

## Comparative Study of The Potential of Phyto-larvicide Extracts from Soursop Leaves and Garlic Bulbs against *Aedes aegypti* Larvae

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### Abstract

Dengue Hemorrhagic Fever (DHF) is a disease caused by the bite of *Aedes* sp. mosquitoes carrying the dengue virus. One of the current efforts to control DHF is through abatement using temephos. However, long-term use of temephos can lead to the development of insecticide resistance, thereby reducing its effectiveness. As an alternative, efforts are needed to develop larvicides derived from natural plant-based ingredients, such as soursop leaves and garlic bulbs. This study aimed to compare the effectiveness of ethanol extracts of soursop leaves and garlic bulbs on the mortality of *Aedes aegypti* larvae. This study was a true experimental study with a post-test only control group design. The experiment was conducted in three replications using 25 *Aedes aegypti* instar III–IV larvae in 12 groups, consisting of one positive control group, one negative control group, and five treatment groups for each extract at concentrations of 0.05%, 0.1%, 0.2%, 0.4%, and 0.8%. Based on the Post Hoc Mann-Whitney statistical analysis, each tested extract showed the highest larvicidal effect at a concentration of 0.8%, with comparisons to the positive control showing a p-value > 0.05. The LC<sub>50</sub> values of the soursop leaf and garlic bulb extracts were 0.102 and 0.116, respectively. Thus, it can be concluded that ethanol extracts of soursop leaves and garlic bulbs are effective as phytolarvicidal agents against *Aedes aegypti* larvae; however, the ethanol extract of soursop leaves had a stronger effect than that of garlic bulbs.

**Keyword:** Larvicides, Soursop Leaves, Garlic Bulbs, *Aedes aegypti*.

### INTRODUCTION

Dengue hemorrhagic fever (DHF) remains one of the greatest public health challenges, particularly in tropical and subtropical regions, including Indonesia, making continuous surveillance and research highly important. This disease has complex epidemiological characteristics because it is caused by four different dengue virus serotypes (DEN-1, DEN-2, DEN-3, and DEN-4), which can circulate simultaneously within a population. Infection with different serotypes may increase the risk of severe dengue due to an immunological mechanism known as antibody-dependent enhancement (ADE). This condition makes dengue transmission patterns difficult to predict and subject to change over time. Additionally, high population mobility, urbanization, and environmental changes continue to influence transmission patterns, making accurate monitoring data and up-to-

date scientific research essential for risk mapping. (1–3)

The high incidence of dengue infection in Indonesia continues to make it a country with a high prevalence of dengue fever. (4,5) Data from the 2023 Indonesia Health Survey indicate that dengue infection has spread across all provinces, with an average prevalence of 0.64%, the highest incidence occurring among children aged 5–14 years, and a higher prevalence in urban areas. (6) In response to this situation, the Indonesian government has set a target of achieving “Zero Dengue Deaths in Indonesia” by 2030. To achieve this target, the government has formulated six key strategies, including strengthening effective, safe, and sustainable vector control. (4)

On the other hand, dengue vectors—*Aedes aegypti* and *Aedes albopictus*—have demonstrated remarkable environmental adaptability. Global climate change, such as

rising temperatures and unstable rainfall patterns, has expanded mosquito habitats and prolonged the transmission season. Insecticide resistance in mosquitoes, particularly to temephos, an organophosphate-based insecticide, which has been frequently reported in various regions, also reduces the effectiveness of conventional vector control methods. To date, no specific antiviral therapy for dengue is available, and the effectiveness of vaccines remains limited to certain population groups. (4,7,8)

Given this situation, research on larvicides derived from natural resources is increasingly being conducted, as these agents are biodegradable and considered more environmentally friendly and safer for humans. (9,10) As a tropical country, Indonesia has great potential to provide a variety of natural resources as the basis for alternative larvicides. Traditionally, several plants have long been used by communities as natural agents to repel and kill mosquitoes. This practice is common in many tropical regions, including Indonesia. Some of these plants include soursop leaves (*Annona muricata* L.) and garlic bulbs (*Allium sativum* L.), whose bioactive compounds are believed to possess both toxic and repellent effects against insects. In addition, both materials are readily available, inexpensive, and derived from natural sources, making them relatively more environmentally friendly than synthetic chemical larvicides. (11–13)

Previous studies have shown that soursop seed salt extract is able to kill *Aedes aegypti* larvae at a lethal concentration ( $LC_{50}$ ) of 0.009 mg/mL, while soursop leaf hydroethanolic extract is effective as a larvicide against *Culex quinquefasciatus* with an  $LC_{50}$  of 210.17  $\mu$ g/mL. (14,15) Another study showed that garlic essential oil had a stronger larvicidal effect than essential oils extracted from Bali orange leaves or peel against *Culex quinquefasciatus* larvae. (16) Garlic ethanol extract has also been shown to be effective in killing *Anopheles* sp. larvae. (17) Based on these positive findings, both plants have similar potential as natural larvicides; however, their active compounds have different mechanisms of ac-

tion, which may result in varying effectiveness against mosquito larvae. Therefore, further study is needed to evaluate the effects of ethanol extracts of soursop leaves and garlic bulbs on the mortality of *Aedes aegypti* larvae. This comparison is important for identifying natural compounds that are more effective, economical, and have the potential to be developed as safer and more environmentally friendly alternatives to conventional larvicides.

## METHODS

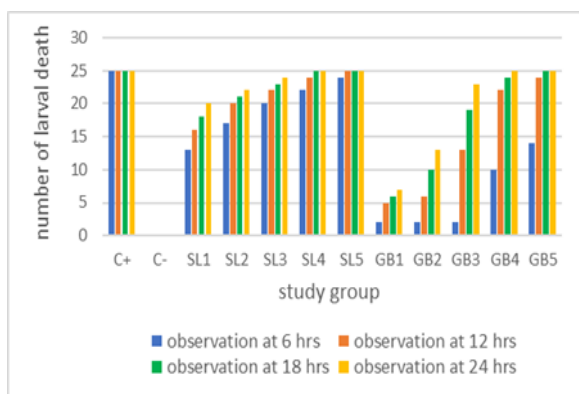
This was a laboratory-based experimental study using 96% ethanol extracts of soursop leaves (*Annona muricata* L.) and garlic bulbs (*Allium sativum* L.) as test materials. These extracts were obtained through a maceration process using 96% ethanol and had previously passed quality control testing at the UPF Traditional Health Services Testing Laboratory in Tawangmangu, under certificate number TL.02.04/D.XI.6/133.059/2024. Each test material was prepared in five different concentration levels: 0.05%, 0.1%, 0.2%, 0.4%, and 0.8%. The positive control used was 1% temephos, while PEG 400, which also served as a diluent, was used as the negative control. The experiment was replicated three times for each treatment group. The study population consisted of instar III–IV *Aedes aegypti* larvae, with 25 larvae used in each study group. (18) The study was conducted after obtaining approval from the Ethics Committee under number 5175/A.1/KEPC-FKUMS/I/2024.

Larvicidal tests were conducted in accordance with laboratory bioassay guidelines. Each concentration of the test materials, as well as the positive and negative controls, was prepared in a container with a test volume of 200 mL, into which 25 larvae were introduced. The larvae were exposed for 24 hours, with observations performed every 6 hours and mortality recorded at each interval. Larvae were considered dead if they showed no response or movement when gently touched, or if they were unable to rise to the surface. (18) Mortality data were statistically analyzed using the Kruskal-Wallis test, followed by the Mann-

Whitney post hoc test, and probit analysis was performed to determine the LC50 with a 95% confidence interval. The extract preparation procedure was conducted at the Pharmacology Laboratory, while the larvicidal tests were carried out at the Parasitology Laboratory, Faculty of Medicine, Universitas Muhammadiyah Surakarta.

**RESULTS**

The initial observation involved counting the number of *Aedes aegypti* larvae that died every 6 hours over a 24-hour observation period. The results are presented in Figure 1.



**Figure 1.** Graph showing the number of larval deaths every 6 hours of observation

Notes:

- C+ : 1% temephos positive control group
- C- : PEG 400 negative control group
- SL1 : 0.05% soursop leaf extract group
- SL2 : 0.1% soursop leaf extract group
- SL3 : 0.2% soursop leaf extract group
- SL4 : 0.4% soursop leaf extract group
- SL5 : 0.8% soursop leaf extract group
- GB1 : 0.05% garlic bulb extract group
- GB2 : 0.1% garlic bulb extract group
- GB3 : 0.2% garlic bulb extract group
- GB4 : 0.4% garlic bulb extract group
- GB5 : 0.8% garlic bulb extract group

The data presented in Figure 1 were subsequently analyzed statistically. The results of the normality test using the Shapiro–Wilk test yielded a p-value of 0.002, while the heterogeneity test using Levene’s test yielded a p-value of 0.008. To determine whether there were significant differences among the data groups, the data were further analyzed using the Kruskal–Wallis test, which yielded a p-value of 0.001. To identify pairs of groups with significant differences, the analysis was continued using the Mann–Whitney post hoc test, the results of which are presented in Table 1.

The final analysis involved a Probit test to determine the LC50 values. The results of the Probit test for each 6-hour observation period are presented in Table 2.

**Table 1.** Results of the Mann–Whitney post hoc test

	C+	C-	SL1	SL2	SL3	SL4	SL5	GB1	GB2	GB3	GB4	GB5
C+		<b>0,025*</b>	<b>0,034*</b>	<b>0,037*</b>	0,121	1,000	1,000	<b>0,037*</b>	<b>0,034*</b>	0,121	1,000	1,000
C-	<b>0,025*</b>		<b>0,034*</b>	<b>0,037*</b>	<b>0,037*</b>	<b>0,025*</b>	<b>0,025*</b>	<b>0,037*</b>	<b>0,037*</b>	<b>0,037*</b>	<b>0,034*</b>	<b>0,025*</b>
SL1	<b>0,034*</b>	<b>0,034*</b>		<b>0,043*</b>	<b>0,046*</b>	<b>0,034*</b>	<b>0,034*</b>	<b>0,046*</b>	<b>0,046*</b>	0,487	<b>0,043*</b>	<b>0,034*</b>
SL2	<b>0,037*</b>	<b>0,037*</b>	<b>0,043*</b>		0,77	<b>0,037*</b>	<b>0,037*</b>	0,050	<b>0,046*</b>	0,376	<b>0,037*</b>	<b>0,037*</b>
SL3	0,121	<b>0,037*</b>	<b>0,046*</b>	0,77		0,121	0,121	0,050	<b>0,046*</b>	0,822	0,121	0,121
SL4	1,000	<b>0,025*</b>	<b>0,034*</b>	<b>0,037*</b>	0,121		1,000	<b>0,037*</b>	<b>0,034*</b>	0,121	1,000	1,000
SL5	1,000	<b>0,025*</b>	<b>0,034*</b>	<b>0,037*</b>	0,121	1,000		<b>0,037*</b>	<b>0,034*</b>	0,121	1,000	1,000
GB1	<b>0,037*</b>	<b>0,037*</b>	<b>0,046*</b>	0,050	0,050	<b>0,037*</b>	<b>0,037*</b>		<b>0,046*</b>	0,050	<b>0,037*</b>	<b>0,037*</b>
GB2	<b>0,034*</b>	<b>0,037*</b>	<b>0,046*</b>	<b>0,046*</b>	<b>0,046*</b>	<b>0,034*</b>	<b>0,034*</b>	<b>0,046*</b>		<b>0,046*</b>	<b>0,034*</b>	<b>0,034*</b>
GB3	0,121	<b>0,037*</b>	0,487	0,822	0,822	0,121	0,121	0,050	<b>0,046*</b>		0,121	0,121
GB4	1,000	<b>0,034*</b>	<b>0,043*</b>	0,121	0,121	1,000	1,000	<b>0,037*</b>	<b>0,034*</b>	0,121		1,000
GB5	1,000	<b>0,025*</b>	<b>0,034*</b>	0,121	0,121	1,000	1,000	<b>0,037*</b>	<b>0,034*</b>	0,121	1,000	

**Table 2.** Probit test results for each study group at 6-hour observation intervals

Study group	LC <sub>50</sub> at 6hrs	LC <sub>50</sub> at 12hrs	LC <sub>50</sub> at 18hrs	LC <sub>50</sub> at 24 hrs
Soursop leaves	0.137	0.128	0.117	0.102
Garlic bulb	0.686	0.229	0.144	0.116

## DISCUSSION

Preliminary results from the larvicidal test presented in Figure 1 indicate that each treatment group exhibited different levels of effectiveness against *Aedes aegypti* larval mortality over a 6- to 24-hour observation period. In the positive control group (C+), mortality reached 100% within 6 hours of observation, confirming that the test procedure was valid and that the larvae were responsive to the standard larvicide. (19) In contrast, the negative control group (C-) showed no mortality, indicating that neither the medium nor the solvent used exerted any toxic effects. (20,21)

In the soursop leaf (SL) extract groups (SL1–SL5), a consistent pattern of increasing mortality was observed as the concentration increased. The SL5 treatment, which used the highest concentration, showed a mortality rate approaching that of the positive control group by the 24th hour, indicating strong larvicidal potential. Mortality in the SL groups generally began to rise sharply after the 12th hour and peaked at the 24th hour, suggesting that the extract acts gradually through a toxicity mechanism that requires a certain exposure time to affect larval physiology. This finding is consistent with several previous studies on phyto-larvicides in Indonesia, which have shown that the larvicidal effects of natural substances require time to develop and increase with prolonged larval exposure to the substance. (22,23)

The garlic bulb (GB) groups (GB1–GB5) showed lower efficacy compared to the soursop leaf groups at equivalent concentrations. Mortality in GB1 and GB2 remained relatively low even after 24 hours,

whereas GB4 and GB5 showed a significant increase, with mortality rates of 60–90% at the 24-hour mark. These findings indicate that garlic extract possesses larvicidal activity but requires higher concentrations to achieve efficacy comparable to that of soursop leaf extract.

Overall, the cumulative mortality patterns indicate that both extracts exhibit larvicidal activity, although with differing potencies. Soursop leaves demonstrated a stronger and more consistent spectrum of efficacy, whereas garlic exhibited moderate activity that was more dependent on increasing concentrations. The progressive increase in mortality from the 6th to the 24th hour, which corresponded with increasing extract concentrations, further supports the hypothesis that the mechanisms of action of both extracts are time-dependent and dose-dependent. When the exposure duration is brief or the extract concentration remains low, only a small number of active molecules are able to reach and damage key targets in the larvae, resulting in a relatively low mortality rate. However, when the exposure duration is extended or the dose is increased, the number of active molecules interacting with larval membranes, enzymes, and structural components becomes greater, resulting in more severe damage. (24,25) These findings support the use of natural substances as alternative larvicides, with soursop leaves showing the most promising potential for controlling the *Aedes aegypti* vector.

The results of the Mann–Whitney post hoc test indicated significant differences among most treatment groups, both between the control groups and among the concentrations of soursop leaf and garlic bulb extracts. Comparisons between the positive control (C+) and nearly all treatment groups yielded p-values < 0.05, confirming that the extract treatments, particularly at medium to high concentrations, produced significantly different larvicidal effects compared to the positive control. Conversely, comparisons between C+ and SL4, SL5, GB4, and GB5 yielded p-values > 0.05, indicating that the efficacy of both

extracts at high concentrations approached that of the positive control.

The negative control (C-) consistently showed significant differences compared to nearly all treatment groups ( $p < 0.05$ ), indicating that larval mortality in the treatment groups was influenced by extract administration. In the soursop leaf groups (SL1–SL5), the pattern of significance indicated that increasing concentrations correlated with increased efficacy. Lower concentrations (SL1–SL2) still showed significant differences compared to several other groups, whereas medium to high concentrations (SL4–SL5) tended to be statistically indistinguishable from the positive control, indicating strong larvicidal activity.

In the garlic bulb groups (GB1–GB5), a similar pattern was observed. GB1 and GB2 showed significant differences compared to many other groups, indicating relatively low to moderate effects. Meanwhile, GB4 and GB5 showed non-significant p-values when compared to the positive control and high concentrations of soursop leaf extract, indicating that high concentrations of garlic bulb extract were also capable of producing comparable larvicidal effects.

Overall, this analysis indicates that both soursop leaf extract and garlic bulb extract exhibit significant larvicidal activity, with potency increasing as concentration increases. The highest concentrations of both extracts produced effects comparable to the positive control, suggesting their potential for development as alternative phyto-larvicides. Furthermore, the significant differences between groups confirm that each dose increase resulted in a measurable change in *Aedes aegypti* larval mortality, reinforcing the validity of the dose-response relationship observed in this study.

In the soursop leaf extract, the  $LC_{50}$  value remained relatively stable throughout the observation period, indicating consistent toxicity from the 6th to the 24th hour. Meanwhile, the garlic bulb extract showed a marked decrease in  $LC_{50}$  value from 0.686 at the 6th hour to 0.116 at the 24th hour, indicating that its effectiveness

increased over time. However, despite this increase in toxicity, the larvicidal efficacy of garlic bulb remained lower than that of soursop leaves at all observation points.

This difference reinforces the finding that soursop leaves contain active compounds that act more rapidly and possess greater potency, such as acetogenins, flavonoids, and annonaceous compounds, which are known to be toxic to insects. In contrast, sulfur compounds in garlic bulb, such as allicin, exhibit maximum larvicidal effects only after a longer contact period. Thus, from the perspective of natural-based vector control potential, soursop leaves demonstrate superior larvicidal efficacy compared to garlic bulb.

Analysis of the  $LC_{50}$  values at various observation times showed that soursop leaf extract has greater larvicidal potential than garlic bulb extract against *Aedes aegypti* larvae. This is indicated by the  $LC_{50}$  values for soursop leaves, which ranged from 0.102 to 0.137—substantially lower than those of garlic bulb extract, which ranged from 0.116 to 0.686. A lower  $LC_{50}$  value indicates that a lower concentration is required to kill 50% of the larval population, thereby demonstrating greater efficacy.

Soursop leaves are known to contain various secondary metabolites believed to possess larvicidal activity, particularly acetogenins, alkaloids, tannins, flavonoids, and saponins. Among these compounds, acetogenins (such as annonacin, squamocin, and bullatacin) are the dominant components and are primarily responsible for the toxic effects on mosquito larvae. Acetogenins act by inhibiting complex I in the electron transport chain of larval mitochondria, thereby disrupting ATP production. This inhibition causes cellular energy deficiency, ultimately leading to larval death through cellular dysfunction. (26)

In addition, saponins act as natural surfactants that disrupt the integrity of larval cell membranes. Saponins reduce surface tension and cause cell lysis by increasing the permeability of intestinal membranes and the integument. Flavonoids and alkaloids also contribute by inducing oxidative stress and disrupting the larval nerv-

ous system, including through inhibition of the enzyme acetylcholinesterase. Tannins, which possess astringent properties, can bind to proteins on the larval body surface and disrupt respiration by blocking the spiracles. (27)

Synergistically, the combination of these active compounds causes multifactorial disruptions, ranging from membrane damage and metabolic disorders to nervous system dysfunction and internal tissue damage. Therefore, soursop leaf extract demonstrates strong larvicidal potential and is effective in reducing the viability of *Aedes aegypti* larvae, making it a promising candidate for development as a natural-based vector control alternative.

Garlic bulb is also known to contain various bioactive compounds with larvicidal potential, particularly allicin, ajoene, organosulfur compounds (such as diallyl disulfide and diallyl trisulfide), and flavonoids. Among these compounds, allicin is the primary component formed when garlic bulb is crushed or extracted through the action of the enzyme alliinase. Allicin is known to possess toxic properties against insects, including mosquito larvae, through mechanisms that target several key biological processes. (28)

The primary mechanism of action of allicin and other sulfur compounds involves the inhibition of essential enzymes through reactions with thiol groups (-SH) on larval proteins and enzymes, particularly those involved in cellular respiration. This disruption leads to metabolic dysfunction, structural protein damage, and ultimately cellular apoptosis. Additionally, organosulfur compounds can penetrate larval cell membranes, causing membrane destabilization, ion leakage, and damage to integumentary tissue. Allicin is also thought to induce oxidative stress, increase free radical production, and disrupt the larval antioxidant defense system. These conditions lead to mitochondrial damage, reduced ATP production, and cellular dysfunction. (29) Meanwhile, flavonoids in garlic bulb exert neurotoxic effects through inhibition of acetylcholinesterase, thereby disrupting nerve impulse transmission, which ulti-

mately leads to paralysis and larval death. (27)

The combination of toxic properties, tissue-damaging effects, and disruption of cellular metabolism makes garlic bulb extract an effective natural larvicide against *Aedes aegypti*. This potential supports the use of garlic as a natural and environmentally friendly alternative to synthetic chemical insecticides for vector control.

## CONCLUSION

A 96% ethanol extract of soursop leaves and garlic bulb was found to exhibit time-dependent and dose-dependent larvicidal effects against *Aedes aegypti* larvae. A concentration of 0.8% was the most effective, producing results nearly equivalent to those of temephos. However, the efficacy of soursop leaf extract against *Aedes aegypti* larvae was found to be greater than that of garlic bulb extract. The findings of this study indicate that ethanol extracts of soursop leaves and garlic bulb have potential as natural and environmentally friendly larvicides against *Aedes aegypti*. These extracts may serve as alternative vector control agents to reduce reliance on synthetic larvicides such as temephos. Future studies are needed to evaluate their safety, stability, active compounds, and effectiveness under field conditions.

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