

RESEARCH ARTICLE

Effectiveness of the repellent of kawista leaf extract (*Limonia acidissima*) against *Aedes aegypti* mosquitoes

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Article history:

Received : 2023/05/17

Revised : 2023/06/09

Accepted : 2023/08/15

Available Online : 2023/08/31

DOI : [10.33086/etm.v3i2.6390](https://doi.org/10.33086/etm.v3i2.6390)

Abstract

Synthetic insecticides containing highly effective active substances such as DEET (N,N-diethyl-met-toluamide) to prevent and control Dengue Hemorrhagic Fever can have adverse effects on health and the environment. Kawista leaves (*Limonia acidissima*) have the potential as a natural insecticide against *Aedes aegypti* mosquitoes because they contain secondary metabolite compounds namely phenols, flavonoids, saponins, and terpenoids. This study aims to determine the effectiveness of the repellent power of kawista leaf extract (*Limonia acidissima*) against *Aedes aegypti* mosquitoes. This study used experimental method with post test only control group design. The sample used was 25 adult *Aedes aegypti* mosquitoes in each treatment. The ethanol extract of kawista leaves was made in the form of a spray with a concentration of 25%, 50% concentration, and 75% concentration. The 25% concentration, 50% concentration, and 75% concentration of kawista leaf extract have an effective repellent power against *Aedes aegypti* mosquitoes, namely 15.22%, 46.20%, and 70.08%, respectively. The results of the analysis of kawista leaf extract against *Aedes aegypti* mosquitoes obtained ED₅₀ (Effective Dose 50) at a concentration of 60.343% and ED₉₉ (Effective Dose 99) at a concentration of 124.452%. From the results of the study it can be concluded that kawista leaves have the ability to repel *Aedes aegypti* mosquitoes.

Keywords: Kawista Leaf Extract, *Aedes aegypti*, Repellency

INTRODUCTION

Dengue Hemorrhagic Fever (DHF) remains a significant health problem in tropical countries such as Indonesia. That factors contribute to the spread of this virus are rising weather temperatures and changes in the rainy and dry seasons which increase the risk of Dengue virus transmission (Hurint, 2021). Based on data from the Kemenkopmk, 2023 DHF cases in Indonesia in 2021 were 73,518 cases with a death rate of 705 people. In 2022 there were 143,266 cases with a death rate of 1,237 people. In 2023, the period January–August reached 57,884 cases (IR 21.06/100,000 population) with 422 deaths (CFR 0.73%). The use of synthetic insecticides such as mosquito repellents such as fuel, electric, and spray is commonly used by the public at this time as an effort to prevent and

control Dengue Hemorrhagic Fever (DHF). These insecticides contain active substances such as DEET (N,N-diethyl-meta-toluamide), permethrin, pyrethroids, carbamates, and organophosphates that function as repellents to reduce contact with *Aedes aegypti* mosquitoes. However, long-term use of synthetic insecticides that are not in accordance with the rules can have adverse effects on health and the environment (Putra, 2020). Moving from the use of synthetic insecticides to natural insecticides is a good step to eradicate *Aedes aegypti* mosquitoes in a way that is safer for health and the environment. Natural insecticides are insecticides derived from plants that have a group of secondary metabolites with active compounds such as alkaloids, phenolics, saponins, and other secondary metabolites (Erlina, 2015). Kawista leaf (*Limonia acidissima*) is one of the plants known to contain secondary metabolite compounds that have potential as natural insecticides. The content of compounds such as terpenoids, flavonoids, tannins, saponins, and phenols makes kawista leaves effective in insect control (Ridwanuloh and Mursal, 2018). Flavonoid compounds can cause changes in the nervous system in insects and saponins function as stomach poisons, inhibit the work of mosquito cholinesterase enzymes and reduce the activity of digestive enzymes and food absorption (Yunus et al., 2022).

Research conducted by Fitri et al., 2022 was to test the repellency ability of 70% ethanol extract from kawista fruit (*Limonia acidissima*) in the form of lotion against *Aedes aegypti* mosquitoes with formulation 1 containing 1% kawsita fruit extract has 60% repellency, fomulation 2 containing 3% kawista fruit extract has 85% repellency and positive control with soffel has 100% repellency against *Aedes aegypti* mosquitoes. Then research conducted by Boesri et al., 2015 tested the repellency (repelling power) of several plant extracts against *Aedes aegypti* mosquito bites of Dengue Hemorrhagic Fever vectors on suren leaves, a 100% dose was only able to reject 63.7% of mosquito bites for 1 hour, 45% dose rejected mosquitoes for 2 hours, 44% dose rejected mosquitoes for 3 hours, 39.2% dose rejected mosquitoes for 4 hours, 36.6% dose rejected mosquitoes for 5 hours, and 32.9% dose rejected mosquitoes for 6 hours.

In the previous study, it was shown that the preparation used was lotion and suren leaf extract was also less effective as a repellent because the 100% dose was only able to reject 63.7% of mosquito bites in the first hour and in the second hour to the sixth hour the repellency was below 50% and there is a need for further research on the breakdown of active ingredients from plant extracts that are very effective in rejecting *Aedes aegypti* mosquito bites.

Based on this description, this study aims to determine the effectiveness of the repellent power of kawista (*Limonia acidissima*) leaf extract against *Aedes aegypti* mosquitoes as an alternative prevention of Dengue fever disease transmission with natural insecticides because synthetic insecticides contain DEET which is toxic. This study showed that kawista fruit extract can be used as a natural repellent. The novelty of this study compared to previous studies is the difference in extracts and preparations of kawista extract used, namely kawista leaf extract in spray preparations with a concentration variation of 25%, 50%, and 75%. The purpose of this study was to determine the effectiveness of the repellent power of kawista leaf extract (*Limonia acidissima*) against *Aedes aegypti* mosquitoes.

MATERIALS AND METHODS

Materials

The materials used in this study were kawista leaves, female *Aedes aegypti* mosquitoes, commercial mosquito repellent spray, 96% ethanol, 70% alcohol, distilled water.

Data Collection Procedure

The data collected were the results of experimental tests obtained from the number of *Aedes aegypti* mosquitoes that landed on the arm in 30 seconds of observation. Observations were made from the first hour to the sixth hour repeatedly for all concentration variations 5 times.

1. Method of Extract Preparation

Kawista leaf extract is made by washing kawista leaves with clean water and drying them for 4 days. The dried kawista leaves were then finely chopped or blended without water and filtered. The powder is placed in a dry container and stored at room temperature. The powder was weighed first as much as 1000 grams and then macerated with 96% ethanol as much as 4000 ml, macerated for 3 days and the extract was stirred periodically. The solution was filtered using filter paper. The next process is evaporated on the extract to remove the ethanol content so that the final result is obtained in the form of 100% concentration of kawista leaf extract repellent in thick form. Then the 100% concentration of kawista leaf extract was diluted into 75%, 50%, and 25% concentration solutions.

2. Testing Repellent Spray against *Aedes aegypti* Mosquitoes

Before testing the forearm was rinsed with clean water and dried, the right arm as a positive control was sprayed with 3 ml of soffel spray. Insert the right arm into the test cage. Observe and record the number of mosquitoes that landed within a 30 second time period. After 30 seconds the arm was carefully removed from the test cage. The left arm as a negative control was sprayed with 3 ml of distilled water. Insert the left arm into the test cage. Observe and record the number of mosquitoes that landed within 30 seconds. If >10 mosquitoes landed then the test can be started. After 30 seconds the arm is carefully removed from the test cage. Then the arm is smeared with a dose of ethanol extract of kawista leaves starting from the lowest extract of 25%, 50%, and 75%. Put the arm back into the cage to be observed for 30 seconds. The arm should not move during the test. This procedure is repeated for each increase in concentration 5 times.

Data Analysis Procedure

Analyze the data obtained in the form of a percentage of repelling power obtained from the following formula:

$$R(\%) = \frac{K - P}{K} \times 100\% \quad (1)$$

Description

DT : Repellency (%)

K : Number of mosquitoes that landed on the negative control arm

P : Number of mosquitoes that landed on the arm of the test group

The data were statistically tested using SPSS (Statistical Package For Social Science). The bivariate analysis used in this study is ED50 and ED99 probit regression analysis which aims to determine the effective dose to obtain a percentage of repellency of 50% and 99%. Data obtained from the observation of *Aedes aegypti* mosquitoes were analyzed with the SPSS program. If the sig value is obtained. <0.05, it can be concluded that there is a repellent ability of kawista leaf extract (*Limonia acidissima*) against *Aedes aegypti* mosquitoes.

RESULTS AND DISCUSSION

Effectiveness Test of Kawista Leaf Extract (*Limonia acidissima*)

This study tested the effectiveness of kawista leaf extract against *Aedes aegypti* mosquitoes with positive control using soffel spray, negative control using distilled water, and repellent spray of kawista leaf extract with concentrations of 25%, 50%, and 75%.

Table 1. Total number of *Aedes aegypti* mosquitoes that landed on the arm

Treatment	Number of Mosquitoes Used	Total Mosquito Stays	Average Mosquito Stays
Positive Control	25	50	8
Negative Control	25	682	114
25% Concentration	25	578	96
50% Concentration	25	367	61
75% Concentration	25	204	34

Table 1 shows the results of the average number of *Aedes aegypti* mosquitoes that landed on the arm from the first replication to the fifth replication observed in the first hour to the sixth hour. The average number of mosquitoes that landed on the arm from highest to lowest was in the negative control with 114 mosquitoes that landed on the arm, 25% concentration with 96 mosquitoes that landed on the arm, 50% concentration with 61 mosquitoes that landed on the arm, 75% concentration with 34 mosquitoes that landed on the arm, and positive control with 114 mosquitoes that landed on the arm.

The results showed that the average number of *Aedes aegypti* mosquitoes on the arm varied for each concentration of kawista leaf extract used. As seen in Table 1, shows that the higher the concentration of extract used, the fewer mosquitoes that land on the arm. This is due to the content of secondary metabolite compounds in kawista leaf extract. So the higher and greater the concentration of kawista leaf extract, the more chemicals it contains. Therefore, at high concentrations mosquitoes are not interested in landing on the arm because the chemical compounds in kawista leaf extract can cause the mosquito's respiratory system to be disrupted (Devitria, 2021).

Based on Table 2, the positive control has an average repellent power for 6 hours reaching 92.67%. The negative control showed no detectable repellency during the same period. The 25% concentration had an average repellency over 6 hours of 15.22%. The 50% concentration had an average repellency over 6 hours of 46.20%. While at 75% concentration the average repellency reached 70.08% in 6 hours.

Table 2. Results of repellency of kawista leaf extract against *Aedes aegypti* mosquitoes

Treatment	Positive Control	Negative Control	25% Concentration	50% Concentration	75% Concentration
First Hour	94,83%	0,00%	27,59%	50,00%	77,59%
Second Hour	93,81%	0,00%	15,04%	46,02%	75,22%
Third Hour	91,82%	0,00%	11,82%	48,18%	70,00%
Fourth Hour	90,35%	0,00%	15,79%	46,49%	68,42%
Fifth Hour	93,81%	0,00%	14,16%	44,25%	65,49%
Sixth Hour	91,38%	0,00%	6,90%	42,24%	63,79%
Average Repellency	92,67%	0,00%	15,22%	46,20%	70,08%

The results of the calculation of the repellency of kawista leaf extract against *Aedes aegypti* mosquitoes at each concentration show different variations in repellency. Tests using repellent spray of kawista leaf extract at concentrations of 25%, 50%, 75%, and positive control showed that the highest repellency occurred at the beginning of use. As time passed, the repellency of each concentration tended to decrease, although some concentrations experienced an increase in certain hours. For example, the 25% concentration showed an increase in repellency from the third to the fourth hour, while the 50% concentration experienced an increase from the second to the third hour.

Figure 1. shows that the negative control had the lowest average repellency against mosquitoes at 0.00%, while the positive control had the highest average repellency at 92.6%.

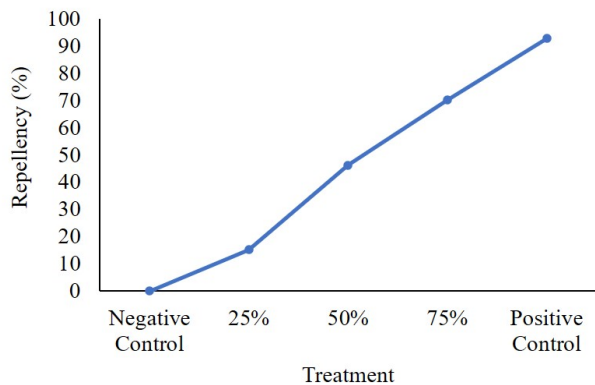


Figure 1. Average repellency of kawista leaf extract against *Aedes aegypti* mosquitoes

In this research, the effective concentration of kawista leaf extract was at a concentration of 75%, which had the highest repellency of 77.59% in the first hour and an average repellency of 70.08% for 6 hours of observation. According to the standards of the Department of Agriculture’s Pesticide Commission (1995) in Organization and Agriculture, 2007 a repellent is said to be effective if it is able to provide at least 90% repellency for 6 hours of exposure. Thus, these results indicate that the repellency effect of kawista leaf extract has not reached the desired standard, as it did not reach the minimum repellency level of 90% over a 6-hour period.

The repellency of kawista leaf extract at each hour fluctuates due to the evaporation of chemical compounds. Factors that influence this include the durability of the extract aroma which is not stable during testing, the activity of *Aedes aegypti* mosquitoes which decreases over time, the lower body temperature of volunteers because mosquitoes prefer a warm atmosphere, and also the activity of volunteers during the experiment can affect repellency (Mufidah et al., 2020).

Similar research conducted by Wahyuni and Nafi’ah, 2021 tested the repellent effectiveness of kaffir lime leaf extract (*Citrus hystrix d.c*) against *Aedes aegypti* mosquitoes with a repellent power of 100% at a concentration of 40% and a repellent power of 96.67% at a concentration of 30%. Research conducted by Boesri et al., 2015 on the repellent test of several plant extracts against *Aedes aegypti* mosquito bites, the vector of Dengue Fever, showed that suren leaf extract at a dose of 100% was less effective in being used as a repellent because it was only able to reject 63.7% of mosquito bites in the first hour and its repellency was below 50% in the second hour to the sixth hour.

Based on this explanation, there is still a possibility that kawista leaf extract has potential as a natural repellent against *Aedes aegypti* mosquitoes. In addition, its use is likely safer than synthetic insecticides because it comes from natural ingredients. Nonetheless, more research needs to be done to optimize the formulation and concentration of the extract to achieve the desired effectiveness.

Data Analysis

The observations from this study were analyzed using probit regression to estimate the concentration of kawista leaf extract that has 50% protection against *Aedes aegypti* mosquitoes and to estimate the concentration needed to achieve 99% protection.

Table 3. Significance results of probit regression analysis

Probit	Parameter	Sig.
	Concentration	<0.001

In Table 3. probit regression analysis obtained a P-Value <0.001 so that there is an ability to repel kawista leaf extract against *Aedes aegypti* mosquitoes.

Table 4. Probit analysis results of ED50 (Effective Dose 50) and ED99 (Effective Dose 99)

Effective Dose (%)	Concentration (%)	Confidence Level (%)	Lower Limit (%)	Upper Limit (%)
50	60,343	95,00	57,736	63,183
99	124,452	95,00	115,709	135,836

Based on the results of probit regression analysis, it was found that kawista leaf extract has 50% inhibition against *Aedes aegypti* mosquitoes at a concentration of 60,343%, with a lower limit of 57,736% and an upper limit of 63,183%. Meanwhile, probit regression analysis showed that to achieve 99% inhibition against *Aedes aegypti* mosquitoes, a concentration of kawista leaf extract of 124,452% was required, with a lower limit of 115,709% and an upper limit of 124,452%.

CONCLUSIONS

From the results of the research, it is known that kawista leaf extract (*Limonia acidissima*) shows repellent ability as a repellent spray against *Aedes aegypti* mosquitoes. The repellency produced at 25% concentration was 15.22%, 50% concentration was 46.20%, and 75% concentration had the most effective repellency ability of 70.08%. The concentration of kawista leaf extract that has a repellent power of 50% (ED50) is a concentration of 60.343%, while the concentration of kawista leaf extract that has a repellent power of 99% (ED99) is a concentration of 124.452%. Further research needs to be done on the administration of kawista leaf extract (*Limonia acidissima*) against *Aedes aegypti* mosquitoes with higher concentrations and using different types of mosquito species.

Author contributions

Nabilah Qotrinnada: Conceptualization, writing draft, writing review and editing; Yauwan Tobing L, Endah Prayekti, Ersalina Nidianti: Data curation, formal analysis.

Conflict of Interest

There is no conflict of interest in this study.

Acknowledgment

Thank you to all parties involved in the completion of this research. This research would not have been possible without the tremendous support of all contributing authors especially my mentor Mr. Tobing for his enthusiasm, knowledge, and meticulous attention to detail which has been my inspiration and kept my work on track. Thank you also to Mrs. Endah and Mrs. Ersal for their input and suggestions for improvement during the research. We also thank the Entomology Laboratory of Surabaya Polytechnic for providing laboratory facilities for the implementation of this research.

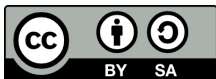
Data availability

We thank all respondents involved in this research project.

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