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RESEARCH ARTICLE

The effect of giving soursop leaves extract (*Annona muricata L.*) on *Aedes aegypti* instar iii larvae

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Abstract

Aedes aegypti is a mosquito that is a vector for various diseases, including Dengue Hemorrhagic Fever (DHF). Mosquito control can be done in various ways, one of which is using synthetic insecticides, using natural ingredients derived from plants. Soursop leaves are a plant that grows widely in Indonesia and can be used as a natural larvicide. This study aims to determine the effect of giving soursop leaf extract on the death of third instar *Aedes aegypti* mosquito larvae. The type of research used in this research is experimental with design in this research using the post test only control design method. The samples used were 25 third instar *Aedes aegypti* larvae in each of 5 treatments using; negative control (aquadest), positive control (1% abate), soursop leaf extract concentration 25%, concentration 50%, and concentration 75%. With observation times of 3 hours, 6 hours and 24 hours. The data obtained was analyzed using SPSS, a probit test obtained a 3 hours LC50 was 97.653, a 6 hours LC¬50 was 87.697, and a 24 hours LC50 was 53.727. The value of LT50 25% concentration is 42.357, LT50 50% concentration is 24.783, and LT50 75% concentration is 12.996, from the parametric test carried out the results were normal and homogeneous, then the ANOVA test was continued and showed a p-value of 0.02 < 0.05 (significant), then H_0 is rejected and H_1 is accepted. So it can be said that there is an effect of giving soursop leaf extract on the death of third instar *Aedes aegypti* mosquito larvae. Researchers are expected to be able to utilize soursop leaves as a natural larvicide that can kill *Aedes aegypti* larvae.

Keywords: *Aedes aegypti*, soursop leaves (*Annona muricata L.*), LC_{50}, LT_{50}.

INTRODUCTION

Dengue fever Dengue (DHF) is a disease caused by Dengue virus infection by a vector mosquito *Aedes aegypti* this disease, is one of disease infectious dangerous and can result death time short (Kusumawati et al., 2018). Mosquito control can done through various method that is control in a way mechanical, biological, and chemical. One of method for control mosquito the simplest and most common method used is method chemistry or use pesticide. However, that's it lots method

eradicate mosquito with use insecticide chemistry give rise to impact negative, and so on. Increasing resistance mosquitoes, pollution environment, poisoning, and death non- residual existence (Susiwati, 2015).

Based on that problem, it is needed to find a plant-based larvicide that is environmentally friendly, easy attainable and effective kill mosquito reason dengue fever. One of them is utilization plants in the environment settlement as larvicide vegetable to mosquito Not yet adults (larvae) or mosquito adults (Boesri et al., 2015).

in Indonesia there are many natural ingredients that have the potential to be utilized as bio-larvicides to replace chemical larvicides (Lukiyono et al., 2023). One of material natural the is soursop leaf (*Annona muricata L.*). Soursop (*Annona muricata L.*) are lots of plants growing in Indonesia. Apart from the fruit, it can direct consumed, other parts of tree soursop like skin wood, leaves, seeds and roots can utilized as plant drug for treat various diseases, insecticides, larvicides, molluscides, antimicrobials and others.

Based on urinate the research this aim for know influence giving extract leaf soursop (*Annona muricata L.*) against death of mosquito larvae *Aedes aegypti* third instar. Recency this study is use different concentrations that is variation concentrations of 25%, 50%, and 75%.

MATERIALS AND METHODS

Materials

The tools used in this research were: plastic tray, oven, blender, scales, measuring cup, test cup, pipette, beaker glass, funnel, spatula, stirring rod, filter paper, label paper.

Procedure Data Collection

Collected data is results from the experimental tests obtained from number of dead *Aedes aegypti* larvae in observation for 3 hours, 6 hours, and 24 hours, in each group treatment from soursop leaves extract.

1. Preparation of Test Solution

The test solution in this study was soursop leaves extract (*Annona muricata L.*) using a 1% abate positive control and using a negative control with distilled water. Soursop extract (*Annona muricata L.*) was made by selecting fresh soursop leaves, which were not wilted or dry, then washed thoroughly with running water to remove dirt attached to the leaves, then the leaves were separated from the stems. Soursop leaves that had been washed thoroughly were then dried in the oven. After that, the dried soursop leaves were mashed without water using a blender. The blended soursop leaves were placed in a dry container and stored at room temperature. Soursop leaves powder was weighed as much as 500 grams, then macerated with 1000 mL of 96% ethanol. The mixture of soursop leaves and 96% ethanol was stirred periodically and left for 24 hours. Filter the solution using filter paper. The remaining pulp obtained was macerated again 1 time with the aim that all substances contained in the soursop leaves were extracted. The filtrate obtained was evaporated at a temperature of 60°C so that the ethanol could evaporate and a thick soursop leaves extract was obtained. Then the soursop leaves extract with a concentration of 100% was diluted to a concentration of 25%, 50%, and 75% using the formula:

$$M_1.V_1 = M_2.V_2 \quad (1)$$

Information:
M_1: Concentration available extract (%)
V_1: Volume of solution with required concentration (mL)
M_2: Desired concentration (%)
V_2: Volume of desired concentration (mL)

Control positive on research This using 1% abate with a volume of 100ml, it was made with method weigh 1gr of abate powder , then dissolved with aquadest as much as 100ml. Control negative that is use aquadest without addition whatever with a volume of 100ml.

2. Larvicide Test

Total third instar *Aedes aegypti* larvae used is 25 fish per test glass. In the test glass, the test solution is used that is as much as 100mL. As for as control negative use Aquadest without addition whatever with a volume of 100mL and in the control positive Abate was used with a volume of 100mL. Group control use solution with concentrations of 25%, 50%, and 75% with a volume of 100mL each repetition. Each group control done five repetitions. After The test solution is ready, third instar *Aedes aegypti* larvae are inserted as many as 25 heads to every test glass so that the condition of the media is not too congested. After the larvae are inserted to in test solution, next researcher observing and calculating amount larval death at intervals of 3 hours, 6 hours and 24 hours. Study This done in five repetitions. Observation to this study done in a way observation direct with method take notes capable of time and concentration kill test larvae.

Procedure Data Analysis

The data has been obtained from death of *Aedes aegypti* larvae, next analyzed. Probit analysis was performed to know mark LC50 and LT50 use spss. Effectiveness soursop leaves extract seen from data analysis, begins with knowing normal data distribution or no using the Shapiro Wilk normality test. Next with the Homogeneity test Levene to know homogeneous data variance or no. Study this use more from 2 groups treatment so, if results normal and variant data distribution homogeneous so continued with parametric tests with the One Way ANOVA test, but if condition the no fulfilled so continued with non-parametric tests with the Kruskal Wallis test.

RESULTS AND DISCUSSION

Results

Data processing was obtained from the results of the larvicide test with the death of *Aedes aegypti* instar III larvae, after being exposed to soursop leaves extract with different concentration groups for 3 hours, 6 hours, and 24 hours with 5 repetitions:

Table 1. Larvicide Test Results Soursop Leaf Extract (*Annona muricata L.*)

			Number of Death							
Treatment	Time	Number of *Aedes aegypti* Larvae	Replication					Total Larval Mortality	Mean ± SD	Percent Death of Larvae
			1	2	3	4	5			
Control (-)	3 hours	25	0	0	0	0	0	0	0 ±0	0%
Aquadest	6 hours	25	0	0	0	0	0	0	0 ±0	0%
	24 hours	25	0	0	0	0	0	0	0 ±0	0%
Control (+)	3 hours	25	12	10	12	12	10	56	11.2 ±1.1	44.80%
Abate 1%	6 hours	25	17	17	16	16	17	83	16.6 ±0.5	66.40%
	24 hours	25	23	22	23	22	23	113	22.6 ±0.6	90.40%
Concentration	3 hours	25	2	2	3	3	2	12	2.4 ±0.5	9.60%
25%	6 hours	25	4	5	5	6	5	25	5 ±0.7	16%
	24 hours	25	8	7	8	7	7	37	7.4 ±0.6	19.60%
Concentration	3 hours	25	5	4	4	5	5	23	4.6 ±0.5	14.40%
50%	6 hours	25	8	8	9	8	9	42	8.4 ±0.5	33.60%
	24 hours	25	12	12	11	12	13	60	12 ±0.5	48%
Concentration	3 hours	25	7	9	9	9	9	43	8.6 ±0.9	34.40%
75%	6 hours	25	11	13	11	9	9	53	10.6 ±1.7	46.40%
	24 hours	25	15	17	17	16	16	81	16.2 ±0.9	64.80%

In the table above, it can be seen that at a concentration of 50% it can kill larvae within 3 hours, namely 18.40%, and within 6 hours, namely 33.60%, and within 24 hours, namely 48%.

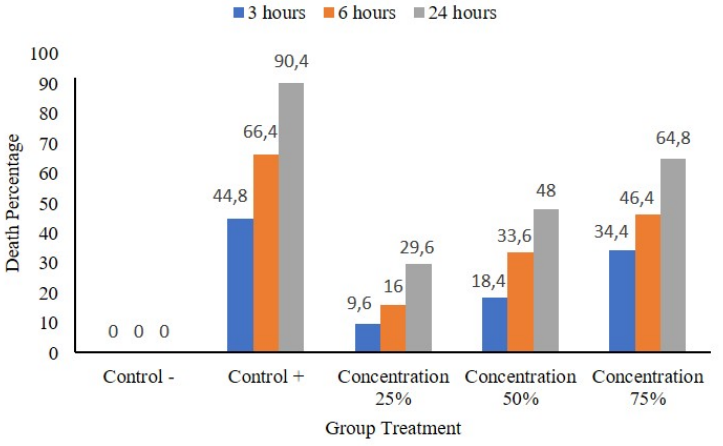

Figure 1. Percentage Larval Mortality %

Table 2. LC_{50} 3 Hours

Mortality (%)	Concentration (%)	Level of confidence (%)	Lower limit (%)	Upper limit (%)
50	97,653	95.0	83,394	129,270

Table 3. LC_{50} 6 Hours

Mortality (%)	Concentration (%)	Level of confidence (%)	Lower limit (%)	Upper limit (%)
50	87,697	95.0	72,728	128,718

Table 4. LC_{50} 24 Hours

Mortality (%)	Concentration (%)	Level of confidence (%)	Lower limit (%)	Upper limit (%)
50	53,727	95.0	46,483	61,921

Table 5. LC_{50} 25% concentration

Mortality (%)	Concentration (%)	Level of confidence (%)	Lower limit (%)	Upper limit (%)
50	42,357	95.0	30,965	79,349

Table 6. LC_{50} 50% concentration

Mortality (%)	Concentration (%)	Level of confidence (%)	Lower limit (%)	Upper limit (%)
50	24,783	95.0	19,481	36,151

Table 7. LC_{50} 75% concentration

Mortality (%)	Concentration (%)	Level of confidence (%)	Lower limit (%)	Upper limit (%)
50	12,996	95.0	9,266	17,502

Based on results data study next testing analysis death of third instar *Aedes aegypti* larvae in the group treatment with use SPSS application.

1. Normality test

Table 8. Normality test results

P-value	Information
0.424	Normal

Based on the results of the normality test using Shapiro-Wilk, the p-value (0.424) > 0.05 is obtained, so it can be assumed that the data is normally distributed.

2. Homogeneity test

Table 9. Homogeneity test results (*Levene*)

p-value	Information
0.259	Homogeneous

Based on homogeneity test results with use *Levene* acquired mark p-value (0.259) > 0.05 so can assumed that the data is homogeneous.

3. ANOVA test

Table 10. ANOVA test result

P-value	Information
0.02	There is a significant influence

Based on ANOVA test results were obtained mark p-value (0.02) < 0.05, so can assumed if the data is significant, then H0 fail is accepted and H1 is accepted, so there is influence giving soursop leaves extract (*Annona muricata L.*) against death of mosquito larvae *Aedes aegypti* third instar.

Discussion

On research Syazana and Porusia, 2022, states that soursop leaves own content the toxic compound for larvae, including flavonoids, tannins, saponins, and alkaloids, where compound the can works as larvicide or bioinsecticide friendly plant-based environment, easy obtained.

Death results for third instar *Aedes aegypti* larvae based on Table 1 were obtained after giving soursop leaves extract (*Annona muricata L.*) was obtained the results at 3 hours of observation were 0% for control (-), 44.8% for control (+), 9.60% at 25% concentration, 18.40% at 50% concentration,

34.40% at 75% concentration. At 6 hours of observation it was 0% in control (-), 66.40% in control (+), 16% at 25% concentration, 33.60% at 50% concentration, 46.40% at 75% concentration. At 24 hour observation it was 0% in control (-), 90.4% in control (+), 29.60% at 25% concentration, 48% at 50% concentration, 64.80% at 75% concentration.

According to WHO, 2005 concentration larvicide considered effective if can cause mortality of test larvae was between 10-95%. Research result This show the more tall concentration extract used so the more there are also many deaths in the larvae. There is an effect giving extract leaf soursop on larvae after exposure for 24 hours, with use concentrations of 25%, 50%, and 75% are available effect the difference tested, where the highest average death of third instar *Aedes aegypti* larvae was found at a concentration of 75%. Based on statement above, yes said that soursop leaves extract effective as larvicide. This study in line with Ekawati et al., 2017 who stated increasingly tall concentration treatment the more Lots number of dead larvae, according to Kolo, 2018 if concentration tall can kills larvae inside short time with level high mortality.

Based on results study This show LC50 soursop can kills third instar *Aedes aegypti* larvae with 50% mortality 3 hour observation test animals, namely at a concentration of ±97.653, 6 hour observation, namely at a concentration of ±87.697, 24 hour observation, namely at a concentration of ±53.727. Besides that observations were also made LT50 soursop leaves extract can kills third instar *Aedes aegypti* larvae with 50% mortality test animals at 25% concentration, namely at ± 42,357 hours, at 50% concentration, namely at ±24,783 hours, at 75% concentration, namely at ± 12,996 hours.

Parametric test results soursop leaves extract in this research using the ANOVA test, because of the data obtained from results this study normally distributed and also homogeneous. Based on Table 10 ANOVA test This show exists significant effect to amount death of *Aedes aegypti* larvae, where obtained mark p-value is 0.02 < 0.05 (significant), then H0 is rejected and H1 is accepted so that there is influence giving soursop leaves extract (*Annona muricata L.*) against death of mosquito larvae *Aedes aegypti* third instar.

CONCLUSIONS

Giving extract leaf soursop (*Annona muricata L.*) against death of mosquito larvae *Aedes aegypti* instar III at a concentration of 25% can be observed for 3 hours killed 9.60%, 6 hour observation, 16%, at 24 hour observation 29.60%, at a concentration of 50% at 3 hour observation could killed 18.40%, at 6 hours observation it was possible killed 33.60%, 24 hour observation was possible kills 48%, at a concentration of 75% at 3 hours observation can killed 34.40%, at 6 hours observation it was possible killed 46.40%, 24 hour observation was possible killed 64.80%. Concentration extract leaf soursop (*Annona muricata L.*) is capable kills 50% of larvae, namely, LC_{50} -3 hours is at a concentration of 97.653%, LC_{50} -6 hours is at a concentration of 87.697%, and LC $_{50}$ -24 hours is at a concentration of 53.727%. For researchers expected can utilise leaf soursop (A*nnona muricata L.*) as larvicide naturally possible kills *Aedes aegypti* larvae. You can also do further use part plant like leaf soursop (*Annona muricata L.*) as larvicide experience.

Author Contribution

Miea Audina: Conceptualization, writing draft, writing review dan editing; Yauwan Tobing Lukiyono, Devyana Dyah Wulandari, Muhammad Afwan Romdloni: Data curation, formal analysis.

Conflict of Interest

There is no conflict of interest in this study.

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Data Availability

We thank all respondents involved in this research project.

REFERENCES

Boesri, H. B., Heriyanto, B., Susanti, L., & Handayani, S. W. (2015). Uji repelen (daya tolak) beberapa ekstrak tumbuhan terhadap gigitan nyamuk aedes aegypti vektor demam berdarah dengue. *Vektora: Jurnal Vektor dan Reservoir Penyakit*, 7(2), 79–84.

Ekawati, E. R., Santoso, S. D., & Purwanti, Y. R. (2017). Pemanfaatan kulit buah jeruk nipis (citrus aurantifolia) sebagai larvasida aedes aegypti instar iii. *Jurnal Biota*, *3*(1), 1–5.

Kolo, S. M. (2018). Efektivitas biolarvasida ekstrak daun sirsak dan serai wangi terhadap larva nyamuk aedes aegypti. *Jurnal Saintek Lahan Kering*, *1*(1), 11–13.

Kusumawati, W. D., Subagiyo, A., & Firdaust, M. (2018). Effect of several doses and types of natural larvicide extracts on death of aedes aegypti mosquito larvae. *Public Health*, *37*(3), 283–295.

Lukiyono, Y. T., Prayekti, E., Setiarsih, D., Zain, S. S., Sari, Y. N. M., Putri, N. A., Putri, E. O. C., & Febrianti, E. R. D. (2023). Sosialisasi penggunaan ekstrak bunga telang (clitoria ternatea l.) dalam upaya penanganan larva aedes sp. di desa simo angin-angin wonoayu sidoarjo. *Community Development Journal: Jurnal Pengabdian Masyarakat*, *4*(3), 6027–6031.

Susiwati, S. (2015). Pengaruh ekstrak daun sirsak (annona muricata zinn) terhadap perkembangan larva aedes aegypti. *JURNAL MEDIA KESEHATAN*, *8*(2), 167–172.

Syazana, N., & Porusia, M. (2022). Kajian literatur efektivitas biolarvasida ekstrak daun sirsak terhadap jentik nyamuk aedes aegypti. *Environmental Occupational Health and Safety Journal*, *2*(2), 203–220.

WHO. (2005). *Guidelines for laboratory and field testing of mosquito larvicides world health organization communicable disease control, prevention and eradication who pesticide evaluation scheme*. World Health Organization.