Eugenol from Clove Leaves Originating from Ambon Island is Effective in Killing *Aedes albopictus* Larvae

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Abstract

Dengue hemorrhagic fever (DHF) is an infectious disease caused by the dengue virus, with *Aedes albopictus* as one of its vectors. Vector control can be conducted using larvicides; however, the uncontrolled use of chemical larvicides may lead to resistance. Therefore, the development of natural larvicides is a promising alternative. Clove (*Syzygium aromaticum*) leaves contain bioactive compounds such as eugenol, which have demonstrated larvicidal activity against mosquito larvae. This study aimed to evaluate the effectiveness of eugenol extracted from clove leaves originating from Ambon Island on the mortality of *Ae. albopictus* larvae at concentrations of 200 µl, 300 µl, and 400 µl. A bioassay method was employed to assess larvicidal activity, and statistical analyses were conducted to determine differences in mortality among the concentrations. The results showed that larval mortality reached 100% at 200 µl within 7 hours, 100% at 300 µl within 6 hours, and 100% at 400 µl within 4 hours. The results of the statistical test using *Kruskal Wallis* showed a P value 0.005. These findings indicate that higher eugenol concentrations lead to faster larval mortality. In conclusion, eugenol from clove leaves has potential as an active ingredient in natural larvicides for the control of *Ae. albopictus*.

I. Introduction

Dengue Hemorrhagic Fever (DHF) is an infectious disease characterized by acute fever and caused by the dengue virus (Akollo, 2023; Padonou et al., 2023). This disease remains a major public health problem worldwide, including in Indonesia (Akollo, 2019). The dengue virus is transmitted to humans through mosquito bites (Akollo, 2019; Akollo et al., 2020). The mosquito species that act as vectors of DHF are *Aedes aegypti* and *Ae. albopictus* (Akollo, 2022; Herath et al., 2024; Watmanlussy et al., 2024).

One of the strategies for controlling dengue fever is vector management through the use of larvicides (Matiadis et al., 2021; Mohd-Nawi et al., 2022; Wira Utami & Roskiana Ahmad, 2016). Larvicides are applied to eliminate *Ae. aegypti* and *Ae. albopictus*larvae, thereby reducing the risk of dengue transmission (Akollo et al., 2023; Palomino et al., 2022). However, continuous and extensive use of larvicides has led to the development of resistance in *Ae. aegypti* and *Ae. albopictus* populations (Davila-Barboza et al., 2024; Dwicahya et al., 2023), making the larvae more difficult to eliminate and consequently hindering effective dengue control (Boyer et al., 2022; Dwicahya et al., 2023).

Larvae of *Ae. aegypti* and *Ae. albopictus* have been reported to develop resistance to synthetic larvicides in several Indonesian islands, including Java, Sumatra, Kalimantan, Sulawesi, and Papua (Dwicahya et al., 2023; Nurmayanti et al., 2020). The emergence of resistant dengue vectors poses a major challenge to effective dengue control. Therefore, the development of natural larvicides is urgently required (Boyer et al., 2022). One plant part with promising potential as a natural larvicide is clove (*Syzygium aromaticum*) leaves (Akollo et al., 2024).

Clove (*Syzygium aromaticum* L.) leaves contain several bioactive compounds with larvicidal properties, one of which is eugenol (Budiman et al., 2022; Ouadi et al., 2022). Eugenol affects the nervous system, leading to mortality in insects (Akollo et al., 2024). The neurotoxic properties of eugenol cause exposed larvae to become immobile and eventually die (Adhikari et al., 2022). In addition, eugenol also induces larval death by disrupting the digestive system (Akollo et al., 2024).

Ambon Island is well known for its spice production, particularly clove. Clove leaves are often regarded as waste by local communities and remain underutilized, despite their potential to be developed as a natural larvicide (Akollo et al., 2024; Lameky et al., 2023). Several previous studies have reported the effectiveness of eugenol in inducing mosquito larval mortality. Research conducted in India demonstrated that eugenol extracted from lemongrass was effective in killing *Ae. aegypti* larvae (Adhikari et al., 2022). Similarly, a study in Iran showed that eugenol derived from clove flowers exhibited larvicidal activity against *Anopheles stephensi* (Osanloo et al., 2018). However, no studies have yet investigated the larvicidal efficacy of eugenol extracted from clove leaves originating from Ambon Island against *Ae. aegypti* and *Ae. albopictus* larvae. This knowledge gap forms the main focus of the present study.

The research question addressed in this study is whether eugenol extracted from clove (*Syzygium aromaticum*) leaves originating from Ambon Island is effective as a natural larvicidal agent against *Ae. aegypti* and *Ae. albopictus*larvae. The objective of this study is to evaluate the larvicidal efficacy of eugenol from Ambon clove leaves against *Ae. albopictus* larvae at concentrations of 200 µL, 300 µL, and 400 µL.

II. Research Method

This study was an experimental research employing a randomized control group design. The research was conducted at three different locations. Essential oil distillation was carried out at PT. Ghema Berkat Abadi. Eugenol isolation was performed at the Organic Chemistry Laboratory, Pattimura University (UNPATTI) and the larvicidal bioassay of eugenol against *Ae. albopictus* larvae was conducted at the Epidemiology Laboratory, Faculty of Medicine, Maluku Indonesia Christian University, Indonesia. This research has passed the ethical test from the Maluku Husada Health College (No: RK. 198/KEPK/STIK/VII/2025).

Eugenol in this study was obtained from clove leaves originating from Ambon Island. The dried clove leaves were collected, sorted, and subjected to a distillation process to produce essential oil (Akollo et al., 2024). The essential oil was then isolated to obtain eugenol through the following procedure. A total of 75 mL of essential oil was mixed with a sodium hydroxide solution and stirred using a magnetic stirrer. The mixture was transferred into a separatory funnel and extracted. The extraction yielded two layers: an aqueous layer containing sodium eugenolate and an oil layer containing caryophyllene. The aqueous sodium eugenolate layer was then acidified with 25% HCl to pH 3, transferred into a separatory funnel, and extracted. The aqueous layer was mixed with 25 mL of ether and re-extracted to separate eugenol from water. The eugenol was washed three times with distilled water, dried over 1 gram of anhydrous Na_2SO_4, and filtered using filter paper. The eugenol was gently heated to evaporate residual ether. The resulting eugenol was subsequently used to kill larvae of Ae. albopictus.

The mosquito population was randomly divided into six groups. The first three treatment groups were exposed to eugenol from clove leaves at concentrations of 200 µL, 300 µL, and 400 µL, respectively, while the remaining three control groups received no treatment and were only provided with distilled water. A total of 810 Ae. albopictuslarvae were used for each concentration, consisting of 648 test larvae and 162 control larvae. All larvae used were in the third instar stage (Adhikari et al., 2022; Akollo et al., 2024).

The larvicidal bioassay of eugenol was conducted as follows (Akollo, et al., 2024; Centers for Disease Control and Prevention, 2010). Five plastic cups were prepared with water, four of which were treated with eugenol to a final volume of 200 mL, while the fifth cup served as a negative control. 18 Ae. albopictus larvae were introduced into each cup. Each test was repeated three times, resulting in 270 larvae per concentration. larval mortality was observed and recorded.

Larvae that did not move, even after being touched, were considered dead. If mortality in the control group was below 10%, the corrected mortality in the test groups was calculated using Abbott's formula. The larvicidal efficacy of eugenol against *Ae. albopictus* larvae was analyzed using one-way ANOVA. If the data were not normally distributed, the Kruskal–Wallis test was used as an alternative (Lameky & Akollo, 2024). The mortality of *control* larvae occurred below 10%, the mortality of the larva trated groups will be calculated following Abbott's formula 1 (Adhikari et al., 2022).

$$\text{Mortality (\%)} = \frac{(C - T)}{C} \times 100\% \qquad (1)$$

Where C = percentage of larvae survived in the control group, T = percentage of larvae survived in the treated group.

III. Result and Discussion

The isolation of eugenol from 75 ml of essential oil originating from Ambon Island yielded 25 ml of eugenol. The isolated eugenol can be seen in Figure 1.

Figure 1. Eugenol

The results of the study indicated that *Ae. albopictus* larval mortality reached 100% within 7 hours at a eugenol concentration of 200 µL. At 300 µL, 100% mortality was achieved within 6 hours, while at 400 µL, complete larval mortality occurred within 4 hours. These findings indicate that eugenol at 400 µL exhibits higher larvicidal activity compared to 200 µL and 300 µL concentrations.

The Kruskal–Wallis test assessing differences in larvicidal efficacy among the 200 µL, 300 µL, and 400 µL concentrations yielded a p-value of 0.005, indicating significant differences in larval mortality among the concentrations. These results demonstrate that increasing the eugenol concentration leads to higher mortality rates of *Ae. albopictus* larvae. The detailed results are presented in Table 1.

Table 1. Differences in the time of death of *Ae. albopictus* larvae at concentrations of 200 µl, 300 µl, and 400 µl

Eugenol concentration	Repetition	Number of larvae	Time of Larval Death									Number of larval deaths	Percentage of larval mortality	Statistical test results
			1 h	2 h	3 h	4 h	5 h	6 h	7 h	8 h	24 h			
200 µl	3	216	97	189	201	201	207	210	216	216	216	216	100	0,005
Control -	3	54	0	0	0	0	0	0	0	0	0	0	0	
300 µl	3	216	87	183	198	207	210	216	216	216	216	216	100	
Control -	3	54	0	0	0	0	0	0	0	0	0	0	0	
400 µl	3	216	87	180	186	216	216	216	216	216	216	216	100	
Control -	3	54	0	0	0	0	0	0	0	0	0	0	0	

Several factors influence the efficacy or lethality of natural insecticides, encompassing both internal and external factors. Internal factors include age, cuticle thickness, and insect species (Anissa & Lamdo, 2025). External factors include the concentration of the compound, frequency and duration of application, application method (Akram et al., 2025), and environmental conditions such as temperature, humidity, and weather (Akram et al., 2025; Anissa & Lamdo, 2025).

The primary factor affecting the lethality of natural insecticides is the chemical concentration of the compound (Akollo et al., 2024; Akram et al., 2025). In this study, the larvicidal efficacy of eugenol was influenced by the concentrations applied. The results demonstrated that higher eugenol concentrations corresponded with increased mortality, whereas lower concentrations resulted in reduced lethality. Increased eugenol concentrations enhance its accumulation within larval bodies, thereby elevating larval mortality (Adhikari et al., 2022; Akollo et al., 2024). Adhikari et al., (2022) reported a direct correlation between higher eugenol concentrations and increased mortality in *Ae. aegypti* larvae. Similarly, a study in Iran showed that eugenol derived from clove flowers exhibited larvicidal activity against *An. stephensi* (Osanloo et al., 2018).

Other factors potentially affecting eugenol lethality include environmental conditions and larval cuticle thickness. Environmental factors, such as temperature, can influence the stability and activity of active compounds in natural insecticides. Elevated temperatures may increase the volatility of active compounds, thereby reducing insecticidal efficacy (Akram et al., 2025). Additionally, cuticle thickness affects the susceptibility of mosquito larvae to natural insecticidal compounds. The sensitivity of insects, including mosquito larvae, to these compounds is determined by the ability of chemicals to penetrate the cuticular barrier and reach the internal body (Anissa & Lamdo, 2025).

The results of this study indicate that eugenol exhibits high larvicidal activity against *Ae. albopictus*larvae. All eugenol concentrations tested achieved 100% larval mortality, although the time required to reach this mortality varied. Achieving complete mortality within a relatively short period indicates that eugenol acts rapidly in inducing larval death.

Eugenol exerts its lethal effect on mosquito larvae by disrupting the digestive and nervous systems (Akollo et al., 2024; Kasma et al., 2019; Sapulette et al., 2019). This compound can cause neurological disruption that ultimately leads to insect death (Akollo et al., 2024; Sapulette et al., 2019). The neurotoxic properties of eugenol result in immobilization and eventual mortality of the larvae (Adhikari et al., 2022).

IV. Conclusions

Eugenol derived from clove leaves from Ambon Island is effective in killing *Ae. albopictus*larvae. Larval mortality reached 100%, although the time to achieve this effect varied depending on the concentration. Higher concentrations of eugenol resulted in greater larval mortality. Eugenol from clove leaves has the potential to be developed as the active ingredient in natural larvicides, serving as an alternative to synthetic insecticides, which often contribute to resistance development and negative environmental impacts.

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References

Adhikari, K., Khanikor, B., & Sarma, R. (2022a). Persistent susceptibility of Aedes aegypti to eugenol. *Scientific Reports*, *12*(1). https://doi.org/10.1038/s41598-022-06302-8

Adhikari, K., Khanikor, B., & Sarma, R. (2022b). Persistent susceptibility of Aedes aegypti to eugenol. *Scientific Reports*, *12*(1). https://doi.org/10.1038/s41598-022-06302-8

Akollo, I. R. (2019). *Deteksi Mutasi Gen Ace-1 sebagai Penyandi Resistensi Insektisida Maltion pada Nyamuk Aedes aegypti di Kota Ambon* [FKKMK, UGM]. https://etd.repository.ugm.ac.id/penelitian/detail/183103

Akollo, I. R. (2022). Pelatihan Pembuatan dan Pengaplikasian Ovitrap Sebagai Upaya Pengendalian Penyakit DBD di Kota Ambon. *Karya Kesehatan Siwalima*, *1*. https://ojs.ukim.ac.id/index.php/KKS

Akollo, I. R. (2023). *Ilmu Dasar Keperawatan: Virologi dan Bakteriologi* (1st ed.). Rajawali Pers. https://www.rajagrafindo.co.id/produk/ilmu-dasar-keperawatan-virologi-dan-bakteriologi-isak-roberth-akollo-s-kep-m-sc/

Akollo, I. R., Salenussa, P. B., Leivitar, E. O., Lameky, V. Y., Ivakdaklam, L. M., & Pattipeluhu, L. (2024). Minyak Atsiri Daun Cengkeh Asal Pulau Ambon Sebagai Larvasida Alami untuk Nyamuk Aedes aegypti. *Jurnal Penelitian Kesehatan "SUARA FORIKES" (Journal of Health Research "Forikes Voice")*. https://doi.org/10.33846/sf15226

Akollo, I. R., Salenussa, P. B., Leivitar, E. O., Lameky, V. Y., Ivakdalam, L. M., & Pattipeluhu, L. (2024). Minyak Atsiri Daun Cengkeh Asal Pulau Ambon Sebagai Larvasida Alami untuk Nyamuk Aedes aegypti. *Jurnal Penelitian Kesehatan Suara Forikes*, *15*, 296–299. https://doi.org/10.33846/sf15226

Akram, A. H., Sudarmawan, A. A. K., & Sudantha, I. M. (2025). Effectiveness of Various Botanical Insecticides for Controlling Liriomyza sp. Pests. *Jurnal Biologi Tropis*, *24*(2b), 397–405. https://doi.org/10.29303/jbt.v24i2b.8161

Anissa, N., & Lamdo, H. (2025). Planta Simbiosa Test of the Vegetable Insecticide of Gadung Tuber and Babadotan Against Brown Planthoppers) on Rice Plants (Oryza sativa L.). *Planta Simbiosa*, *7*(1), 65–70. https://doi.org/10.25181/jplantasimbiosa.vXiX.XXXX

Boyer, S., Maquart, P. O., Chhuoy, K., Suor, K., Chhum, M., Heng, K., Leng, S., Fontenille, D., & Marcombe, S. (2022). Monitoring insecticide resistance of adult and larval Aedes aegypti (Diptera: Culicidae) in Phnom Penh, Cambodia. *Parasites and Vectors*, *15*(1). https://doi.org/10.1186/s13071-022-05156-3

Centers for Disease Control and Prevention. (2010). *Guideline for Evaluating Insecticide Resistance in Vectors Using the CDC Bottle Bioassay*. 1–28.

Davila-Barboza, J. A., Gutierrez-Rodriguez, S. M., Juache-Villagrana, A. E., Lopez-Monroy, B., & Flores, A. E. (2024). Widespread Resistance to Temephos in Aedes aegypti (Diptera: Culicidae) from Mexico. *Insects*, *15*(2). https://doi.org/10.3390/insects15020120

Dwicahya, B., Arsunan Arsin, A., Ishak, H., Hamid, F., & Mallongi, A. (2023). Aedes Sp. Mosquito Resistance and the Effectiveness of Biolarvicides on Dengue Vector Mortality. *Pharmacognosy Journal*, *15*(4), 541–546. https://doi.org/10.5530/pj.2023.15.116

Herath, J. M. M. K., De Silva, W. A. P. P., Weeraratne, T. C., & Karunaratne, S. H. P. P. (2024). Breeding Habitat Preference of the Dengue Vector Mosquitoes Aedes aegypti and Aedes albopictus from Urban, Semiurban, and Rural Areas in Kurunegala District, Sri Lanka. *Journal of Tropical Medicine*, *2024*. https://doi.org/10.1155/2024/4123543

Kasma, A. Y., Muftiah, A. T., & M, R. (2019). Efektivitas Ekstrak Daun Pandan Wangi (Pandanus amaryllifolius) Terhadap Mortalitas Larva Aedes sp dan Anopheles. *Jurnal Vektor Penyakit*, *13*(2), 107–114. https://doi.org/10.22435/vektorp.v13i2.465

Lameky, V. Y., & Akollo, I. R. (2024). *Buku Ajar Biostatisik* (1st ed.). PT. Ghema Berkat Abadi. https://www.ghemmaberkatabadi.com/2024/02/buku-ajar-pengantar-biostatistik.html

Lameky, V. Y., Siahaya, G. C., Tandi, D., Maitimu, A. N., & Akollo, I. R. (2023). Minyak Atsiri Daun Cengkeh (Eugenia Caryophyllus)Terbukti Menurunkan Intensitas Nyeri Gigi. *Jurnal Penelitian Kesehatan Suara Forikes*, *14*. https://www.forikes-ejournal.com/index.php/SF/article/view/sf14nk105

Matiadis, D., Liggri, P. G. V., Kritsi, E., Tzioumaki, N., Zoumpoulakis, P., Papachristos, D. P., Balatsos, G., Sagnou, M., & Michaelakis, A. (2021). Curcumin derivatives as potential mosquito larvicidal agents against two mosquito vectors, culex pipiens and aedes albopictus. *International Journal of Molecular Sciences*, *22*(16). https://doi.org/10.3390/ijms22168915

Mohd-Nawi, F., Dom, N. C., Ramli, S., Rambely, A. S., Haron, N., Emida, M., & Camalxaman, S. N. (2022). Larvicidal Potential of Essential

Oils Extracted from Syzygium aromaticum Against Aedes albopictus in Malaysia: A Preliminary Study. *Malaysian Journal of Medicine and Health Sciences*, *18*, 9–13. https://doi.org/10.47836/mjmhs18.s15.2

Osanloo, M., Sedaghat, M. M., Esmaeili, F., & Amani, A. (2018). Larvicidal Activity of Essential Oil of Syzygium aromaticum (Clove) in Comparison with Its Major Constituent, Eugenol, against Anopheles stephensi. *J Arthropod-Borne Dis*, *12*(4), 361. https://jad.tums.ac.ir/index.php/jad/article/view/1019

Ouadi, S., Sierro, N., Goepfert, S., Bovet, L., Glauser, G., Vallat, A., Peitsch, M. C., Kessler, F., & Ivanov, N. V. (2022). The clove (Syzygium aromaticum) genome provides insights into the eugenol biosynthesis pathway. *Communications Biology*, *5*(1). https://doi.org/10.1038/s42003-022-03618-z

Palomino, M., Pinto, J., Yañez, P., Cornelio, A., Dias, L., Amorim, Q., Martins, A. J., Lenhart, A., & Lima, J. B. P. (2022). First national-scale evaluation of temephos resistance in Aedes aegypti in Peru. *Parasites and Vectors*, *15*(1). https://doi.org/10.1186/s13071-022-05310-x

Sapulette, F. V, A Unitly, A. J., Moniharapon, D. D., Violenta Sapulette, F., Jems Akiles Unitly, A., & Moniharapon, D. D. (2019). Aktivitas Larvasida Seduhan Daun Cengkeh(Syzygium aromaticum L.) terhadap Mortalitas Larva Nyamuk Anopheles sp. *RUMPHIUS PATTIMURA BIOLOGICAL JOURNAL*, *1*(2), 5.

Watmanlussy, G. J., Akollo, I. R., Talarima, B., Lameky, F., & Pattipeluhu, L. (2024). Preferensi Tempat Perindukan Nyamuk Aedes spp. Pada Jenis dan Letak Kontainer di Wilayah Kerja Puskesmas Lateri, Kota Ambon. *Journal of Medical Laboratory in Infectious and Degenerative Diseases*, *1*(2). https://jmid.uds.ac.id/jmlid/article/view/16/14

Wira Utami, W., & Roskiana Ahmad, A. (2016). Uji Aktivitas Larvasida Ekstrak Daun Jarak Kepyar (Ricinus communis L.) Terhadap Larva Nyamuk Aedes aegypti. *Jurnal Fitofarmaka Indonesia*, *3*(1).