ORIGINAL RESEARCH REPORT

Effectiveness of the Larvacide Ethanol Extract of Soursop (*Annona muricata* L.) Leaves against *Aedes aegypti* Larva

Selvi Marcellia[1*], Tiara Inggrit Margareta[2], Agustina Retnaningsih[2]
[1]Medical Education Study Program, Faculty of Medicine, Universitas Lampung, Lampung, Indonesia.
[2]Department of Pharmacy, Faculty of Health Sciences, Universitas Malahayati, Lampung, Indonesia.

Article Info

Article history:
Received 29-11-2023
Revised 27-12-2023
Accepted 30-12-2023
Published 10-01-2024

Keywords:
Aedes aegypti
Dengue
Soursop leaves
Larvacides

***Corresponding author**:
Selvi Marcellia
selvicellia@gmail.com

ABSTRACT

Background: Dengue Hemorrhagic Fever (DHF) is an infectious disease caused by one of the four serotypes of dengue virus transmitted through mosquitoes, especially *Aedes aegypti* and *Aedes albopictus*. As the *Aedes aegypti* mosquito-borne DHF illness is still in its larval stage, it can be prevented by utilizing larvicides. A more secure and efficient method to eliminate mosquitoes is by using natural larvicides. Underutilized soursop leaves contain secondary metabolite components that can potentially be larvicides, such as annonins, saponins, flavonoids, and tannins. **Objective:** This study was to determine the effectiveness of soursop leaf extract as a larvicide in controlling *Aedes aegypti* vectors and to determine the most effective concentration of soursop leaf extract as an *Aedes aegypti* larvicide. **Material and Method:** The study was an analytic observational study utilizing a cross-sectional methodology. This study was carried out from March to May 2022. Soursop leaves were extracted using the percolation method, employing a solvent of 96% ethanol with alkaloids, flavonoids, saponins, and tannins. **Result:** The soursop leaves extract yielded in this study was as much as 131.22 grams (13.12%). The most effective concentration of soursop (*Annona muricata* L.) leaves was 3%, with a mortality value of 100%, not much different from the value resulting from 1% temephos. The LC50 results obtained a value of 0.163%. **Conclusion:** Soursop leaf extract was effective as a larvicide against *Aedes aegypti* mosquito larvae.

How to cite:
Marcellia, S., Margareta, T. I., Retnaningsih, A. 2024. Effectiveness of the Larvacide Ethanol Extract of Soursop Leaves (*Annona muricata* L.) against *Aedes aegypti* Larva. Majalah Biomorfologi-Biomorphology Journal, 34(1): 45-51.

Majalah Biomorfologi (Biomorphology Journal) p.ISSN:0215-8833, e.ISSN: 2716-0920
doi: 10.20473/mbiom.v34i1.2024.45-51

Highlights

1. Dengue fever, caused by mosquitoes known as *Aedes aegypti*, can be avoided by applying larvicides while the insects are still in their larval stage.
2. The extract from soursop leaves at a concentration of 3% is the most effective for *Aedes aegypti* larvae, resulting in 100% mortality.

BACKGROUND

Dengue hemorrhagic fever (DHF) is an infectious disease caused by one of the four serotypes of dengue virus transmitted through mosquitoes, especially *Aedes aegypti* and *Aedes albopictus* (Kularatne & Dalugama, 2022). Dengue hemorrhagic fever (DHF) can be transmitted to humans through mosquito bites from *Aedes aegypti*, which remains a significant public health issue in Indonesia and frequently leads to outbreaks (Jumaina & Gani, 2019). The prevalence of DHF continues to rise, with a 30-fold increase observed in 50 to 100 million cases of dengue across 100 nations where dengue is prevalent (Soekiman, 2012). DHF emerges rapidly, which can lead to concern among Indonesians due to its potential to cause fatalities and a quick spread. Therefore, it is crucial to provide appropriate medical care for dengue fever patients in this situation (Minister of Health of The Republic of Indonesia, 2021).

Temephos is the predominant larvicide employed for managing *Aedes aegypti* L. larvae. Indonesia has used temephos (Abate 1SG) as a mosquito control since 1976. Since 1980, temephos has been widely utilized to manage *Aedes aegypti* mosquitoes (Adyatma, et al., 2021). Extensive utilization of melatonin and temephos insecticides has been conducted in Indonesia for over 25 years to manage *Aedes aegypti*, resulting in the rapid development of resistance in this species (Rahmayanti, et al., 2016).

Larvicides exhibit several adverse consequences, including the development of mosquito and larvae resistance, the potential for water and food contamination, and the possibility of chemical residue accumulation in flora, fauna, soil, and the surrounding environment (Brühl, et al., 2020; Meier, et al., 2022). Efforts to mitigate the adverse effects of chemical pesticides can employ safer alternative methods. A more secure option is using natural larvicides as a more productive means of exterminating mosquitoes. *Aedes aegypti* larvae can be derived from safe and environmentally friendly plants. These plants can degrade specifically to the target. The larval phase has limited movement compared to adult mosquitoes (Dhavan & Jadhav 2020).

Soursop leaves are frequently utilized in Indonesia as a potential source of larvicides. Scientific evidence has demonstrated the efficacy of soursop plants in eliminating mosquito larvae. Moreover, soursop leaves do not harm humans or other organisms (Parthiban, et al., 2020). Furthermore, soursop leaves are readily accessible and anticipated to exert a beneficial influence on human well-being. Soursop leaves possess insecticidal and larvicidal properties, making them effective insect repellents (Santos, et al., 2023).

In a previous study by Bestari, et al., (2020), the effectiveness of soursop leaf extract in eliminating *Aedes aegypti* mosquito larvae was demonstrated. The results showed that using soursop leaf extract at a dosage of 0.6% successfully eradicated *Aedes aegypti* larvae. In addition, a concentration of 0.12% showed a notable 100% effectiveness rate.

OBJECTIVE

The objective of this study was to determine the concentration of soursop leaf extract that was effective as a larvicide for controlling *Aedes aegypti* vectors and the LC_{50} value of soursop leaf extract for exterminating *Aedes aegypti* larvae.

MATERIAL AND METHOD

The study was an analytical observational study with a cross-sectional design. The research was carried out from March to May 2022. The collection and analysis of larvicides were conducted in the FMIPA Chemical Laboratory of The University of Lampung, Indonesia. The sample in this study used 1000 g of soursop leaves.

The population in this study consisted of *Aedes aegypti* larvae in their third and fourth developmental stages. *Aedes aegypti* eggs were obtained in dry form and on paper media. *Aedes aegypti* mosquito larvae eggs were obtained from the Research Center and Pangandaran Health Development (-7.666884816624908, 108.68046687514536). The sample used in this study was collected from young soursop leaves (*Annona muricata* L.). Three leaves were counted, starting from the stem of the soursop plant. Soursop leaves were collected from plantations in the Gedongtataan area, Pesawaran. Soursop leaves were in good condition and free from pests, and the undamaged/fresh leaves were chosen.

Meanwhile, during the early stage of development of *Aedes aegypti*, known as instar III, the larvae are usually 3-5 days old and are actively living (Hermawan & Amirullah, 2016; Anggrain & Cahyati, 2017).

The equipment utilized for testing a beaker glass includes a measuring glass, an Erlenmeyer flask, a pipette for measuring drops and volume, a stirrer, paper strain stems, a paper label, a plastic vessel, an oven, a blender for simplistic, a tray, spatula, and funnel. Additionally, analytical scales and rotary evaporators are also considered essential tools.

The materials utilized in this study included leaves from the Soursop plant (*Annona muricata* L.), larvae of the *Aedes aegypti* mosquito, and 96% Ethanol made in Indonesia by PT. Karya Muda Indochem (210114.001), the Abate, distilled water, HCL made in Indonesia by Braco (2208195445), $FeCl_3$ made in Indonesia by Merck (1.07174.1000), the Mg made in Indonesia by Braco (263/MP/0617), H_2SO_4 made in Indonesia by PT. Smart Lab Indonesia (109502072001), Mayers reagent, Dragenroff's reagent, Wagner's reagent made in Indonesia by PT. Palapa Muda Perkasa (8085038811), Chloroform and Ammonia.

The method employed in this study was the maceration method for extraction. The extraction procedure was carried out by soaking the powdered plant material in a liquid and gently stirring it at average room temperature. The maceration method was chosen because of its benefits, such as its straightforward work procedure, simple tools, and compatibility with materials that cannot withstand heat (Wilson, et al., 2022).

The results of this study were analyzed using the Shapiro-Wilk, Probit test, and ANOVA statistical tests. The significant level is considered if $p<0.000$ The hypothesis was examined statistically using IBM SPSS Statistics for Windows, version 25 (IBM Corp., Armonk, N.Y., USA). The Shapiro-Wilk test is used for valid and efficient normality tests on small samples. The probit test is used to determine the LC_{50} value obtained from the results of this study. In this case, ANOVA is used to analyze independent and dependent variables to determine the efficacy of ethanol extracts as *Aedes aegypti* larvicides.

RESULT

In the control group, alternative names were used for the negative and the positive control groups for the temephos dissolved in water until it reached a volume of 100 ml. The experimental group utilized a 4% concentration (b/v) of ethanol extract derived from soursop leaves, with concentrations of 0.50%, 0.75%, 1%, and 3%. The dissolved component was added to the distilled water slowly until the amount reacheed 100 ml. Every time, there were 25 immature *Aedes* present in the solution.

Table 1. Treatment plan for 96% ethanol extract of soursop leaves on *Aedes aegypti* larvae.

Treatment	Concentration	Number of larvae x repetition	Total
% Control (-)	Aquades	25 larvae x 5	125 larvae
% Control (+) *Temefos*	1%	25 larvae x 5	125 larvae
P1	0.50%	25 larvae x 5	125 larvae
P2	0.75%	25 larvae x 5	125 larvae
P3	1%	25 larvae x 5	125 larvae
P4	3%	25 larvae x 5	125 larvae
	Total		750 larvae

An ethanol-free test was performed on soursop leaf extract (*Annona muricata* L.) to release the extract from ethanol and yield a pure extract free of contamination. It is ethanol-free if the extract does not smell like Iodoform and no yellow precipitate occurs (Sumiati, 2014). This was also found in this study, as can be seen in Table 2, that the ethanol-free experiment involved the combination of the extract with NaOH 1 N and iodine 0.1 N.

Table 2. Ethanol-free test results on ethanol extract of soursop leaves.

Identification	Procedure	Result
Ethanol-free test	Extraction + NaOH 1 N + Iodine 0.1 N	It did not produce an iodoform odor or form a yellow precipitate.

Table 3 shows the phytochemical screening testing of the soursop (*Annona muricata* L.) leaves using ethanol solvent, indicating that this sample has an Alkaloid, Flavonoid, Tannin, and Saponins. Phytochemical tests on soursop leaf extract were done to determine the presence of secondary metabolites (Asfahani, et al., 2022).

Table 3. Phytochemical test results of ethanol extract of soursop leaves (*Annona muricata* L.).

Qualitative Test	Observation	Result
Alkaloids	The solution was red-brown, and there was a white residue	Positive
Flavonoids	Brick-red solution	Positive
Saponins	The solution was brick-red, and stable foam was formed	Positive
Tannins	Greenish-black solution	Positive

The toxicity test extract of soursop leaves (*Annona muricata* L.) was processed, and the LC_{50} value of the data was obtained (Table 4). Regarding the larvae of *Aedes aegypti*, the results demonstrated that the lethal concentration required to kill 50% of the test animals was 0.163%. Larvicide concentration is deemed effective when it results in 10-95% mortality rates for the larvae (World Health Organization, 2011). Based on the test results, it can be concluded that the extract from soursop leaves is an effective larvicide. This effectiveness was demonstrated at concentrations of 0.50%, resulting in a larval mortality rate of 94.4%. An ANOVA test yielded a statistically significant result ($p<0.000$). This indicated a statistically significant difference ($p< 0.05$) in the number of deaths among the *Aedes aegypti* larvae in each treatment. LSD test results showed that a 0.50% concentration had better larvicide effectiveness.

Table 4. Test results for the effectiveness of larval mortality.

Concentration (%)	Death of larvae (tail)	Mortality (%) (10 Hours)	LC_{50} (%)	p-value
0.50%	118	94.4%		0.000
0.75%	121	96.8%		0.000
1%	122	97.6%	0.163%	0.000
3%	125	100%		0.000
Control +	125	100%		0.000
Control -	0	0		0.000

The result of the LSD (Least Significant Differences) test showed that concentrations of 0.50% had better effectiveness for the larva. This can be seen at a concentration of 0.50%, which has significant differences when compared to concentrations of 0.75%, 1%, and 3% in killing the larvae of the Aedes aegypti mosquito. 0.50% concentration has better larvicide effectiveness.

Table 5. Results of Least Significant Differences (LSD) at 10^{th} hours.

Treatment	0,5%	0.75%	1%	3%	K+	K-
0.5%		0.081	0.012	0.000	0.000	0.000
0.75%	0.081		0.372	0.012	0.012	0.000
1%	0.012	0.372		0.081	0.081	0.000
3%	0.000	0.012	0.081		1.000	0.000
K+	0.000	0.012	0.081	1.000		0.000
K-	0.000	0.000	0.000	0.000	0.000	

DISCUSSION

The alkaloid compound supposedly inhibits the AchE enzyme, which causes acetylcholine to accumulate, disrupting the impulse transmission system to muscle cells. This results in larvae experiencing constant convulsions and eventually paralysis, and if this condition continues, it can lead to larval death. Flavonoids work by entering the larva's body through the respiratory system, resulting in nerve swelling and damage to the breathing system, causing larvae to be unable to breathe and eventually die. Saponins work by interfering with the development and disturbance of the molting of the larvae so that they will not be able to develop to the next stage. Tanin compounds can bind protease enzymes to the enzyme by the tannin. The work of the enzyme will be inhibited so that the metabolism of the cells can be disrupted, and the larva will lack nutrients, resulting in the inhibition of larval growth. If this process occurs continuously, it will affect larval death (Rahmayanti, et al., 2016).

This study used *Aedes aegypti* larval instars III and IV. The third and fourth instar larvae were chosen because they were similar in size and structure to those in Trisnawati & Rahayuningsih, (2009) which found that instar larvae I and II were developing digestive tract cells, particularly in the middle intestine, almost identically to instars III and IV. Third and fourth-stage larvae have structures and complete components such as the number of internal epithelial cells and digestive tracts. This makes the larvae more resistant to toxin attack, and they are also almost the same size. Therefore, choosing third- and fourth-stage larvae is important to avoid mistakes when selecting test larvae. In this study, the larvae were separated into six groups: the positive control group with a 1% concentration of temephos, the negative control group, and the soursop leaf extract treatment groups with concentrations of 0.50%, 0.75%, 1%, and 3%. Every treatment group included 25 *Aedes aegypti* mosquito larvae, with the amount of larvae established by the (World Health Organization, 2011).

The LC_{50} test measured the lethal dose at which 50% of the larvae in the test population died. The LC_{50} probit analysis demonstrated that the ethanol extract of soursop leaves (*Annona muricata* L.) was effective at 0.163%. These findings indicate that the soursop leaf extract can eradicate 50% of Aedes aegypti mosquito larvae when used at this dose. Hence, the extract derived from soursop leaves has highly toxic characteristics against *Aedes aegypti* larvae. According to Ismatullah, (2014) the level of toxicity was categorized as highly hazardous if below 1%, toxic if between 1-10%, moderately harmful if between 10-50%, slightly hazardous if between 50-99%, and non-toxic if beyond 100%. Value is more effective in killing experimental animals since it takes a lower dosage to deliver deadly effects over a more extended period (Erhirhie, et al., 2018).

In a study conducted by Bestari, et al., (2020) the effectiveness of the extracts from soursop leaves (*Annona muricta* L.) and ketapang leaves (*Terminalia catappa* L.) in eliminating *Aedes aegypti* larvae was examined. The chemicals present in these extracts include alkaloids, flavonoids, saponins, and tannins. The LC_{50} findings were obtained at a concentration of 0.015%. These findings indicate that the research conducted on the extraction of leaves from the soursop fruit has revealed a higher toxicity level than prior studies. The variability in the LC_{50} results is attributable to differences in the larvae's ability to withstand the effects of the test chemical. Additionally, a number of plant-derived factors may also be relevant, including the plant's geographical origin, the time of harvest, the storage conditions of the plant material, the type and quantity of active chemicals the plant contains, and the extraction techniques used (Rosmayanti, 2014).

Strength and limitations

This research was helpful as a reference regarding larvicide against mosquito larvae. For this study, we utilized a low concentration of mosquito larvae. In future studies, we recommend using greater dosages to accelerate the death rate of mosquito larvae.

CONCLUSION

Soursop (*Annona muricata* L.) leaf extract has a larvicidal activity against *Aedes aegypti* larvae. It has a larvicidal efficacy class based on the LC_{50} value produced.

Acknowledgment
The authors would like to thank the staff of the FMIPA Chemical Laboratory of the University of Lampung, Indonesia, for assisting in testing larvicides to explore this topic and providing the necessities for this article.

Conflict of Interest
All authors have no conflict of interest.

Ethic Consideration
This research has been approved by the ethical committee of the Faculty of Health Sciences University Malahayati with ethics certification no. 2445/EC/KEPK-UNMAL/lll/2022 on 28-03-2022.

Funding Disclosure
This research was self-funded by the authors.

Author Contribution
TIM contributes to conception and design, analysis and interpretation of the data, drafting the article, provision of study materials or patients, administrative, technical, or logistic support, and collection and assembly of the data. AR contributes to the conception and design, critical revision of the article for important intellectual content, final approval of the article, and statistical expertise. SM contributes to conception and design; critical revision of the article for important intellectual content; and final approval of the article.

REFERENCES

Adyatma, I. B. P., Damayanti, P. A. A., Swastika, I. K. 2021. Status resistensi larva nyamuk *Aedes aegypti* terhadap temefos di Desa Peguyangan Kaja, Kota Denpasar tahun 2020. Intisari Sains Medis, 12(1): 294–297. doi: 10.15562/ism.v12i1.944.

Anggrain, T. S., Cahyati, W. H. 2017. Perkembangan *Aedes aegypti* pada berbagai pH air dan salinitas air. Higeia Journal of Public Health Research and Development, 1(3): 1–10. Available at: https://journal.unnes.ac.id/sju/index.php/higeia/article/download/15165/8441/.

Asfahani, F., Halimatussakdiah., Amna, U. 2022. Analisis fitokimia ekstrak daun sirsak (*Annona muricata* Linn.) dari Kota Langsa. Quimica: Jurnal Kimia Sains dan Terapan, 4(2): 18–22. Available at: https://ejurnalunsam.id/index.php/JQ/article/download/6530/3819/.

Bestari, R. S., Felina, S., Hidayatullah, M., et al. 2020. Perbedaan uji efektivitas ekstrak daun sirsak (*Annona muricta* L.) dan daun ketapang (*Terminalia catappa* L.) dalam membunuh larva *Aedes aegypti*. Prosiding University Research Colloquium, 389–396. Available at: http://repository.urecol.org/index.php/proceeding/article/view/1086.

Brühl, C. A., Després, L., Frör, O., et al. 2020. Environmental and socioeconomic effects of mosquito control in Europe using the biocide *Bacillus thuringiensis* subsp. israelensis (Bti). Science of The Total Environment, 724: 137800. doi: 10.1016/j.scitotenv.2020.137800.

Dhavan, P. P., Jadhav, B. L. 2020. Eco-friendly approach to control dengue vector *Aedes aegypti* larvae with their enzyme modulation by Lumnitzera racemosa fabricated zinc oxide nanorods. SN Applied Sciences, 2(5): 843. doi: 10.1007/s42452-020-2636-0.

Erhirhie, E. O., Ihekwereme, C. P., Ilodigwe, E. E. 2018. Advances in acute toxicity testing: strengths, weaknesses and regulatory acceptance. Interdisciplinary Toxicology, 11(1): 5–12. doi: 10.2478/intox-2018-0001.

Hermawan, S, Amirullah, A. 2016. Metode penelitian bisnis pendekatan kuantitatif & kualitatif. Malang: Media Nusa Creative. Available at: http://eprints.umsida.ac.id/id/eprint/6233.

Ismatullah, A. 2014. Uji efektivitas larvasida ekstrak daun binahong (*Anredera cordifolia* (Ten.) Steenis) terhadap larva Aedes aegypti instar III. Medical Journal of Lampung University, 3(5). Available at: https://juke.kedokteran.unila.ac.id/index.php/majority/article/view/259.

Jumaina, J., Gani, A. 2019. Determinants of the incidence of dengue hemorrhagic fever in the work area of Kunciran Health Center, Tangerang, Banten. in Strengthening Hospital Competitiveness to

Improve Patient Satisfaction and Better Health Outcomes. Masters Program in Public Health, Universitas Sebelas Maret: 59–67. doi: 10.26911/the6thicph-FP.01.08.

Kularatne, S. A., Dalugama, C. 2022. Dengue infection: Global importance, immunopathology and management. Clinical Medicine, 22(1): 9–13. doi: 10.7861/clinmed.2021-0791.

Meier, C. J., Rouhier, M. F., Hillyer, J. F. 2022. Chemical control of mosquitoes and the pesticide treadmill: A case for photosensitive insecticides as larvicides, Insect, 13(12): 1093. doi: 10.3390/insects13121093.

Minister of Health of The Republic of Indonesia. 2021. Strategi nasional penanggulangan dengue 2021-2025. Jakarta: Kementerian Kesehatan Republik Indonesia. Available at: https://p2pm.kemkes.go.id/storage/publikasi/media/file_1631494745.pdf.

Parthiban, E., Arokiyaraj, C., Ramanibai, R. 2020. *Annona muricata*: An alternate mosquito control agent with special reference to inhibition of detoxifying enzymes in Aedes aegypti. Ecotoxicology and Environmental Safety, 189: 110050. doi: 10.1016/j.ecoenv.2019.110050.

Rahmayanti, R., Putri, S. K., Fajarna, F. 2016. Uji potensi kulit bawang bombay (*Allium Cepa*) sebagai larvasida terhadap kematian larva nyamuk *Aedes aegypti*. Jurnal Edukasi dan Sains Biologi, 5(1). Available at: https://www.neliti.com/publications/77794/uji-potensi-kulit-bawang-bombay-allium-cepa-sebagai-larvasida-terhadap-kematian#cite.

Rosmayanti, K. 2014. Uji efektivitas ekstrak biji sirsak (*Annona muricata* L) sebagai larvasida pada larva *Aedes aegypti* instar III/IV). Universiras Islam Negeri Syarif Hidayatullah. Available at: https://repository.uinjkt.ac.id/dspace/bitstream/123456789/25762/1/KIKI ROSMAYANTI.pdf.

Santos, I. L., Rodrigues, A. M. C., Amante, E. R., et al. 2023. Soursop (*Annona muricata*) properties and perspectives for integral valorization. Foods, 12(7): 1448. doi: 10.3390/foods12071448.

Soekiman, S. 2012. Demam berdarah dengue. Jakarta: Sagung Seto.

Sumiati, E. 2014. Uji aktivitas antibakteri ekstrak kloroform dan ekstrak etanol biji bidara laut (*Strychnos ligustrina* Bl) terhadap *Staphylococcus aureus* ATCC 25923 dan *Salmonella thypi*. Biogenesis: Jurnal Ilmiah Biologi, 2(1): 1–10. doi: 10.24252/bio.v2i1.461.

Trisnawati, A. G., Rahayuningsih, F. B. 2009. Analisis faktor risiko kejadian demam berdarah dengue di Desa Mojosongo Kabupaten Boyolali. Universitas Muhammadiyah Surakarta. Available at: https://publikasiilmiah.ums.ac.id/bitstream/handle/11617/2612/2009_dm_azizah_depan.pdf?isAllowed=y&sequence=9.

Wilson, J., Simpson, T., Spelman, K. 2022. Total cannabidiol (CBD) concentrations and yields from traditional extraction methods: Percolation vs. maceration. Frontiers in Pharmacology, 13. doi: 10.3389/fphar.2022.886993.

World Health Organization. 2011. Comprehensive guidelines for prevention and control of dengue and dengue haemorrhagic fever. WHO. Available at: https://www.aedescost.eu/sites/default/files/2023-01/WHO_SEA-Comprehensive_Guidelines_Prev%26Control_Dengue-2011.pdf.