INTERNATIONAL JOURNAL OF MULTIDISCIPLINARY: APPLIED BUSINESS AND EDUCATION RESEARCH
2024, Vol. 5, No. 9, 3609 – 3614
http://dx.doi.org/10.11594/ijmaber.05.09.20

Research Article

Larvicidal Activity of *Zingiber purpureum* Roscoe Essential Oil from East Kalimantan in Preventing Dengue Hemorrhagic Fever

Efficacy Test of *Zingiber purpureum* Roscoe Essential Oil as a Natural Larvicide

Lirana Imute Syahdumawarta[1], Vera Madonna Lumban Toruan[2], Swandari Paramita[3]*, Mona Zubaidah[4]

[1]Faculty of Medicine Mulawarman University Samarinda, 75123, Indonesia
[2]Laboratory of Dermatology-Venereology Faculty of Medicine Mulawarman University Samarinda, 75123, Indonesia
[3]Laboratory of Public Health Faculty of Medicine Mulawarman University Samarinda, 75123, Indonesia
[4]Laboratory of Parasitology Faculty of Medicine Mulawarman University Samarinda, 75123, Indonesia

Article history:
Submission 12 June 2024
Revised 07 September 2024
Accepted 23 September 2024

**Corresponding author:*
E-mail:
s.paramita@fk.unmul.ac.id

ABSTRACT

Temephos is extensively employed currently to eliminate *Aedes aegypti* mosquito larvae, thus preventing the spread of dengue fever. Raising the level of temephos application can lead to health issues for individuals and long-term detrimental impacts on the environment. This research aims to demonstrate that *Z. purpureum* Rosc. essential oil effective in eliminating more than 70% of *Aedes aegypti* mosquito larvae. *Z. purpureum* Rosc.essential oil was diluted with Tween 80 to achieve various concentrations, namely 12.5 µg/mL, 25 µg/mL, 50 µg/mL, and 100 µg/mL. A positive control was established using temephos at a concentration of 0.012 mg/L, while a negative control was prepared using clean water. The larvae were subjected to these treatments for a duration of 48 hours, with three repetitions conducted. The data were analyzed using the *Kruskal Wallis Test* that shows a significant difference between treatment groups, with a p value <0.05. A concentration of 12.5 µg/mL of *Z. purpureum* Rosc. essential oil was found to effectively eliminate 76.67% of *Aedes aegypti* mosquito larvae. A concentration of 25 µg/mL of *Z. purpureum* Rosc. essential oil was found to effectively eliminate 86.67% of *Aedes aegypti* mosquito larvae. A concentration of 50 µg/mL of *Z. purpureum* Rosc. essential oil was found to effectively eliminate 96.67% of *Aedes aegypti* mosquito larvae. A concentration of 100 µg/mL of *Z. purpureum* Rosc. essential oil was found to effectively eliminate 100% of *Aedes aegypti* mosquito larvae. The overall findings show that more

How to cite:
Syahdumawarta, L. I., Toruan, V. M. L., Paramita, S., & Zubaidah, M. (2024). Larvicidal Activity of *Zingiber purpureum* Roscoe Essential Oil from East Kalimantan in Preventing Dengue Hemorrhagic Fever. *International Journal of Multidisciplinary: Applied Business and Education Research*. *5*(9), 3609 – 3614. doi: 10.11594/ijmaber.05.09.20

than 70% of *Aedes aegypti* mosquito larvae are effectively killed by *Z. purpureum* Rosc. essential oil at a concentration of 12.5 µg/mL.

Keywords: *Aedes aegypti, Dengue Hemorraghic Fever, Essential Oil, Zingiber purpureum Roscoe*

Introduction

Dengue hemorrhagic fever is a disease characterized by fever and bleeding caused by the dengue virus and transmitted to humans through the bite of the *Aedes aegypti* mosquito (Schaefer, 2022). Temephos is a synthetic larvicide in powder form used to kill mosquito larvae (Dinkes, 2017). Organophosphates contained in temephos work by binding and damaging the cholinesterase enzyme, resulting in the accumulation of acetylcholine in the larvae's nervous tissue, leading to continuous muscle contractions, convulsions, and eventual death of the larvae (Suparyati, 2020). Reports of *Aedes aegypti* larvae resistance to temephos have been found in several countries, including Indonesia (Istiana, Farida, & Isnaini, 2012). The use of temephos at increased doses can result in human poisoning, presenting symptoms such as dizziness, nausea, and other nervous disorders (Suparyati, 2020; Yang et al., 2020). Additionally, the low biodegradability of this synthetic larvicide can have long-term adverse effects on the environment (Suparyati, 2020; Yang et al., 2020).

Utilization of alternatives in the form of natural larvicides derived from plants can help address these problems (Pratiwi, 2013). One such ingredient is the essential oil content found in *Zingiber purpureum* Rosc. rhizomes (Artini, 2013). *Zingiber purpureum* Roscoe was chosen because its essential oil contains dichloromethane with ovicidal effects, as well as alkaloids, flavonoids, saponins, tannins, and triterpenoids, all of which exhibit larvicidal activity against *Aedes aegypti* mosquitoes (Devkota et al., 2021; Minarni et al., 2013).

Saponins damage the gastrointestinal wall and destroy the protective wax layer of the larvae (Dewi, Wahyuni, & Suratno, 2014). Tannins can degrade proteins necessary for larval survival (Prakoso, Aulung, & Citrawati, 2017). Flavonoids can disrupt nerve and muscle function in the respiratory system (Paju et al., 2013). Alkaloids and triterpenoids act as stomach poisons with antifeedant effects, and alkaloids can also inhibit the cholinesterase enzyme in larvae (Idris, 2015).

Methods

Plant Collection

The rhizomes of *Zingiber purpureum* Roscoe were collected from the Faculty of Agriculture, Mulawarman University.

Mosquito Larvae

Aedes aegypti mosquito larvae are obtained from the process of hatching *Aedes aegypti* mosquito eggs using thinwall containing clean water. *Aedes aegypti* mosquito eggs were obtained from Bionas Research Laboratory in Gresik, East Java. The stages of mosquito egg hatching were carried out until all the eggs were found to turn into *Aedes aegypti* larvae with the inclusion criteria being active third instar larvae. The number of larvae that will be tested in each treatment and repetition group is ten larvae.

Time and Place

Research was carried out at the Field Laboratory of the Faculty of Agriculture, Mulawarman University for refining *Z. purpureum* Rosc. rhizome essential oil, the Parasitology Laboratory of the Faculty of Medicine, Mulawarman University for collecting *Aedes aegypti* larvae and testing the effectiveness of natural larvicides. Essential oil dilution was carried out at the Faculty of Forestry, Mulawarman University and determining temephos levels in the Pharmacology Laboratory Mulawarman University Faculty of Medicine. The natural larvicide test was carried out at the Parasitology Laboratory, Faculty of Medicine, Mulawarman University on 13 November – 8 December 2023.

Zingiber purpureum Roscoe Essential Oil

Zingiber purpureum Roscoe rhizomes were obtained from the Faculty of Agriculture, Mulawarman University. The rhizomes were collected and thoroughly washed. They were then sliced into 3-5 mm thick pieces and placed into a still. The distillation method employed was a combination of steam and water distillation. Following the distillation process, a distillate containing both essential oil and water was obtained, which was then separated using a separating funnel. After ensuring complete separation of the water, the essential oil was stored in vials coated with aluminum foil and kept at 4°C (Pratiwi, 2013; Dewi, Wahyuni, & Suratno, 2014).

Dilution of Essential Oils

Zingiber purpureum Roscoe rhizome essential oil concentrate is made by diluting the essential oil using Tween 80 solution until four concentrations are obtained which are placed in different test tubes. Each reaction tube is labeled with a concentration of 12.5 µg/mL, 25 µg/mL, 50 µg/mL, and 100 µg/mL (Putri, 2015).

Larvicidal Test

Thin walls containing ten *Aedes aegypti* larvae were marked on the outside to differentiate treatment groups with *Zingiber purpureum* Rosc. rhizome essential oil concentrations of 12.5 µg/mL, 25 µg/mL, 50 µg/mL, and 100 µg/mL, positive control, and negative control. Each group was given ten active third instar larvae, marked with repetition numbers I, II, and III. The positive control group received temephos at 0.012 mg/L of water, while clean water was used for the negative control. Larvicidal testing was carried out by applying diluted essential oil to each thin wall according to the markings. Testing was conducted within 48 hours with three repetitions (Schaefer et al., 2022). The assessment involved counting the number of dead larvae in each treatment group and repetition. A natural larvicide is considered effective if more than 70% of the larvae die. Characteristics of dead larvae include no movement after being stimulated by touch or movement in the water (Dinkes NTB, 2017).

Statistical Analysis

This research is experimental research. The research design used Post-Test Only Control Group Design, where this study only assessed the test results at the end after the research subjects were given treatment. The first statistical data test carried out was *Shapirowilk* to test the normality of the data. Continued with *one-way ANOVA* test if the previous test shows the data is normally distributed. The *one-way ANOVA* test is conducted to determine whether there is a difference between the test group and the control group based on the data obtained. The difference is said to be significant if the p value <0.05. If the *Shapirowilk* test shows the data is not normally distributed, it can be continued with the *Kruskal Wallis* test.

Result and Discussion

Larvicidal Test

Efficacy test of *Zingiber purpureum* Roscoe essential oil as a natural larvicide used active third instar *Aedes aegypti* mosquito larvae with a total of ten larvae in each treatment and repetition group. *Aedes aegypti* mosquito larvae are obtained from the process of hatching *Aedes aegypti* eggs for 2-3 weeks. This study had six types of treatment given, namely four concentrations of *Z. purpureum* Rosc. rhizomes essential oil (12.5 µg/mL, 25 µg/mL, 50 µg/mL, and 100 µg/mL), positive control with temephos (0.012 mg/L), and negative control with clean water. Each treatment was repeated three times. Evaluation of the effectiveness of larvicide was carried out by counting the number of larvae that died after being treated for 48 hours.

Table 1. Differences results of the larvicidal activity of Zingiber purpureum Roscoe essential oil on the death of Aedes aegypti mosquito larvae

Type of Treatment	Concentration	Number of Dead Larvae			Average of Dead Larvae
		Repetition			
		I	II	III	
Zingiber purpureum Roscoe essential oil	12,5 µg/mL	5	9	9	76,67%
	25 µg/mL	8	8	10	86,67%
	50 µg/mL	9	10	10	96,67%
	100 µg/mL	10	10	10	100%
Positive control (temephos)	0,012 mg/L	10	10	10	100%
Negative control (clean water)	-	0	0	0	0%

Table 1 shows the number of larvae that died after exposure to *Z. purpureum* Rosc. essential oil, starting from the lowest concentration of 12.5 µg/mL to the highest concentration of 100 µg/mL. The table also compares the positive and negative control groups. Dead larvae were observed in all repetitions at a concentration of 12.5 µg/mL. The highest mortality was observed at a concentration of 100 µg/mL and in the positive control, while no dead larvae were found in the negative control group. Based on standard methods for testing insecticide efficacy, natural larvicide from *Z. purpureum* Rosc. essential oil at concentrations of 12.5 µg/mL, 25 µg/mL, 50 µg/mL, and 100 µg/mL is classified as effective if it kills more than 70% of *Aedes aegypti* larvae (Schaefer et al., 2022).

From the research data that has been obtained, a *Shapirowilk* normality test is carried out to determine whether the data is normally distributed or not. The normality test results show that a p value <0.05, which means that the data is not normally distributed. Furthermore *Levene* homogeneity test was carried out to find out whether the data was homogeneous or not. The results of the homogeneity test show that the data is not homogeneous with a p value <0.05. Therefore the data were not normally distributed and not homogeneous, a non-parametric Kruskal Wallis test was carried out which show that there were significant differences between treatment groups, with a p value <0.05.

In research, Devkota (2021) explain that in the essential oil of bangle *(Zingiber montanum)* rhizomes there are dichloromethane compounds with ovicidal effects as well as alkaloids, flavonoids, saponins, tannins and triterpenoids with larvicidal effects against the *Aedes aegypti* mosquito. In research, Artini (2013) stated that these ingredients have a vegetable larvicide effect which can kill *Aedes aegypti* larvae. This opinion is also supported by Rahardjo (2004) who explain that bangle (*Zingiber purpureum* Roxb.) can be used as a larvicide.

Saponins have foaming properties, can dissolve in polar solvents, and possess various pharmacological effects (Abdelrahman & Jogaiah, 2020). They irritate the larva's digestive tract, causing appetite suppression and death. Tannins disrupt growth and reduce appetite by inhibiting digestive enzyme activity (Tandi, 2010). Flavonoids act as respiratory toxins (Izzah et al., 2019), while alkaloids inhibit acetylcholinesterase in larvae (Funayama & Cordell, 2014). Triterpenoids possess antifeedant properties, leading to starvation and death (Armyandi et al., 2022)..

Tannins have toxic properties that result in stunted growth and reduce insect appetite by inhibiting the activity of digestive enzymes (Schaefer et al., 2022; Dinkes NTB, 2017). Tannins also decrease the activity of protease enzymes responsible for converting amino acids (Suparyati, 2020). This disruption in cellular metabolic processes leads to nutritional deficiencies in larvae (Suparyati, 2020). Furthermore, tannins bind to proteins in the digestive system that are necessary for larval growth (Suparyati, 2020). Continuous exposure can result in the death of *Aedes aegypti* larvae (Suparyati, 2020).

Flavonoids act as respiratory toxins, leading to larval death (Schaefer et al., 2022; Dinkes NTB, 2017). They enter the mosquito's respiratory tract, weakening the nerves and respiratory muscles, which impairs the mosquito's ability to breathe, ultimately causing death (Istiana et al., 2012). Meanwhile, alkaloid compounds relax the striated muscles of larvae, act as stomach poisons, and inhibit the action of acetylcholinesterase in larvae (Yang et al., 2020; Pratiwi, 2013).

Triterpenoids possess antifeedant properties, causing larvae to die due to inadequate nutritional intake (Devkota et al., 2021; Minarni et al., 2013). At certain levels, triterpenoids can also induce stomach poisoning; when these compounds enter the larvae's body, they disrupt digestive processes, leading to a failure to recognize food and eventual starvation (Devkota et al., 2021; Minarni et al., 2013).

Based on the results of this research, *Zingiber purpureum* Rosc. rhizome essential oil at the lowest concentration of 12.5 µg/mL can effectively kill *Aedes aegypti* mosquito larvae, with an average mortality rate of 76.67%. Observations indicated that several dead larvae showed significant body damage. Minarni et al. (2013) noted that this damage occurs due to saponins in *Z. purpureum*, which can disrupt the protective wax layer of the larvae. In contrast, larvae treated with the positive control (0.012 mg/L temephos) exhibited intact bodies post-mortem.

Conclusion

Based on the results of the research conducted, it can be concluded that *Zingiber purpureum* Roscoe rhizome essential oil with a concentration 12.5 µg/mL is effective in killing more than 70% of *Aedes aegypti* mosquito larvae.

Acknowledgement

This research received funding from Faculty of Medicine, Mulawarman University in 2023.

References

Abdelrahman, M., & Jogaiah, S. (2020). Bioactive molecules in plant defense. In Bioactive molecules in plant defense. Springer International Publishing.

Armyandi, A., Wahyuni, D., & Kamalia, F. (2022). Toksisitas ekstrak terpurifikasi dengan n-heksan buah kecubung (Datura metel L.) terhadap mortalitas larva nyamuk Aedes aegypti. Saintifika, 24(1), 55-67.

Artini, P. E. U. D. (2013). Uji fitokimia etil asetat rimpang bangle. Jurnal Kefarmasian, Udayana University.

Baharun, K., Rukmi, I., Lunggani, A. T., & Fachriyah, E. (2013). Rimpang temu hitam (Curcuma aeruginosa Roxb.) terhadap Bacillus subtilis dan Staphylococcus aureus secara in vitro. Jurnal Biologi, 2(24), 16-24.

Devkota, H. P., Paudel, K. R., Hassan, M. M., Dirar, A. I., Das, N., Devkota, A. A., ... & Dua, K. (2021). Bioactive compounds from Zingiber montanum and their pharmacological activities with focus on zerumbone. Applied Sciences, 11(21).

Dewi, D. P., Wahyuni, & Suratno. (2014). Toksisitas granula ekstrak biji alpukat (Persea americana Mill) terhadap mortalitas larva nyamuk Aedes aegypti. Artikel Ilmiah, 1-5.

Dinkes NTB. (2017). Obat pembunuh jentik nyamuk (Abate). Datin Litbangkes. https://dinkes.ntbprov.go.id/artikel/obat-pembunuh-jentik-nyamuk-abate/

Directorate of Fertilizer and Pesticide. (2012). Metode standar pengujian efikasi insektisida. Ministry of Agriculture.

Du, J. R., Long, F. Y., & Chen, C. (2014). Research progress on natural triterpenoid saponins in the chemoprevention and chemotherapy of cancer. Enzymes, 36, 95–130.

Funayama, S., & Cordell, G. A. (2014). Alkaloids: A treasury of poisons and medicines. Alkaloids: A Treasury of Poisons and Medicines, 1–284.

Huong, L. T. (2020). Chemical composition and larvicidal activity of essential oils from Zingiber montanum (J. Koenig) Link ex. Dietr. against three mosquito vectors. Boletin Latinoamericano y Del Caribe de Plantas Medicinales y Aromáticas, 57, 1-10.

Idris, H. (2015). Tanaman kecubung (Datura metel L.) sebagai bahan baku insektisida botanis untuk mengendalikan hama Aspidomorpha miliaris F. Jurnal Littri, 21(1), 41-46.

Indrawati, E. T., Arung, E. T., & Irawan, W. K. (2011). Analisis fitokimia dari beberapa tumbuhan hutan dari Kebun Raya Balikpapan Provinsi Kalimantan Timur. Prosiding Seminar Nasional, 290-293.

Istiana, H., Farida, H., & Isnaini. (2012). Resistance status of Aedes aegypti larvae to temephos in West Banjarmasin. Jurnal BUSKI, 4(2), 53-58.

Izzah, N., Kadang, & Permatasari. (2019). Uji identifikasi senyawa alkaloid ekstrak metanol daun kelor (Moringa oleifera Lamk) dari Kabupaten Ende Nusa Tenggara Timur secara kromatografi lapis tipis. Jurnal Farmasi Sandi Karsa, 5(1), 52-55.

Juang, Y. P., & Liang, P. H. (2020). Biological and pharmacological effects of synthetic saponins. Molecules, 25(21), 4974.

Ludwiczuk, A., Skalicka-Woźniak, K., & Georgiev, M. I. (2017). Terpenoids. In Pharmacognosy: Fundamentals, Applications and Strategy (pp. 233–266).

Minarni, E., Armansyah, T., & Hanafiah, M. (2013). Daya larvasida ekstrak etil asetat daun kemuning (Murraya paniculata (L) Jack) terhadap larva nyamuk Aedes aegypti. Jurnal Medika Veterinaria, 7(1), 27–29.

Paju, N., Yamlean, P. V. Y., & Kojong, N. (2013). Uji efektivitas salep ekstrak daun binahong (Anredera cordifolia (Ten.) Steenis) pada kelinci (Oryctolagus cuniculus) yang terinfeksi bakteri Staphylococcus aureus. Pharmacon, 12(1), 51-61.

Pardosi, D. R. S., Purnamasari, C. B., Paramita, S., Astuti, L. A., Masyhudi, M., & Arung, E. T. (2022). Antibacterial activity of bangle rhizoma essential oil (Zingiber montanum) against Streptococcus mutans. Journal of Dento Maxillofacial Science, 7(1), 5-8.

Prakoso, G., Aulung, A., & Citrawati, M. (2017). Uji efektivitas ekstrak buah pare (Momordica charantia) pada mortalitas larva Aedes aegypti. Jurnal Profesi Medika: Jurnal Kedokteran dan Kesehatan, 10(1).

Pratiwi, A. (2013). Studi deskriptif penerimaan masyarakat terhadap larvasida alami. Thesis, Semarang State University.

Schaefer, T. J., Panda, P. K., & Wolford, R. W. (2022). Dengue fever. StatPearls. https://www.ncbi.nlm.nih.gov/books/NBK430732/

Suparyati, S. (2020). Uji daya bunuh abate berdasarkan dosis dan waktu terhadap kematian larva nyamuk Aedes sp. dan Culex sp. Pena Jurnal Ilmu Pengetahuan Dan Teknologi, 34(2), 1.

Tandi, E. (2010). Pengaruh tanin terhadap aktivitas enzim protease. Journal of Science.

Yang, S., Bai, M., Yang, J., Yuan, Y., Zhang, Y., Qin, J., & Sampietro, D. A. (2020). Chemical composition and larvicidal activity of essential oils from Peganum harmala, Nepeta cataria, and Phellodendron amurense against Aedes aegypti (Diptera: Culicidae). Saudi Pharmaceutical Journal, 28(5), 560–564.

Yassi, M. R., & Harsanti. (2018). Uji daya larvasida ekstrak daun kelor (Moringa oleifera) terhadap mortalitas larva Aedes aegypti. Journal of Agromedicine and Medical Sciences, 4(3), 159-163.