} value obtained is 453.071ppm and the LC90 value obtained is 1737.760 ppm. So, it can be assumed that the Bintaro seed extract can cause the death of 50% of the total mosquitoes at a concentration of 453.071ppm and can cause the death of 90% of the total mosquitoes at a concentration of 453.071ppm. The lower the value of LC_{50} and LC_{90}, the higher the toxicity level of an insecticide. It can be concluded that an extract can be said to be toxic if the LC_{50} value is <1000ppm. As previously explained, Bintaro also contains saponins, terpenoids, alkaloids, phenolic acids, flavonoids, and cardiac glycosides [33–37–40]. Saponin works as an insecticide by providing a toxic effect on insect pests. They have been found to have repellent or deterrent activity, causing reduced food intake, digestive disorders, weight loss, developmental retardation, reduced reproductive rates, and death in insects [33–41–45] . Saponins are surface-active compounds with high molecular weight present in many plants. They have a non-polar aglycone moiety combined with a polar sugar molecule. This compound has been shown to be effective against a variety of insect pests, including diamond moth larvae, aphids [43], mosquito larvae [45], caterpillars [42], and Japanese beetles [44]. According to this secondary plant metabolites, have been found to have insecticidal activity by causing cell membrane permeation and damage to the insect midgut epithelium [46].

On the other hand, terpenoids act as insecticides through various mechanisms. They can inhibit detoxification enzymes in insects, making them effective in formulating botanical insecticides [47]. Additionally, terpenoids can synergize with other insecticides, increasing their effectiveness [48]. Furthermore, terpenoids can penetrate the integument of larvae, increasing their insecticidal activity [49]. Overall, terpenoids act as insecticides by directly influencing pest physiology or by increasing the effectiveness of other insecticides. While alkaloids have been investigated for their potential use as insecticides on mosquitoes. Natural alkaloids were evaluated for their inhibitory effects on AChE in *Aedes aegypti*, with alpha-solanine identified as a potential inhibitor [50]. Amaryllidaceae alkaloids from Nerine sarniensis tubers show adulticidal activity against *Aedes aegypti* [51]. These findings indicate that alkaloids in Bintaro also have the potential to be used as insecticides for mosquito control.

Moreover, flavonoids work as insecticides in mosquitoes by inhibiting the activity of certain enzymes and disrupting hormonal processes in insects. They have been shown to inhibit the activity of glutathione S-transferase Noppera-BO (Nobo), an enzyme involved in the biosynthesis of the insect steroid hormone ecdysone [52]. In addition, flavonoids can act as general toxins, growth inhibitors, reproductive inhibitors, repellents, and oviposition deterrents [53]. The larvicidal activity of flavonoids has been observed in various mosquito species, including *Aedes aegypti*, *Aedes albopictus*, and *Culex pipiens* pallens [54]. However, the exact way flavonoids work as mosquito adulticides is not fully understood

Cerbera manghas fruit extract, which contains cerberin [37–40], has been shown to have bio-larvicidal activity against *Sitophilus oryzae*, a type of beetle [55]. It is possible that cerberin, similar to other cardiac glycosides (CG), disrupts the normal function of the insect's nervous system or other vital processes, leading to paralysis or death [33–37–39–40–56]. CG act as insecticides by inhibiting the sodium-potassium pump (Na+/K+-ATPase), which disrupts ionic homeostasis in cells and causes increased calcium concentration and cell death. Insects that can absorb CG in their tissues are protected from predation due to the toxicity of this compound [57]. The mechanism of action of CG involves binding to specific receptor sites on the sodium pump [58]. Binding of these receptors leads to the activation of second messengers and can even function as a growth factor at physiological concentrations [59]. However, at higher concentrations reached after consuming exogenous cardenolides, CG inhibits the pump and produces toxic consequences [60]. Thus, the cerberin content in *Cerbera manghas* has great potential to be used as a natural insecticide against Aedes aegypti. Overall, the active compound content in Bintaro seed extract (saponins, terpenoids, alkaloids, flavonoids, and cerberin) has a potential mortality effect on the Aedes aegypti mosquito.

4 Conclusion

Based on the results of research and discussion about the effectiveness of Bintaro seed extract as adulticide against *Aedes aegypti* mosquitoes. There is an effect of Bintaro seed extract on the mortality of *Aedes aegypti* mosquitoes at a concentration of 600ppm, but each treatment concentration shows that it is not significantly different. This occurs because the determination of the concentration range used is not high enough, only 50ppm, and the temperature when treating *Aedes aegypti* mosquito larvae until they become adults is less stable. The LC_{50} value of Bintaro seed extract on *Aedes aegypti* mosquito mortality is 453.071ppm while the LC90 value of Bintaro seed extract on *Aedes aegypti* mosquito mortality is 1737.760ppm, Bintaro seed extract is said to be toxic because it has an LC_{50} value <1000ppm.

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