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Title and Abstract

Title	The Potential of Srigading Plants (Nyctanthes arbor-tristis) as Aedes aegypti Larvicides
Abstract	Dengue Hemorrhagic Fever (DHF) is an endemic disease that causes high morbidity and mortality rates. The prevalence of DHF in Indonesia (2018) reached 24.75 per 100,000, and in South Kalimantan (2019) was 56.83 per 100,000 population. One of the efforts to control dengue vectors is the use of larvicides. The purpose of this study was to determine the potential of Srigading Plants (Nyctanthes arbor-tristis) as Aedes aegypti larvae as vectors of DHF in Wetlands. This study used a true experimental research design using the post-test-only controlled group design to determine the effectiveness of Srigading extract (Nyctanthes arbor-tristis) against the death of Aedes aegypti instar IV mosquito larvae. Based on the results, a significance value or p-value of 0.0001 < 0.05 was obtained. It indicated a significant difference between the number of mosquito larvae deaths and the concentration of Srigading extract given.

Indexing

Keywords Aedes Aegypti, Dengue Hemorrhagic Fever, Larvacides, Srigading plants, Vector
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References

Adnyana, I. M. D. M., Sudaryati, N. L. G., & Sitepu, I. (2021). Toxicity of Legiayu incense as Insecticide and Larvicide against Aedes aegypti Mosquitoes Mortality. Indonesian Journal of Pharmacy, 32(4), 514–521.

Araujo, A. F. D. O., Ribeiro-Paes, J. T., Deus, J. T. D., Cavalcanti, S. C. D. H., Nunes, R. D. S., Alves, P. B., & Macoris, M. D. L. D. G. (2016). Larvicidal activity of Syzygium aromaticum (L.) Merr and Citrus sinensis (L.) Osbeck essential oils and their antagonistic effects with temephos in resistant populations of Aedes aegypti. Memórias Do Instituto Oswaldo Cruz, 111(1), 443–449.

Bhalakiya, H., & Modi, N. R. (2019). Traditional medicinal uses, phytochemical profile and pharmacological activities of Nyctanthes arbortris. RJLBPCS, 5(1), 1003–1023.

Bowman, L. R., Donegan, S., & McCall, P. J. (2016). Is dengue vector control deficient in effectiveness or evidence?: Systematic review and meta-analysis. PLoS Neglected Tropical Diseases, 10(3), 1–24.

Dinas Kesehatan Provinsi Kalimantan Selatan. (2020). Data Demam Berdarah Dengeu (DBD) Provinsi Kalimantan Selatan. Dinas Kesehatan Provinsi Kalimantan Selatan.

Doughari, J. (2015). An overview of plant immunity. J. Plant Pathol. Microbiol, 6(11), 4110–4172.

Espinal, M. A., Andrus, J. K., Jauregui, B., Waterman, S. H., Morens, D. M., Santos, J. I., & Olson, D. (2019). Emerging and reemerging Aedes-transmitted arbovirus infections in the region of the Americas: implications for health policy. American Journal of Public Health, 109(3), 387–392.

Faisal, A. P. (2018). Identifikasi Metabolit Sekunder dan Aktivitas Larvasida dari Daun Matahari (Helianthus annuus L.) terhadap Larva Aedes aegypti Sp. Mahakam Medical Laboratory Technology Journal, 2(2), 80–90.

Fatimah, G., & Hasmiwati, R. R. (2020). Lethal concentration (LC50, 90, and 98) and lethal time (LT50, 90, and 98) at various temephos concentrations of Aedes aegypti L. larvae. International Journal of Mosquito Research, 7(1), 1–3.

Jogawat, A., Yadav, B., Lakra, N., Singh, A. K., & Narayan, O. P. (2021). Crosstalk between phytohormones and secondary metabolites in the drought stress tolerance of crop plants: a review. Physiologia Plantarum, 17(2), 1106–1132.

Khameneh, B., Iranshahy, M., Soheili, V., & Fazly Bazzaz, B. S. (2019). Review on plant antimicrobials: a mechanistic viewpoint. Antimicrobial Resistance & Infection Control, 8(1), 1–28.

Kurniawan, M. E., Mohamed, A. M. D., Siyam, N., Fatikha, N., & Fitriani, N. A. (2017). Relation Between Knowledge and Attitude Regarding DHF with PSN Behavior Among the Community Around the Campus. KEMAS: Jurnal Kesehatan Masyarakat, 13(2), 145–151.

Lesmana, S. D., Maryanti, E., Susanty, E., Afandi, D., Harmas, W., Octaviani, D. N., & Mislindawati, M. (2022). Organophosphate Resistance in Aedes aegypti: Study from Dengue Hemorrhagic Fever Endemic Subdistrict in Riau, Indonesia. Reports of Biochemistry & Molecular Biology, 10(4), 589–596.

Mandasari, N., Hazar, S., & Sadiyah, E. R. (2016). Uji Aktivitas Sitotoksik Ekstrak Metanol Daun Srigading (Nyctanthes arbor-tristis L.). Prosiding Farmasi, 717–722.

Minarti, M., Anwar, C., Irfannuddin, I., & Irsan, C. (2021). Community Knowledge and Attitudes about the Transmission of Dengue Haemorrhagic Fever and Its Relationship to Prevention Behaviour in Palembang, South Sumatra, Indonesia. Open Access Macedonian Journal of Medical Sciences, 9(5), 1534–1543.

Nisrina, H. (2022). Toxicity Assessment Of Avovo Leaf Extract (Persea Americana Miller) On Mortality Of Aedes Aegypti Larva. International Journal of Health, Education & Social (IJHES), 5(5), 1–7.

Patel, M. I., Patel, A., Patel, A., Patel, S., & Padsala, S. (2018). Study of haematological, biochemical profile and clinical presentation in dengue positive patients: 82 cases. International Journal of Research in Medical Sciences, 6(6), 2099–2105.

Rohmah, E. A., Subekti, S., & Rudyanto, M. (2020). Larvicidal Activity and Histopathological Effect of Averrhoa bilimbi Fruit Extract on Aedes aegypti from Surabaya, Indonesia. Journal of Parasitology Research, 1–5.

Şengül Demirak, M. Ş., & Canpolat, E. (2022). Plant-based bioinsecticides for mosquito control: Impact on insecticide resistance and disease transmission. Insects. Insects, 13(2), 1–24.

Suryani, A. I., Hariani, N., Majid, A. F., & Amalia, D. N. (2020). Histological changes in the midgut of Spodoptera litura larvae exposured by the extract of Mirabilis jalapa leaves. In IOP Conference Series: Earth and Environmental Science. IOP Publishing, 484(1).

Tlak Gajger, I., & Dar, S. A. (2021). Plant allelochemicals as sources of insecticides. Insects, 12(3), 1–21.

Wahyuni, E. (2021). Potensi Eksudat Daun Sirih Merah (Piper ornatum L.) sebagai Insektisida Herbal terhadap Mortalitas Semut Hitam. Hydrogen: Jurnal Kependidikan Kimia, 9(2), 97–104.

Wang, W. H., Urbina, A. N., Chang, M. R., Assavalapsakul, W., Lu, P. L., Chen, Y. H., & Wang, S. F. (2020). Dengue hemorrhagic fever–A systemic literature review of current perspectives on pathogenesis, prevention and control. Journal of Microbiology, Immunology and Infection, 53(6), 963–978.

Wanti, W., Sila, O., Irfan, I., & Sinaga, E. (2016). Transovarial transmission and dengue virus serotypes in Aedes aegypti in Kupang. Kemas: Jurnal Kesehatan Masyarakat, 12(1), 131–138.

World Health Organization. (2020). Dengue and Severe Dengue. Dengue Data Application. World Health Organization Press. https://ntdhq.shinyapps.io/dengue5/

Yousaf, M. Z., Siddique, A., Ashfaq, U. A., & Ali, M. (2018). Scenario of dengue infection & its control in Pakistan: An up—date and way forward. Asian Pacific Journal of Tropical Medicine, 11(1), 15–23.

Yusuf, Y., Efendi, K., & Diantasari, S. (2020). Larvicidal Activity Test of Ethanolic Extract of (Euphorbia tirucalli Linn) Stem on Aedes aegypti Larvae. Systematic Reviews in Pharmacy, 11(3).

Zaynab, M., Fatima, M., Abbas, S., Sharif, Y., Umair, M., Zafar, M. H., & Bahadar, K. (2018). Role of secondary metabolites in plant defense against pathogens. Microbial Pathogenesis, 124(1), 198–202.

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The Potential of Srigading Plants (Nyctanthes arbor-tristis) as Aedes aegypti Larvicides as Dengue Hemorrhagic Fever Vectors in Wetlands

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ABSTRACT

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Dengue Hemorrhagic Fever (DHF) is an endemic disease that causes high morbidity and mortality rates. Globally, dengue fever is reported to range from 1-3 million cases while the number of deaths ranges from 2-4 thousand in the last decade. The prevalence of DHF in Indonesia in 2018 reached 24.75 per 100,000 and in South Kalimantan in 2019 it reached 56.83 per 100,000 population. One of the efforts to control dengue vectors is the use of larvicides. The purpose of this study was to determine the potential of Srigading Plants (*Nyctanthes arbor-tristis*) as Aedes aegypti larvae as vectors of DHF in Wetlands. This study used a true experimental research design using the post test only controlled group design to determine the effectiveness of Srigading extract (Nyctanthes arbor-tristis) against the death of Aedes aegypti instar IV mosquito larvae. Based on the results of the one way ANOVA test, a significance value or p-value of 0.0001 < 0.05 was obtained, which indicated that there was a significant difference between the number of mosquito larvae deaths and the concentration of Srigading extract given.

Based on the results of the post-hoc test using LSD, it was found that there was a significant difference between the Srigading extract and the control group. The comparison found between the negative control and the concentration was found at a concentration of 3588 ppm, at that concentration there was a significant difference. There is one concentration of Srigading leaf extract (Nycthanthes arbor-tritis) which has larvicidal activity, that is a concentration of 3588 ppm.

Keywords: Aedes Aegypti, Dengue Hemorrhagic Fever, Larvacides, Srigading plants, Vector

INTRODUCTION

Dengue Hemorrhagic Fever (DHF) is a disease caused by a virus from the flaviviridae family which is transmitted through mosquito bites (arthropod borne viruses/arbovirus) namely Aedes aegypti and Aedes albopictus mosquitoes with clinical manifestations of fever, muscle or joint pain accompanied by leukopenia, rash, lymphodenopathy, and thrombocytopenia. The serotypes DEN-1, DEN-2, DEN-3 and DEN-4 are the cause of Dengue Hemorrhagic Fever which is transmitted by Aedes aegypti and Aedes albopictus mosquitoes that have been infected with the dengue virus. The four serotypes can be found in various regions in Indonesia. Each percentage of infection incidence of the four serotypes, namely DEN-1 by 8%, DEN-2 by 65%, DEN-3 by 15% and DEN-4 by 12%. DEN-2 serotype is the highest serotype and is most often associated with severe cases (1, 2).

Globally, Dengue Hemorrhagic Fever is reported to range from 1-3 million cases while the number of deaths ranges from 2-4 thousand in the last decade. According to data from the Ministry of Health of the Republic of Indonesia, the prevalence of Dengue Hemorrhagic Fever in 2016 was 78.85 per 100,000 population, in 2017 and 2018 it decreased to 26.1 per 100,000 population, and again decreased to 24.75 per 100,000. Ministry of Health, 2019). The morbidity rate of Dengue Hemorrhagic Fever according to data from the Health Office of South Kalimantan Province, namely in 2016 amounted to 106.21 per 100,000 population, then in 2017 there was a decrease to 13.49 per 100,000 population but increased again in 2018 by 47.91 and in 2019 by 56.83 per 100,000 population (3, 4).

Vector control of Dengue Hemorrhagic Fever in Indonesia has not yet reached the program target. The target of the vector control program is 95%. The indicator of the dengue hemorrhagic fever vector control program is the larva-free rate (ABJ). Nationally, ABJ in 2016 was 67.6%, in 2017 it decreased to 46.7% and then decreased again in 2018 which was 31.5%. Based on data from the South Kalimantan Provincial Health Office, ABJ from 2015-2019 fluctuated with an increasing trend. The lowest ABJ in the five years was in 2016 at 83.85% and the highest was in 2017 at 89.08%. The lower the ABJ or below the program target, it means that the mosquito density will be higher. If the mosquito density is high, it will be a threat to increase the transmission of Dengue Hemorrhagic Fever in the future (5, 6).

Vector control of Dengue Hemorrhagic Fever can be done in various ways, one of which is by using temephos. Temephos is still part of the government's program for eradicating mosquito nests, especially the Aedes aegypti mosquito with 3M plus. The use of temephos for a long time will cause negative impacts such as environmental pollution and cause resistance. On the other hand, this resistance results in ineffective vector control. Reports of resistance to Aedes aegypti larvae, where administration of temephos can harm humans by causing cancer in a number of body parts. Another chemical control that is also commonly used in the community is fumigation or fogging, which targets the adult Aedes aegypti mosquito. This method also has the same negative impact as the use of temephos, especially in the human lungs when inhaled directly (7, 8, 9).

The World Health Organization (WHO) recommends the development of natural vector control that is environmentally friendly because it will be safer for the environment and human health. Therefore, natural larvicidation efforts continue to be developed from various plants that have potential as larvicides. Natural larvicides are obtained from secondary metabolites that function as a means of self-defense from attack and are known to be able to kill nuisance organisms. Indonesia is a country that is rich in various types of plants. One of these plants is the Srigading plant (Nyctanthes arbor-tristis L). This plant usually thrives on the islands of Java and

Kalimantan as an ornamental plant in tombs or graves. Apart from being an ornamental plant, Srigading plant is also used as a medicinal plant. Srigading contains high antioxidants. This Srigading plant contains secondary metabolites which include saponins, tannins, terpenoids and steroids (10, 11, 12).

Saponins can cause cell damage, interfere with metabolic processes and damage the outer protective barrier so that mosquito larvae will lose a lot of fluids. Tannins can cause nutritional disturbances by reducing the activity of digestive enzymes in mosquito larvae. Terpenoids function as disruptors of cell membranes and tissues in mosquito larvae. Steroids function as growth hormones that work to inhibit growth by influencing skin turnover in mosquito larvae (13). So far as literature searches have been carried out, there has been no research on the effect of Srigading extract (Nyctanthes arbor-tristis L) as a larvicide on Aedes aegypti mosquito larvae. Therefore, it is necessary to conduct research on Srigading extract (Nyctanthes arbor-tristis L) as a natural larvicide that can kill Aedes aegypti mosquito larvae as the basis for this research.

METHODS

This study used a true experimental research design using the post test only controlled group design to determine the effectiveness of Srigading extract (Nyctanthes arbor-tristis) against the death of Aedes aegypti instar IV mosquito larvae. In this study, five treatments were tested with concentrations of 448.5 ppm, 879 ppm, 1794 ppm, 3588 ppm, 7176 ppm and using two controls (positive and negative). In research, the selection of larval age is a very important activity. The age of mosquito larvae has a very influential factor on the resistance of the larvae to exposure to chemical substances. The age range of 2-5 days is the best age range and is in accordance with the Guidelines for Biological Insecticide Testing. The longer the age of mosquito larvae, the higher their resistance and the more mature their physical condition. Therefore, the subjects in this study were Aedes aegypti mosquito larvae instar IV 5 days old and had signs of life such as active movement. The fourth instar Aedes aegypti mosquito larvae were obtained from the Center for the Development and Research of Seasoned Soil Disease Vectors, South Kalimantan (14, 15).

The research materials used were Srigading (Nyctanthes arbor-tristis), aquades, 70% ethanol, temephos, fish food and Aedes aegypti instar IV mosquito larvae which were colonized at the Entomology Laboratory of the Lokalitbang Center for the Development and Research of Spice Soil Disease Vector Reservoirs, South Kalimantan. The Srigading plant (Nyctanthes arbor-tristis) will be identified at the Laboratory of the Faculty of Medicine, University of Lambung Mangkurat Banjarbaru, this is done to avoid errors in the selection of plant species so that they can be accounted for. Extracts of Srigading (Nyctanthes arbor-tristis) were made by researchers Together with laboratory analysts of Agricultural Industrial Engineering (TIP) Faculty of Agriculture, University of Lambung Mangkurat Banjarbaru using the maceration method. Preparation of stock solutions with One part per million (ppm) is a concentration of 1 mg of solute 1000 ml . then to make a stock solution of 13,903.5 ppm is by weighing the extract 13,903.5 mg of solute 1000 ml.

The results of the calculation of the number of deaths of Aedes aegypti mosquito larvae from seven treatments were tabulated in tabular form. The data were statistically analyzed using a computer program. Normality test was calculated using Shapiro-Wilk and homogeneity test was calculated using Levene's test. If the data is normally distributed and homogeneous, then the One Way ANOVA test is carried out with post Hoc Bonferroni. If the data is not normally distributed, the Kruskal-Wallis test is performed. If there is a difference in Kruskal-Wallis, then

it is continued with the Mann-Whitney test. Determining the LC50 and LT50 values of Srigading Leaf extract was carried out using probit analysis with a 95% confidence level (16, 17).

RESULTS AND DISCUSSION

The concentration of Srigading leaf extract (Nyctanthes arbor-tristis) in the larvicidal test was 448.5 ppm, 879 ppm, 1794 ppm, 3588 ppm, 7176 ppm, aquadest as a negative control and temephos as a positive control, which were exposed to Aedes aegypti larvae instar IV during 24 hours.

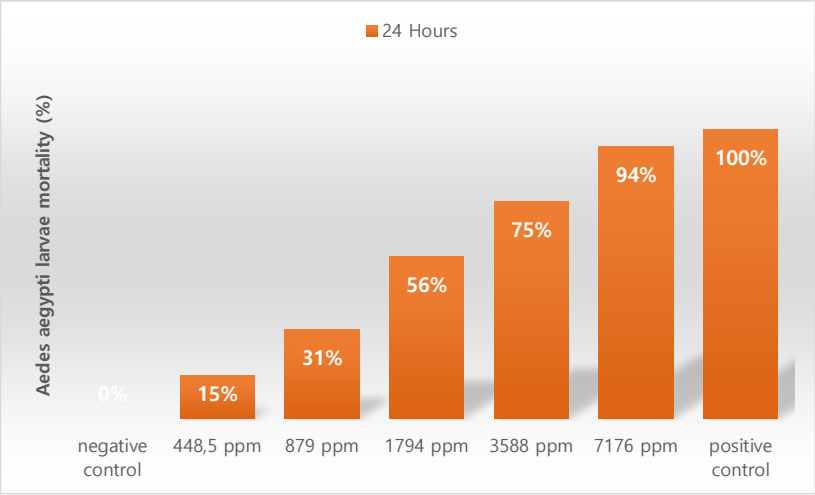

Figure 1. Effectiveness of Srigading Leaf Extract (Nyctanthes arbor-tristis) against fourth instar Aedes aegypti larvae for 24 hours of exposure

Figure 1 shows a graphic depiction of the percentage of mortality of fourth instar Aedes aegypti larvae after 24 hours of exposure to Srigading leaf extract (Nyctanthes arbor-tristis). In the negative control, there was no larval death so there was no need to correct the calculation of larval mortality using the Abbot formula. In the positive control using 1% temephos there was larval mortality with an average percentage of 100%. There was an increase in the mortality of Aedes aegypti larvae instar IV along with the increasing concentration of Srigading leaf extract (Nyctanthes arbor-tristis). The average larval mortality at a concentration of 448.5 ppm was 15%, at a concentration of 879 ppm was 31%, at a concentration of 3588 ppm was 56%, at a concentration of 1794 ppm was 75% and at a concentration of 7176 ppm, Aedes aegypti larvae mortality was 94%.

Table 1. ANOVA test results

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1937.333	5	387.467	149.987	**.000**
Within Groups	46.500	18	2.583		
Total	1983.833	23			

Based on table 1, it can be seen that the mortality of Aedes aegypty larvae using the one way ANOVA test obtained a significance value or p-value of 0.0001 <0.05 which indicates that there is a significant difference between the number of mosquito larvae deaths and the concentration of Srigading extract given. Then a post-hoc Least Signification Difference (LSD) test was carried out to determine the location of the difference in the average larval mortality at each concentration and used as a reference in determining whether the average of the two treatment concentrations was statistically different or not, so that the concentration could be determined. which has larvicidal activity.

Table 2. LSD post-hoc test results

Consentration		*p-value*	Meaning
Negative Control	With 448,5 ppm	0,203	Not significant
	With 897 ppm	0,828	Not significant
	With 1794 ppm	1,000	Not significant
	With 3588 ppm	0,0001	Significant
	Positive Control	0,0001	Significant
448,5 ppm	Negative Control	0,203	Not significant
	With 897 ppm	0,286	Not significant
	With 1794 ppm	0,203	Not significant
	With 3588 pm	0,002	Significant
	Positive Control	0,0001	Significant
897 ppm	Negative Control	0,828	Not significant
	With 448,5 ppm	0,286	Not significant
	With 1794 ppm	0,828	Not significant
	With 3588 ppm	0,0001	Significant
	Positive Control	0,0001	Significant
1794 ppm	Negative Control	1,000	Not significant
	With 448,5 ppm	0,203	Not significant
	With 897 ppm	0,828	Not significant
	With 3588 ppm	0,0001	Significant
	Positive Control	0,0001	Significant
3588 ppm	Negative Control	0,0001	Significant
	With 448,5 ppm	0,002	Significant
	With 897 ppm	0,0001	Significant
	With 1794 ppm	0,0001	Significant
	Positive Control	0,0001	Significant
Positive Control	Negative Control	0,0001	Significant
	With 448,5 ppm	0,0001	Significant
	With 897 ppm	0,0001	Significant
	With 1794 ppm	0,0001	Significant
	With 3588 ppm	0,0001	Significant

Based on the results of the one way ANOVA test, a significance value or p-value of 0.0001 < 0.05 was obtained, which indicated that there was a significant difference between the number of mosquito larvae deaths and the concentration of Srigading extract given. Based on the results

of the post-hoc test using LSD, it was found that there was a significant difference between the Srigading extract and the control group. The comparison found between the negative control and the concentration was found at a concentration of 3588 ppm, at that concentration there was a significant difference, this can be seen from the p-value or the significance value of 0.0001 compared to the value of <0.05. From these results it can be concluded that there is one concentration of Srigading leaf extract (Nycthanthes arbor-tritis) which has larvicidal activity.

Then, for the comparison between the positive control with concentrations of 448.5 ppm, 897 ppm, 1794 ppm, and 3588 ppm had the same significance value or p-value, namely 0.0001 with a value of <0.05, which means there is a significant difference. . So, it can be concluded that at concentrations of 448.5 ppm, 897 ppm, 1794 ppm, and 3588 ppm, it does not have the same effectiveness as 1% temephos. The average percentage of Aedes aegypti larvae mortality was not affected by increasing concentration, so the higher concentration of Srigading leaf extract did not affect the mortality of the test larvae.

Death of Aedes agypti larvae occurred after treatment in the form of Srigading (Nycthanthes arbor-tritis) leaf extract, this was due to the larvicidal effect of Srigading (Nycthanthes arbor-tritis) leaves which contain secondary metabolites. The secondary metabolites contained in Srigading consist of alkaloids, flavonoids, saponins, tannins, and triterpenoids. Alkaloid secondary metabolites are one of a group of compounds that can be found in most types of plants. The way alkaloids work as insecticides is by inhibiting the activity of the acetylcholinesterase enzyme. This compound works by stimulating the endocrine glands to secrete juvenile hormones, this increase can cause metamorphosis failure in mosquito larvae resulting in abnormal death in mosquito larvae. In addition, in inhibiting cell mitosis, alkaloids can synergize with triterpenoid compounds (27).

The secondary metabolites of flavonoids are compounds that can damage the cytoplasmic membrane and cause cell leakage and can turn off the enzyme system. This of course can result in phospholipids being unable to maintain the shape of the cytoplasmic membrane itself until it will eventually burst and the larvae themselves will die (27). Saponin secondary metabolites are compounds that work by lowering the surface tension on the mucous membrane of the larval digestive tract. This will inhibit the rate of nutrient absorption by the larvae. In addition, saponins have another effect, namely destroying the chitin layer on the surface of the larvae so that the extract can easily enter the body of the larvae (27). The secondary metabolites of tannins are polyphenolic compounds that work by causing astringency in plant parts that can enter mosquito larvae through the body wall, causing disturbances in the larval muscles. As a result, the larvae will experience weakness in the muscles of movement, so that the movement of the larvae will slow down. In addition to entering through the body wall, tannins can enter through the digestive tract of the larvae, this can cause interference with protein absorption in the larvae's intestines by reducing the activity of digestive enzymes and food absorption, so that the larvae will experience nutritional deficiencies and can cause death (28). Triterpenoid secondary metabolites are compounds that work by remembering free sterols in digestion where sterols act as precursors of the hormone ecdysone, so that a decrease in the amount of free sterols will disrupt the process of skin turnover in insects. In addition, these compounds can cause a decrease in the activity of digestive enzymes and affect the process of food absorption (28).

Determining the LC50 and LC90 values from the ethanol extract of Srigading leaves (Nyctanthes arbor-tristis) was performed using Probit Analysis with a 95% confidence level. Probit analysis for LC50 and LC90 can be seen in table 3 as follows

Table 3. LC50 and LC90 values from the results of probit analysis of Srigading leaf ethanol extract (Nyctanthes arbor-tristis) against IV instar Aedes aegypti larvae after 24 hours

Mortality (%)	Estimate (%)	Confidence Level (%)	Confidence Interval	
			Lower Limit	Upper Limit
50	2.527	95	-9.649	3.507
90	5.460	95	4.389	20.775

Based on table 3, it can be seen that the results of the Probit analysis on the mortality rate of Aedes aegypti larvae obtained an LC50 value of 2.527%, this indicates that at a concentration of 2.527% within 24 hours it was able to kill 50% of the test larvae. While the obtained LC90 value of 5.460%, this shows that at a concentration of 5.460% within 24 hours it is able to kill 90% of the test larvae. Based on the results of the Probit analysis in table 3, the 24-hour LC50 value of Srigading leaf ethanol extract (Nyctanthes arbor-tristis) on the mortality of Aedes aegypti mosquito larvae was obtained at a concentration of 2.527%, which means that at a concentration of 2.527% ethanol extract of Srigading leaves (Nyctanthes arbor-tristis)) was able to kill 50% of Aedes aegypti larvae which were exposed for 24 hours, so it can be stated that the ethanol extract of Srigading leaves (Nyctanthes arbor-tristis) was effective against Aedes aegypti mosquito larvae with an LC50 value at 24 hours. While the 24-hour LC90 value of the ethanol extract of Srigading leaves (Nyctanthes arbor-tristis) on the mortality of Aedes aegypti mosquito larvae was obtained at a concentration of 5.460%, which means that at a concentration of 5.460% the ethanol extract of Srigading leaves (Nyctanthes arbor-tristis) was able to kill 90% of Aedes larvae. aegypti which was exposed for 24 hours, so it can be stated that the ethanol extract of Srigading leaves (Nyctanthes arbor-tristis) was effective against Aedes aegypti mosquito larvae with an LC90 value at 24 hours.

The results of this study showed that the ethanol extract of the leaves of Srigading (Nyctanthes arbor-tristis) had larvicidal activity against the larvae of the Aedes aegypti mosquito. However, according to previous studies, it is said that an extract of natural ingredients is effective as a pesticide if the LC50 is not more than 1000 ppm (0.1%). The LC50 value of the ethanol extract of Srigading leaves (Nyctanthes arbor-tristis) obtained was 2.527% or 25.270 ppm (conversion 1% = 10000 ppm), so it can be concluded that the ethanol extract of Srigading leaves (Nyctanthes arbor-tristis) still has high killing power. less against Aedes aegypti larvae. This may be caused by the form of the extract which is still in the form of crude extract or not in the form of pure compounds, so it is necessary to purify the compounds before they can be used as larvicides to obtain more optimal results (29).

The ethanol extract of Srigading leaves (Nyctanthes arbor-tristis) has larvicidal activity against Aedes aegypti mosquito larvae. Although the ability of Srigading leaf ethanol extract (Nyctanthes arbor-tristis) was still under positive control (temephos 1%), the ability of Srigading leaf ethanol extract (Nyctanthes arbor-tristis) could cause mortality of Aedes aegypti mosquito larvae, which was able to kill 50% of Aedes larvae. aegypti which was exposed for 24 hours with a concentration of 2.527%, this makes Srigading leaves (Nyctanthes arbor-tristis) have the potential to be used as biolarvicides and are relatively safer for the environment and not persistent in nature, in contrast to temephos 1% which has the potential to cause pollution and the occurrence of resistance in larvae if not using the appropriate dose (30).

Conclusion

Srigading leaf extract (Nyctanthes arbor-tristis) has the effect of larvacide Aedes aegypti instar IV at a concentration of 448.5 ppm, 879 ppm, 3588 ppm, 1794 ppm and at a concentration of 7176 ppm it has the equivalent effectiveness of 1% temephos. With the discovery of natural ingredients from the Srigading plant (Nyctanthes arbor-tristis L) which functions as a natural larvicide, it will help the community to carry out 3M activities in overcoming the problem of Dengue Hemorrhagic Fever (DHF) outbreak, especially for rural areas that are difficult to get access to. buy abate powder.

REFERENCES

1. Cahyati, Hary W, Siyam N. Perilaku Masyarakat dalam Penggunaan *Temephos. Higeia Journal Of Public Health Research and Development*. 2019;3(1):155–63.
2. Faizal AP. Identifikasi Metabolit Sekunder dan Aktivitas Larvasida dari Daun Matahari (*Helianthus annuus* L.) terhadap Larva *Aedes aegypti* Sp. *Mahakam Medical Laboratory Technology Journal*. 2017;2(2):80–90.
3. Firdhayani IN, Alamsjah MA, Subekti S. Eksplorasi Bahan Aktif Rumput Laut Coklat (*Phaeophyceae*) sebagai Biolarvasida *Aedes aegypti*. Jurnal Ilmiah Perikanan dan Kelautan. 2014;6(2):187–92.
4. Handayani SW, Prastowo D, Boesri H, Oktsariyanti A, Joharina AS. Efektivitas Ekstrak Daun Tembakau (*Nicotiana tabacum* L) dari Semarang, Temanggung, dan Kendal sebagai Larvasida *Aedes aegypti* L. Jurnal BALABA. 2018;14(1):23–30.
5. Hasbullah ML, Nurdian Y, Abrori C. Potensi Ekstrak Etanol Daun Meniran (*Phyllantus niruri* L.) sebagai Larvasidal Nyamuk *Aedes aegypti*. *Journal of Agromedicine and Medical Sciences*. 2019;5(1):1–6.
6. Henrique MO, Neto LS, Assis JB, Barros MS, Capurro ML, Lepique AP, *et al*. *Evaluation of Inflammatory Skin Infiltrate Following Aedes aegypti Bites in Sensitized and Non-Sensitized Mice Reveals Saliva-Dependent and Immune-Dependent Phenotypes. Immunology.* 2019;158(1):47–59.
7. Kartikasari D, Novitasari M. Uji Aktivitas Larvasida Perasan Herba Seledri (*Apium Graveolensl*.) terhadap Larva *Aedes aegypti*. Jurnal As-Syifaa. 2018;10(2):152–60.
8. Kementerian Kesehatan RI. Profil Kesehatan Indonesia Tahun 2018. Jakarta: Kementrian Kesehatan RI; 2019.
9. Khusna AM. Toksisitas Campuran Ekstrak Buah Srikaya (*Annona squamosa* L.) dan Ekstrak Buah Ketapang (*Terminalia catappa* L.) terhadap mortalitas Larva Nyamuk *Aedes aegypti* serta Pemanfaatannya. Universitas Jember; 2017
10. Koneri R, Pontororing HH. Uji Ekstrak Biji Mahoni (*Swietenia macrophylla*) terhadap Larva *Aedes aegypti* Vektor Penyakit Deman Berdarah. Jurnal Media Kesehatan Masyarakat Indonesia. 2016;12(4):216–24.
11. Loren I. Toksisitas Campuran Ekstrak Daun Sirih (*Piper betle* L.) dan Ekstrak Biji Srikaya (*Annona squamosa* L.) terhadap Mortalitas Larva Nyamuk *Aedes aegypti* L. Universitas Jember; 2016.
12. Merisia. Uji Ekstrak Batang Sereh (*Cymbpogon nardus* (L.) Rendle) dalam Membunuh Larva *Aedes aegypti*. STIKes Insan Cendekia Medika; 2018.
13. Nyctanthes arbor-tristis in Flora of Pakistan @ efloras.org". *www.efloras.org*. Diakses tanggal 2019-08-30.

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14. Pambudi BC, Martini, Tarwotjo A, Hestiningsih R. Efektivitas *Temephos* sebagai Larvasida pada Stadium Pupa *Aedes aegypti*. Jurnal Kesehatan Masyarakat (*e-Journal*). 2018;6(1):381–8.
15. Puri, A., Saxena, R., Saxena, R. P., Saxena, K. C., Srivastava, V., & Tandon, J. S. Immunostimulant activity of Nyctanthes arbor-tristis L. *J. Ethnopharmacol*. 42 (1): 31-37.
16. Putranta NR, Musyabiq S. Efektifitas Ekstrak Kulit Duku (Lansium domesticum corr) sebagai Larvasida *Aedes aegypti*. Jurnal Medusa. 2018;7(5):165–70.
17. Rawani A, Pal S, Chandra G. *Evaluation of Antimicrobial Properties of Four Plant Extracts Againts Human Pathogen. Asian Pacific Journal Of Tropical Biomedicine*. 2011;1(1):S71–5.
18. Ramadhan MRF. Toksisitas Campuran Ekstrak Biji Pepaya (*Carica papaya* L.) dan Biji Srikaya (*Annona squamosa* L.) terhadap Mortalitas Larva Nyamuk *Aedes aegypti*. Universitas Jember; 2016.
19. Rosyid A, Santoso A, Naila IU. Analisis Efektivitas Biaya Terapi Suportif Imunomodulator dan Kapsul Ekstrak Daun Jambu Biji Demam Berdarah Dengue. Jurnal Farmasi Sains dan Praktis. 2019;5(1):1–6.
20. Sandra T, Sofro MA, Suhartono, Martini, Hadisaputro S. Faktor-Faktor yang Berpengaruh terhadap Kejadian Demam Berdarah Dengue Pada Anak Usia 6-12 Tahun Di Kecamatan Tembalang. Jurnal Epidemiologi Kesehatan Komunitas. 2019;4(1):1–10.
21. Seksi Surveilans dan Imunisasi. Data Penyakit Demam Berdarah Dengue. Banjarmasin; 2020.
22. Syadid R. Hu n Pengetahuan, Sikap dan Pendidikan dengan Perilaku Pencegahan Demam Berdarah Dengue. Universitas Lambung Mangkurat; 2017.
23. Tina L, Misnawati, Nirmala F. Uji Perbandingan Efektivitas Ekstrak Daun Sirih Hijau (*Piper betle* Linn) dengan Ekstrak Daun Pandan Wangi (*Pandanus amaryllifolius* Roxb) terhadap Kematian Larva Nyamuk *Aedes aegypti* Tahun 2018. Jurnal Ilmiah Praktisi Kesehatan Masyarakat. 2018;3(1):1–11.
24. World Health Organization. *Dengue and Severe Dengue* [Internet]. *Dengue Data Application*. 2020 [cited 2020 Feb 29]. *Available from: https://ntdhq.shinyapps.io/dengue5/*
25. Widyastuti DA, Rahayu P, Dewi LR. Potensi Ekstrak Sirsak (*Annona muricata*) sebagai Larvasida Pengendali Populasi *Aedes albopictus*. Jurnal Bioeksperimen. 2019;5(1):48–54.
26. Wahyuni D. Toksisitas Ekstrak Tanaman sebagai Bahan Dasar Biopestisida Baru Pembasmi Larva Nyamuk *Aedes aegypti* (Ekstrak Daun Sirih, Ekstrak Biji Pepaya, dan Ekstrak Biji Srikaya) Berdasarkan Hasil Penelitian. Malang: Media Nusa Creative; 2016.
27. Sogandi S, Gunarto F. Efek Larvasida Fraksi Etil Asetat Daun Bangun-bangun (Plectranthus amboinicus) terhadap Mortalitas Larva Aedes aegypti. ASPIRATOR - J Vector-borne Dis Stud. 2020;12(1):27–36.
28. Nadila I, Istiana I, Wydiamala E. AKTIVITAS LARVASIDA EKSTRAK ETANOL DAUN BINJAI (Mangifera caesia) TERHADAP LARVA Aedes aegypti. Berk Kedokt. 2017;13(1):61–8.

29. Maulana H, Wydiamala E, Biworo A. Uji Aktivitas Larvasida Ekstrak Etanol Daun Pucuk Merah (Syzygium myrtifolium Walp.) terhadap Nyamuk Aedes aegypti. Homeostasis. 2021 Dec 29;4(3):567-74.
30. Rodriguez, M.M., *et al*. Detection of insecticide resistence in *Aedes aegypti* (Diptera: Culicidae) from Cuba and Venezuela. Journal of Medical Entomology 2001. 38: 623-628.

The Potential of Srigading Plants (Nyctanthes arbor-tristis) as Aedes aegypti Larvicides

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ABSTRACT

Dengue Hemorrhagic Fever (DHF) is an endemic disease that causes high morbidity and mortality rates. The prevalence of DHF in Indonesia (2018) reached 24.75 per 100,000 and in South Kalimantan (2019) it reached 56.83 per 100,000 population. One of the efforts to control dengue vectors is the use of larvicides. The purpose of this study was to determine the potential of Srigading Plants (*Nyctanthes arbor-tristis*) as Aedes aegypti larvae as vectors of DHF in Wetlands. This study used a true experimental research design using the post test only controlled group design to determine the effectiveness of Srigading extract (Nyctanthes arbor-tristis) against the death of Aedes aegypti instar IV mosquito larvae. Based on the results, a significance value or p-value of 0.0001 < 0.05 was obtained, which indicated that there was a significant difference between the number of mosquito larvae deaths and the concentration of Srigading extract given.

Keywords: Aedes Aegypti, Dengue Hemorrhagic Fever, Larvacides, Srigading plants, Vector

INTRODUCTION

DHF is a disease caused by a virus from the flaviviridae family which is transmitted through mosquito bites (arthropod borne viruses/arbovirus) namely Aedes aegypti and Aedes albopictus mosquitoes and can causing mortality if does not seriously taken care of (Kurniawan et al., 2017; Wang et al., 2020). DHF have clinical manifestations of fever, muscle or joint pain accompanied by leukopenia, rash, lymphodenopathy, and thrombocytopenia (Patel et al., 2018; Wang et al., 2020). The serotypes DEN-1, DEN-2, DEN-3 and DEN-4 are the cause of DHF which is transmitted by Aedes aegypti and Aedes albopictus mosquitoes that have been infected with the dengue virus (Yousaf et al., 2018). DHF is transmitted through the bite of female Aedes sp, where Aedes aegypti is the main vector and Aedes albopictus as the secondary vector in Indonesia, but for other countries such as Costa Rica Aedes albopictus is the main vector. DEN-1 virus can be found only in sporadic and endemic areas (Wanti et al., 2016). DEN-2 serotype is the highest serotype and is most often associated with severe cases (Faisal, 2018).

Globally, DHF is reported to range from 1-3 million cases while the number of deaths ranges from 2-4 thousand in the last decade (Espinal et al., 2019). According to data from the Ministry of Health of the Republic of Indonesia, the prevalence of DHF in 2016 was 78.85 per 100,000 population, in 2017 and 2018 it decreased to 26.1 per 100,000 population, and again decreased to 24.75 per 100,000 (Irma & Swaidatul, 2021). The morbidity rate of DHF according to data from the Health Office of South Kalimantan Province, namely in 2016 amounted to 106.21 per 100,000 population, then in 2017 there was a decrease to 13.49 per 100,000 population but increased again in 2018 by 47.91 and in 2019 by 56.83 per 100,000 population (Dinas Kesehatan Provinsi Kalimantan Selatan, 2020).

Vector control of DHF in Indonesia has not yet reached the program target. The target of the vector control program is 95%. The indicator of the DHF vector control program is the larva-free rate (ABJ). Nationally, ABJ in 2016 was 67.6%, in 2017 it decreased to 46.7% and then decreased again in 2018 which was 31.5% (Arisanti & Suryaningtyas, 2021; Kementerian Kesehatan RI, 2019). Based on data from the South Kalimantan Provincial Health Office, ABJ from 2015-2019 fluctuated with an increasing trend. The lowest ABJ in the five years was in 2016 at 83.85% and the highest was in 2017 at 89.08%. The lower the ABJ or below the program target, it means that the mosquito density will be higher. If the mosquito density is high, it will be a threat to increase the transmission of DHF in the future (Dinas Kesehatan Provinsi Kalimantan Selatan, 2020).

Vector control of DHF can be done in various ways, one of which is by using temephos (Fatimah & Hasmiwati, 2020). Temephos is still part of the government's program for eradicating mosquito nests, especially the Aedes aegypti mosquito with 3M plus (Minarti et al., 2021). The use of temephos for a long time will cause negative impacts such as environmental pollution and cause resistance (Araujo et al., 2016). On the other hand, this resistance results in ineffective vector control. Reports of resistance to Aedes aegypti larvae, where administration of temephos can harm humans by causing cancer in a number of body parts (Lesmana et al., 2022). Another chemical control that is also commonly used in the community is fumigation or fogging, which targets the adult Aedes aegypti mosquito. This method also has the same negative impact as the use of temephos, especially in the human lungs when inhaled directly (Bowman et al., 2016). To reduce this effect, then efforts to use natural larvicides for controlling Aedes Sp. larvae. In general, natural larvicides are defined as pesticides and the basic ingredients come from plants (Pratiwi, 2012).

The World Health Organization (WHO) recommends the development of natural vector control that is environmentally friendly because it will be safer for the environment and human health (World Health Organization, 2020). Therefore, natural larvicidation efforts continue to be developed from various plants that have potential as larvicides. Natural larvicides are obtained from secondary metabolites that function as a means of self-defense from attack and are known to be able to kill nuisance organisms (Wahyuni, 2021). Indonesia is a country that is rich in various types of plants. One of these plants is the Srigading plant (Nyctanthes arbor-tristis L). This plant usually thrives on the islands of Java and Kalimantan as an ornamental plant in tombs or graves. Apart from being an ornamental plant, Srigading plant is also used as a medicinal plant. Srigading contains high antioxidants. This Srigading plant contains secondary metabolites which include saponins, tannins, terpenoids and steroids (Mandasari et al., 2016).

Saponins can cause cell damage, interfere with metabolic processes and damage the outer protective barrier so that mosquito larvae will lose a lot of fluids (Tlak Gajger & Dar, 2021). Tannins can cause nutritional disturbances by reducing the activity of digestive enzymes in mosquito larvae (Nisrina, 2022). Terpenoids function as disruptors of cell membranes and tissues in mosquito larvae. Steroids function as growth hormones that work to inhibit growth by influencing skin turnover in mosquito larvae (Doughari, 2015). So far as literature searches have been carried out, there has been no research on the effect of Srigading extract (Nyctanthes arbor-tristis L) as a larvicide on Aedes aegypti mosquito larvae. Therefore, it is necessary to conduct research on Srigading extract (Nyctanthes arbor-tristis L) as a natural larvicide that can kill Aedes aegypti mosquito larvae as the basis for this research.

METHODS

This study used a true experimental research design using the post test only controlled group design to determine the effectiveness of Srigading extract (Nyctanthes arbor-tristis) against the death of Aedes aegypti instar IV mosquito larvae. In this study, five treatments were tested with concentrations of 448.5 ppm, 879 ppm, 1794 ppm, 3588 ppm, 7176 ppm and using two controls (positive and negative). In research, the selection of larval age is a very important activity. The age of mosquito larvae has a very influential factor on the resistance of the larvae to exposure to chemical substances (Adnyana et al., 2021). The age range of 2-5 days is the best age range and is in accordance with the Guidelines for Biological Insecticide Testing (Rahmawati et al., 2020). The longer the age of mosquito larvae, the higher their resistance and the more mature their physical condition (Şengül Demirak & Canpolat, 2022). Therefore, the subjects in this study were Aedes aegypti mosquito larvae instar IV 5 days old and had signs of life such as active movement. The fourth instar Aedes aegypti mosquito larvae were obtained from the Center for the Development and Research of Seasoned Soil Disease Vectors, South Kalimantan.

The research materials used were Srigading (Nyctanthes arbor-tristis), aquades, 70% ethanol, temephos, fish food and Aedes aegypti instar IV mosquito larvae which were colonized at the Entomology Laboratory of the Lokalitbang Center for the Development and Research of Spice Soil Disease Vector Reservoirs, South Kalimantan. The Srigading plant (Nyctanthes arbor-tristis) will be identified at the Laboratory of the Faculty of Medicine, University of Lambung Mangkurat Banjarbaru, this is done to avoid errors in the selection of plant species so that they can be accounted for. Extracts of Srigading (Nyctanthes arbor-tristis) were made by researchers Together with laboratory analysts of Agricultural Industrial Engineering (TIP) Faculty of Agriculture, University of Lambung Mangkurat Banjarbaru using the maceration

method. Preparation of stock solutions with One part per million (ppm) is a concentration of 1 mg of solute 1000 ml, then to make a stock solution of 13,903.5 ppm is by weighing the extract 13,903.5 mg of solute 1000 ml.

The results of the calculation of the number of deaths of Aedes aegypti mosquito larvae from seven treatments were tabulated in tabular form. The data were statistically analyzed using a computer program. Normality test was calculated using Shapiro-Wilk and homogeneity test was calculated using Levene's test. If the data is normally distributed and homogeneous, then the One Way ANOVA test is carried out with post Hoc Bonferroni. If the data is not normally distributed, the Kruskal-Wallis test is performed. If there is a difference in Kruskal-Wallis, then it is continued with the Mann-Whitney test. Determining the LC50 and LT50 values of Srigading Leaf extract was carried out using probit analysis with a 95% confidence level.

RESULTS AND DISCUSSION

The concentration of Srigading leaf extract (Nyctanthes arbor-tristis) in the larvicidal test was 448.5 ppm, 879 ppm, 1794 ppm, 3588 ppm, 7176 ppm, aquadest as a negative control and temephos as a positive control, which were exposed to Aedes aegypti larvae instar IV during 24 hours.

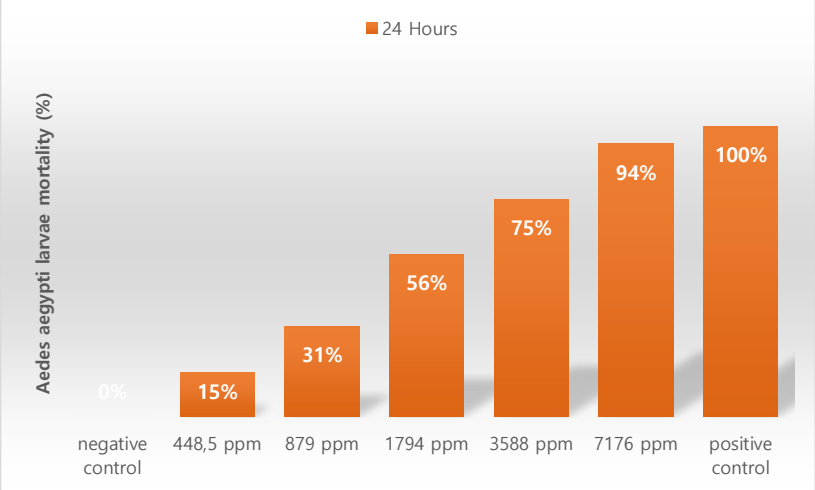

Figure 1. Effectiveness of Srigading Leaf Extract (Nyctanthes arbor-tristis) against fourth instar Aedes aegypti larvae for 24 hours of exposure

Figure 1 shows a graphic depiction of the percentage of mortality of fourth instar Aedes aegypti larvae after 24 hours of exposure to Srigading leaf extract (Nyctanthes arbor-tristis). In the negative control, there was no larval death so there was no need to correct the calculation of larval mortality using the Abbot formula. In the positive control using 1% temephos there was larval mortality with an average percentage of 100%. There was an increase in the mortality of Aedes aegypti larvae instar IV along with the increasing concentration of Srigading leaf extract (Nyctanthes arbor-tristis). The average larval mortality at a concentration of 448.5 ppm was 15%, at a concentration of 879 ppm was 31%, at a concentration of 3588 ppm was 56%, at a

concentration of 1794 ppm was 75% and at a concentration of 7176 ppm, Aedes aegypti larvae mortality was 94%.

Table 1. ANOVA test results

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1937.333	5	387.467	149.987	**.000**
Within Groups	46.500	18	2.583		
Total	1983.833	23			

Based on table 1, it can be seen that the mortality of Aedes aegypty larvae using the one way ANOVA test obtained a significance value or p-value of 0.0001 <0.05 which indicates that there is a significant difference between the number of mosquito larvae deaths and the concentration of Srigading extract given. Then a post-hoc Least Signification Difference (LSD) test was carried out to determine the location of the difference in the average larval mortality at each concentration and used as a reference in determining whether the average of the two treatment concentrations was statistically different or not, so that the concentration could be determined. which has larvicidal activity.

Table 2. LSD post-hoc test results

Consentration		*p-value*	Meaning
Negative Control	With 448,5 ppm	0,203	Not significant
	With 897 ppm	0,828	Not significant
	With 1794 ppm	1,000	Not significant
	With 3588 ppm	0,0001	Significant
	Positive Control	0,0001	Significant
448,5 ppm	Negative Control	0,203	Not significant
	With 897 ppm	0,286	Not significant
	With 1794 ppm	0,203	Not significant
	With 3588 pm	0,002	Significant
	Positive Control	0,0001	Significant
897 ppm	Negative Control	0,828	Not significant
	With 448,5 ppm	0,286	Not significant
	With 1794 ppm	0,828	Not significant
	With 3588 ppm	0,0001	Significant
	Positive Control	0,0001	Significant
1794 ppm	Negative Control	1,000	Not significant
	With 448,5 ppm	0,203	Not significant
	With 897 ppm	0,828	Not significant
	With 3588 ppm	0,0001	Significant
	Positive Control	0,0001	Significant
3588 ppm	Negative Control	0,0001	Significant
	With 448,5 ppm	0,002	Significant
	With 897 ppm	0,0001	Significant
	With 1794 ppm	0,0001	Significant
	Positive Control	0,0001	Significant
Positive Control	Negative Control	0,0001	Significant
	With 448,5 ppm	0,0001	Significant
	With 897 ppm	0,0001	Significant
	With 1794 ppm	0,0001	Significant
	With 3588 ppm	0,0001	Significant

Based on the results of the one way ANOVA test, a significance value or p-value of 0.0001 < 0.05 was obtained, which indicated that there was a significant difference between the number of mosquito larvae deaths and the concentration of Srigading extract given. Based on the results of the post-hoc test using LSD, it was found that there was a significant difference between the Srigading extract and the control group. The comparison found between the negative control and the concentration was found at a concentration of 3588 ppm, at that concentration there was a significant difference, this can be seen from the p-value or the significance value of 0.0001 compared to the value of <0.05. From these results it can be concluded that there is one concentration of Srigading leaf extract (Nycthanthes arbor-tritis) which has larvicidal activity.

Then, for the comparison between the positive control with concentrations of 448.5 ppm, 897 ppm, 1794 ppm, and 3588 ppm had the same significance value or p-value, namely 0.0001 with a value of <0.05, which means there is a significant difference. So, it can be concluded that at concentrations of 448.5 ppm, 897 ppm, 1794 ppm, and 3588 ppm, it does not have the same effectiveness as 1% temephos. The average percentage of Aedes aegypti larvae mortality was not affected by increasing concentration, so the higher concentration of Srigading leaf extract did not affect the mortality of the test larvae.

Death of Aedes agypti larvae occurred after treatment in the form of Srigading (Nycthanthes arbor-tritis) leaf extract, this was due to the larvicidal effect of Srigading (Nycthanthes arbor-tritis) leaves which contain secondary metabolites. The secondary metabolites contained in Srigading consist of alkaloids, flavonoids, saponins, tannins, and triterpenoids (Bhalakiya & Modi, 2019). Alkaloid secondary metabolites are one of a group of compounds that can be found in most types of plants. The way alkaloids work as insecticides is by inhibiting the activity of the acetylcholinesterase enzyme (Zaynab et al., 2018). This compound works by stimulating the endocrine glands to secrete juvenile hormones, this increase can cause metamorphosis failure in mosquito larvae resulting in abnormal death in mosquito larvae. In addition, in inhibiting cell mitosis, alkaloids can synergize with triterpenoid compounds (Khameneh et al., 2019).

The secondary metabolites of flavonoids are compounds that can damage the cytoplasmic membrane and cause cell leakage and can turn off the enzyme system (Jogawat et al., 2021). This of course can result in phospholipids being unable to maintain the shape of the cytoplasmic membrane itself until it will eventually burst and the larvae themselves will die (Turner, 2018). Saponin secondary metabolites are compounds that work by lowering the surface tension on the mucous membrane of the larval digestive tract. This will inhibit the rate of nutrient absorption by the larvae. In addition, saponins have another effect, namely destroying the chitin layer on the surface of the larvae so that the extract can easily enter the body of the larvae (Rohmah et al., 2020). The secondary metabolites of tannins are polyphenolic compounds that work by causing astringency in plant parts that can enter mosquito larvae through the body wall, causing disturbances in the larval muscles (Suryani et al., 2020). As a result, the larvae will experience weakness in the muscles of movement, so that the movement of the larvae will slow down. In addition to entering through the body wall, tannins can enter through the digestive tract of the larvae, this can cause interference with protein absorption in the larvae's intestines by reducing the activity of digestive enzymes and food absorption, so that the larvae will experience nutritional deficiencies and can cause death (Yusuf et al., 2020). Triterpenoid secondary metabolites are compounds that work by remembering free sterols in digestion where sterols act as precursors of the hormone ecdysone, so that a decrease in the amount of free sterols will disrupt the process of skin turnover in insects. In addition, these compounds can cause a decrease

in the activity of digestive enzymes and affect the process of food absorption (Arora & Sandhu, 2017).

Determining the LC50 and LC90 values from the ethanol extract of Srigading leaves (Nyctanthes arbor-tristis) was performed using Probit Analysis with a 95% confidence level. Probit analysis for LC50 and LC90 can be seen in table 3 as follows

Table 3. LC50 and LC90 values from the results of probit analysis of Srigading leaf ethanol extract (Nyctanthes arbor-tristis) against IV instar Aedes aegypti larvae after 24 hours

Mortality (%)	Estimate (%)	Confidence Level (%)	Confidence Interval	
			Lower Limit	Upper Limit
50	2.527	95	-9.649	3.507
90	5.460	95	4.389	20.775

Based on table 3, it can be seen that the results of the Probit analysis on the mortality rate of Aedes aegypti larvae obtained an LC50 value of 2.527%, this indicates that at a concentration of 2.527% within 24 hours it was able to kill 50% of the test larvae. While the obtained LC90 value of 5.460%, this shows that at a concentration of 5.460% within 24 hours it is able to kill 90% of the test larvae. Based on the results of the Probit analysis in table 3, the 24-hour LC50 value of Srigading leaf ethanol extract (Nyctanthes arbor-tristis) on the mortality of Aedes aegypti mosquito larvae was obtained at a concentration of 2.527%, which means that at a concentration of 2.527% ethanol extract of Srigading leaves (Nyctanthes arbor-tristis)) was able to kill 50% of Aedes aegypti larvae which were exposed for 24 hours, so it can be stated that the ethanol extract of Srigading leaves (Nyctanthes arbor-tristis) was effective against Aedes aegypti mosquito larvae with an LC50 value at 24 hours. While the 24-hour LC90 value of the ethanol extract of Srigading leaves (Nyctanthes arbor-tristis) on the mortality of Aedes aegypti mosquito larvae was obtained at a concentration of 5.460%, which means that at a concentration of 5.460% the ethanol extract of Srigading leaves (Nyctanthes arbor-tristis) was able to kill 90% of Aedes larvae. aegypti which was exposed for 24 hours, so it can be stated that the ethanol extract of Srigading leaves (Nyctanthes arbor-tristis) was effective against Aedes aegypti mosquito larvae with an LC90 value at 24 hours.

The results of this study showed that the ethanol extract of the leaves of Srigading (Nyctanthes arbor-tristis) had larvicidal activity against the larvae of the Aedes aegypti mosquito. However, according to previous studies, it is said that an extract of natural ingredients is effective as a pesticide if the LC50 is not more than 1000 ppm (0.1%). The LC50 value of the ethanol extract of Srigading leaves (Nyctanthes arbor-tristis) obtained was 2.527% or 25.270 ppm (conversion 1% = 10000 ppm), so it can be concluded that the ethanol extract of Srigading leaves (Nyctanthes arbor-tristis) still has high killing power, less against Aedes aegypti larvae. This may be caused by the form of the extract which is still in the form of crude extract or not in the form of pure compounds, so it is necessary to purify the compounds before they can be used as larvicides to obtain more optimal results.

The ethanol extract of Srigading leaves (Nyctanthes arbor-tristis) has larvicidal activity against Aedes aegypti mosquito larvae. Although the ability of Srigading leaf ethanol extract (Nyctanthes arbor-tristis) was still under positive control (temephos 1%), the ability of Srigading leaf ethanol extract (Nyctanthes arbor-tristis) could cause mortality of Aedes aegypti mosquito larvae, which was able to kill 50% of Aedes larvae. aegypti which was exposed for 24 hours with a concentration of 2.527%, this makes Srigading leaves (Nyctanthes arbor-tristis) have the

potential to be used as biolarvicides and are relatively safer for the environment and not persistent in nature, in contrast to temephos 1% which has the potential to cause pollution and the occurrence of resistance in larvae if not using the appropriate dose.

Conclusion

Srigading leaf extract (Nyctanthes arbor-tristis) has the effect of larvacide Aedes aegypti instar IV at a concentration of 448.5 ppm, 879 ppm, 3588 ppm, 1794 ppm and at a concentration of 7176 ppm it has the equivalent effectiveness of 1% temephos. With the discovery of natural ingredients from the Srigading plant (Nyctanthes arbor-tristis L) which functions as a natural larvicide, it will help the community to carry out 3M activities in overcoming the problem of DHF outbreak, especially for rural areas that are difficult to get access to. buy abate powder.

REFERENCES

Adnyana, I. M. D. M., Sudaryati, N. L. G., & Sitepu, I. (2021). Toxicity of Legiayu incense as Insecticide and Larvicide against Aedes aegypti Mosquitoes Mortality. *Indonesian Journal of Pharmacy*, *32*(4), 514–521.

Araujo, A. F. D. O., Ribeiro-Paes, J. T., Deus, J. T. D., Cavalcanti, S. C. D. H., Nunes, R. D. S., Alves, P. B., & Macoris, M. D. L. D. G. (2016). Larvicidal activity of Syzygium aromaticum (L.) Merr and Citrus sinensis (L.) Osbeck essential oils and their antagonistic effects with temephos in resistant populations of Aedes aegypti. *Memórias Do Instituto Oswaldo Cruz*, *111*(1), 443–449.

Arisanti, M., & Suryaningtyas, N. H. (2021). Kejadian demam berdarah dengue (DBD) di Indonesia tahun 2010-2019. *Spirakel*, *13*(1), 34–41.

Arora, R., & Sandhu, S. (2017). Insect-plant interrelationships. Breeding insect resistant crops for sustainable agriculture. *Springer*, 1–44.

Bhalakiya, H., & Modi, N. R. (2019). Traditional medicinal uses, phytochemical profile and pharmacological activities of Nyctanthes arbortris. *RJLBPCS*, *5*(1), 1003–1023.

Bowman, L. R., Donegan, S., & McCall, P. J. (2016). Is dengue vector control deficient in effectiveness or evidence?: Systematic review and meta-analysis. *PLoS Neglected Tropical Diseases*, *10*(3), 1–24.

Dinas Kesehatan Provinsi Kalimantan Selatan. (2020). *Data Demam Berdarah Dengeu (DBD) Provinsi Kalimantan Selatan*. Dinas Kesehatan Provinsi Kalimantan Selatan.

Doughari, J. (2015). An overview of plant immunity. *J. Plant Pathol. Microbiol*, *6*(11), 4110–4172.

Espinal, M. A., Andrus, J. K., Jauregui, B., Waterman, S. H., Morens, D. M., Santos, J. I., & Olson, D. (2019). Emerging and reemerging Aedes-transmitted arbovirus infections in the region of the Americas: implications for health policy. *American Journal of Public Health*, *109*(3), 387–392.

Faisal, A. P. (2018). Identifikasi Metabolit Sekunder dan Aktivitas Larvasida dari Daun Matahari (Helianthus annuus L.) terhadap Larva Aedes aegypti Sp. *Mahakam Medical Laboratory Technology Journal*, *2*(2), 80–90.

Fatimah, G., & Hasmiwati, R. R. (2020). Lethal concentration (LC50, 90, and 98) and lethal time (LT50, 90, and 98) at various temephos concentrations of Aedes aegypti L. larvae. *International Journal of Mosquito Research*, *7*(1), 1–3.

Irma, I., & Swaidatul, M. A. F. (2021). Trend Penyakit Demam Berdarah Dengue (DBD) di Sulawesi Tenggara Berbasis Ukuran Epidemiologi. *JUMANTIK (Jurnal Ilmiah Penelitian*

Commented [W1]: At least 80% of the references are international journal articles
Please substract 1 reference which block in yellow in order to meet that requirement

Kesehatan), *6*(1), 70–78.
Jogawat, A., Yadav, B., Lakra, N., Singh, A. K., & Narayan, O. P. (2021). Crosstalk between phytohormones and secondary metabolites in the drought stress tolerance of crop plants: a review. *Physiologia Plantarum*, *17*(2), 1106–1132.
Kementerian Kesehatan RI. (2019). *Profil Kesehatan Indonesia Tahun 2018*. Kementerian Kesehatan RI.
Khameneh, B., Iranshahy, M., Soheili, V., & Fazly Bazzaz, B. S. (2019). Review on plant antimicrobials: a mechanistic viewpoint. *Antimicrobial Resistance & Infection Control*, *8*(1), 1–28.
Kurniawan, M. E., Mohamed, A. M. D., Siyam, N., Fatikha, N., & Fitriani, N. A. (2017). Relation Between Knowledge and Attitude Regarding DHF with PSN Behavior Among the Community Around the Campus. *KEMAS: Jurnal Kesehatan Masyarakat*, *13*(2), 145–151.
Lesmana, S. D., Maryanti, E., Susanty, E., Afandi, D., Harmas, W., Octaviani, D. N., & Mislindawati, M. (2022). Organophosphate Resistance in Aedes aegypti: Study from Dengue Hemorrhagic Fever Endemic Subdistrict in Riau, Indonesia. *Reports of Biochemistry & Molecular Biology*, *10*(4), 589–596.
Mandasari, N., Hazar, S., & Sadiyah, E. R. (2016). Uji Aktivitas Sitotoksik Ekstrak Metanol Daun Srigading (Nyctanthes arbor-tristis L.). *Prosiding Farmasi*, 717–722.
Minarti, M., Anwar, C., Irfannuddin, I., & Irsan, C. (2021). Community Knowledge and Attitudes about the Transmission of Dengue Haemorrhagic Fever and Its Relationship to Prevention Behaviour in Palembang, South Sumatra, Indonesia. *Open Access Macedonian Journal of Medical Sciences*, *9*(5), 1534–1543.
Nisrina, H. (2022). Toxicity Assessment Of Avovo Leaf Extract (Persea Americana Miller) On Mortality Of Aedes Aegypti Larva. *International Journal of Health, Education & Social (IJHES)*, *5*(5), 1–7.
Patel, M. I., Patel, A., Patel, A., Patel, S., & Padsala, S. (2018). Study of haematological, biochemical profile and clinical presentation in dengue positive patients: 82 cases. *International Journal of Research in Medical Sciences*, *6*(6), 2099–2105.
Pratiwi, A. (2012). Penerimaan masyarakat terhadap larvasida alami. *KEMAS: Jurnal Kesehatan*, *8*(1), 88–93.
Rahmawati, U., Gustina, M., & Mirza, R. (2020). Efektivitas Anti Nyamuk Alami Elektrik Mat Serai Wangi (Cymbopogon Nardus) Dalam Mematikan Nyamuk Aedes Aegypti. *Journal of Nursing and Public Health*, *8*(2), 100–107.
Rohmah, E. A., Subekti, S., & Rudyanto, M. (2020). Larvicidal Activity and Histopathological Effect of Averrhoa bilimbi Fruit Extract on Aedes aegypti from Surabaya, Indonesia. *Journal of Parasitology Research*, 1–5.
Şengül Demirak, M. Ş., & Canpolat, E. (2022). Plant-based bioinsecticides for mosquito control: Impact on insecticide resistance and disease transmission. Insects. *Insects*, *13*(2), 1–24.
Suryani, A. I., Hariani, N., Majid, A. F., & Amalia, D. N. (2020). Histological changes in the midgut of Spodoptera litura larvae exposured by the extract of Mirabilis jalapa leaves. *In IOP Conference Series: Earth and Environmental Science*. *IOP Publishing*, *484*(1).
Tlak Gajger, I., & Dar, S. A. (2021). Plant allelochemicals as sources of insecticides. *Insects*, *12*(3), 1–21.
Turner, R. (2018). *Essentials of microbiology. Scientific e-Resources.*
Wahyuni, E. (2021). Potensi Eksudat Daun Sirih Merah (Piper ornatum L.) sebagai Insektisida

Herbal terhadap Mortalitas Semut Hitam. *Hydrogen: Jurnal Kependidikan Kimia*, *9*(2), 97–104.

Wang, W. H., Urbina, A. N., Chang, M. R., Assavalapsakul, W., Lu, P. L., Chen, Y. H., & Wang, S. F. (2020). Dengue hemorrhagic fever–A systemic literature review of current perspectives on pathogenesis, prevention and control. *Journal of Microbiology, Immunology and Infection*, *53*(6), 963–978.

Wanti, W., Sila, O., Irfan, I., & Sinaga, E. (2016). Transovarial transmission and dengue virus serotypes in Aedes aegypti in Kupang. *Kemas: Jurnal Kesehatan Masyarakat*, *12*(1), 131–138.

World Health Organization. (2020). *Dengue and Severe Dengue. Dengue Data Application*. World Health Organization Press. https://ntdhq.shinyapps.io/dengue5/

Yousaf, M. Z., Siddique, A., Ashfaq, U. A., & Ali, M. (2018). Scenario of dengue infection & its control in Pakistan: An up—date and way forward. *Asian Pacific Journal of Tropical Medicine*, *11*(1), 15–23.

Yusuf, Y., Efendi, K., & Diantasari, S. (2020). Larvicidal Activity Test of Ethanolic Extract of (Euphorbia tirucalli Linn) Stem on Aedes aegypti Larvae. *Systematic Reviews in Pharmacy*, *11*(3).

Zaynab, M., Fatima, M., Abbas, S., Sharif, Y., Umair, M., Zafar, M. H., & Bahadar, K. (2018). Role of secondary metabolites in plant defense against pathogens. *Microbial Pathogenesis*, *124*(1), 198–202.

The Potential of Srigading Plants (Nyctanthes arbor-tristis) as Aedes aegypti Larvicides

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ABSTRACT

Dengue Hemorrhagic Fever (DHF) is an endemic disease that causes high morbidity and mortality rates. The prevalence of DHF in Indonesia (2018) reached 24.75 per 100,000 and in South Kalimantan (2019) it reached 56.83 per 100,000 population. One of the efforts to control dengue vectors is the use of larvicides. The purpose of this study was to determine the potential of Srigading Plants (*Nyctanthes arbor-tristis*) as Aedes aegypti larvae as vectors of DHF in Wetlands. This study used a true experimental research design using the post test only controlled group design to determine the effectiveness of Srigading extract (Nyctanthes arbor-tristis) against the death of Aedes aegypti instar IV mosquito larvae. Based on the results, a significance value or p-value of 0.0001 < 0.05 was obtained, which indicated that there was a significant difference between the number of mosquito larvae deaths and the concentration of Srigading extract given.

Keywords: Aedes Aegypti, Dengue Hemorrhagic Fever, Larvacides, Srigading plants, Vector

INTRODUCTION

DHF is a disease caused by a virus from the flaviviridae family which is transmitted through mosquito bites (arthropod borne viruses/arbovirus) namely Aedes aegypti and Aedes albopictus mosquitoes and can causing mortality if does not seriously taken care of (Kurniawan et al., 2017; Wang et al., 2020). DHF have clinical manifestations of fever, muscle or joint pain accompanied by leukopenia, rash, lymphodenopathy, and thrombocytopenia (Patel et al., 2018; Wang et al., 2020). The serotypes DEN-1, DEN-2, DEN-3 and DEN-4 are the cause of DHF which is transmitted by Aedes aegypti and Aedes albopictus mosquitoes that have been infected with the dengue virus (Yousaf et al., 2018). DHF is transmitted through the bite of female Aedes sp, where Aedes aegypti is the main vector and Aedes albopictus as the secondary vector in Indonesia, but for other countries such as Costa Rica Aedes albopictus is the main vector. DEN-1 virus can be found only in sporadic and endemic areas (Wanti et al., 2016). DEN-2 serotype is the highest serotype and is most often associated with severe cases (Faisal, 2018).

Globally, DHF is reported to range from 1-3 million cases while the number of deaths ranges from 2-4 thousand in the last decade (Espinal et al., 2019). According to data from the Ministry of Health of the Republic of Indonesia, the prevalence of DHF in 2016 was 78.85 per 100,000 population, in 2017 and 2018 it decreased to 26.1 per 100,000 population, and again decreased to 24.75 per 100,000 (Bhalakiya, & Modi, 2019). The morbidity rate of DHF according to data from the Health Office of South Kalimantan Province, namely in 2016 amounted to 106.21 per 100,000 population, then in 2017 there was a decrease to 13.49 per 100,000 population but increased again in 2018 by 47.91 and in 2019 by 56.83 per 100,000 population (Dinas Kesehatan Provinsi Kalimantan Selatan, 2020).

Vector control of DHF in Indonesia has not yet reached the program target. The target of the vector control program is 95%. The indicator of the DHF vector control program is the larva-free rate (ABJ). Nationally, ABJ in 2016 was 67.6%, in 2017 it decreased to 46.7% and then decreased again in 2018 which was 31.5% (Bhalakiya & Modi, 2019). Based on data from the South Kalimantan Provincial Health Office, ABJ from 2015-2019 fluctuated with an increasing trend. The lowest ABJ in the five years was in 2016 at 83.85% and the highest was in 2017 at 89.08%. The lower the ABJ or below the program target, it means that the mosquito density will be higher. If the mosquito density is high, it will be a threat to increase the transmission of DHF in the future (Dinas Kesehatan Provinsi Kalimantan Selatan, 2020).

Vector control of DHF can be done in various ways, one of which is by using temephos (Fatimah & Hasmiwati, 2020). Temephos is still part of the government's program for eradicating mosquito nests, especially the Aedes aegypti mosquito with 3M plus (Minarti et al., 2021). The use of temephos for a long time will cause negative impacts such as environmental pollution and cause resistance (Araujo et al., 2016). On the other hand, this resistance results in ineffective vector control. Reports of resistance to Aedes aegypti larvae, where administration of temephos can harm humans by causing cancer in a number of body parts (Lesmana et al., 2022). Another chemical control that is also commonly used in the community is fumigation or fogging, which targets the adult Aedes aegypti mosquito. This method also has the same negative impact as the use of temephos, especially in the human lungs when inhaled directly. To reduce this effect, then efforts to use natural larvicides for controlling Aedes Sp. larvae. In general, natural larvicides are defined as pesticides and the basic ingredients come from plants (Bowman et al., 2016).

The World Health Organization (WHO) recommends the development of natural vector control that is environmentally friendly because it will be safer for the environment and human health (World Health Organization, 2020). Therefore, natural larvicidation efforts continue to be

developed from various plants that have potential as larvicides. Natural larvicides are obtained from secondary metabolites that function as a means of self-defense from attack and are known to be able to kill nuisance organisms (Wahyuni, 2021). Indonesia is a country that is rich in various types of plants. One of these plants is the Srigading plant (Nyctanthes arbor-tristis L). This plant usually thrives on the islands of Java and Kalimantan as an ornamental plant in tombs or graves. Apart from being an ornamental plant, Srigading plant is also used as a medicinal plant. Srigading contains high antioxidants. This Srigading plant contains secondary metabolites which include saponins, tannins, terpenoids and steroids (Mandasari et al., 2016).

Saponins can cause cell damage, interfere with metabolic processes and damage the outer protective barrier so that mosquito larvae will lose a lot of fluids (Tlak Gajger & Dar, 2021). Tannins can cause nutritional disturbances by reducing the activity of digestive enzymes in mosquito larvae (Nisrina, 2022). Terpenoids function as disruptors of cell membranes and tissues in mosquito larvae. Steroids function as growth hormones that work to inhibit growth by influencing skin turnover in mosquito larvae (Doughari, 2015). So far as literature searches have been carried out, there has been no research on the effect of Srigading extract (Nyctanthes arbor-tristis L) as a larvicide on Aedes aegypti mosquito larvae. Therefore, it is necessary to conduct research on Srigading extract (Nyctanthes arbor-tristis L) as a natural larvicide that can kill Aedes aegypti mosquito larvae as the basis for this research.

METHODS

This study used a true experimental research design using the post test only controlled group design to determine the effectiveness of Srigading extract (Nyctanthes arbor-tristis) against the death of Aedes aegypti instar IV mosquito larvae. In this study, five treatments were tested with concentrations of 448.5 ppm, 879 ppm, 1794 ppm, 3588 ppm, 7176 ppm and using two controls (positive and negative). In research, the selection of larval age is a very important activity. The age of mosquito larvae has a very influential factor on the resistance of the larvae to exposure to chemical substances (Adnyana et al., 2021). The age range of 2-5 days is the best age range and is in accordance with the Guidelines for Biological Insecticide Testing (Wahyuni, 2021). The longer the age of mosquito larvae, the higher their resistance and the more mature their physical condition (Şengül Demirak & Canpolat, 2022). Therefore, the subjects in this study were Aedes aegypti mosquito larvae instar IV 5 days old and had signs of life such as active movement. The fourth instar Aedes aegypti mosquito larvae were obtained from the Center for the Development and Research of Seasoned Soil Disease Vectors, South Kalimantan.

The research materials used were Srigading (Nyctanthes arbor-tristis), aquades, 70% ethanol, temephos, fish food and Aedes aegypti instar IV mosquito larvae which were colonized at the Entomology Laboratory of the Lokalitbang Center for the Development and Research of Spice Soil Disease Vector Reservoirs, South Kalimantan. The Srigading plant (Nyctanthes arbor-tristis) will be identified at the Laboratory of the Faculty of Medicine, University of Lambung Mangkurat Banjarbaru, this is done to avoid errors in the selection of plant species so that they can be accounted for. Extracts of Srigading (Nyctanthes arbor-tristis) were made by researchers Together with laboratory analysts of Agricultural Industrial Engineering (TIP) Faculty of Agriculture, University of Lambung Mangkurat Banjarbaru using the maceration method. Preparation of stock solutions with One part per million (ppm) is a concentration of 1 mg of solute 1000 ml, then to make a stock solution of 13,903.5 ppm is by weighing the extract 13,903.5 mg of solute 1000 ml.

The results of the calculation of the number of deaths of Aedes aegypti mosquito larvae from seven treatments were tabulated in tabular form. The data were statistically analyzed using a

computer program. Normality test was calculated using Shapiro-Wilk and homogeneity test was calculated using Levene's test. If the data is normally distributed and homogeneous, then the One Way ANOVA test is carried out with post Hoc Bonferroni. If the data is not normally distributed, the Kruskal-Wallis test is performed. If there is a difference in Kruskal-Wallis, then it is continued with the Mann-Whitney test. Determining the LC50 and LT50 values of Srigading Leaf extract was carried out using probit analysis with a 95% confidence level.

RESULTS AND DISCUSSION

The concentration of Srigading leaf extract (Nyctanthes arbor-tristis) in the larvicidal test was 448.5 ppm, 879 ppm, 1794 ppm, 3588 ppm, 7176 ppm, aquadest as a negative control and temephos as a positive control, which were exposed to Aedes aegypti larvae instar IV during 24 hours.

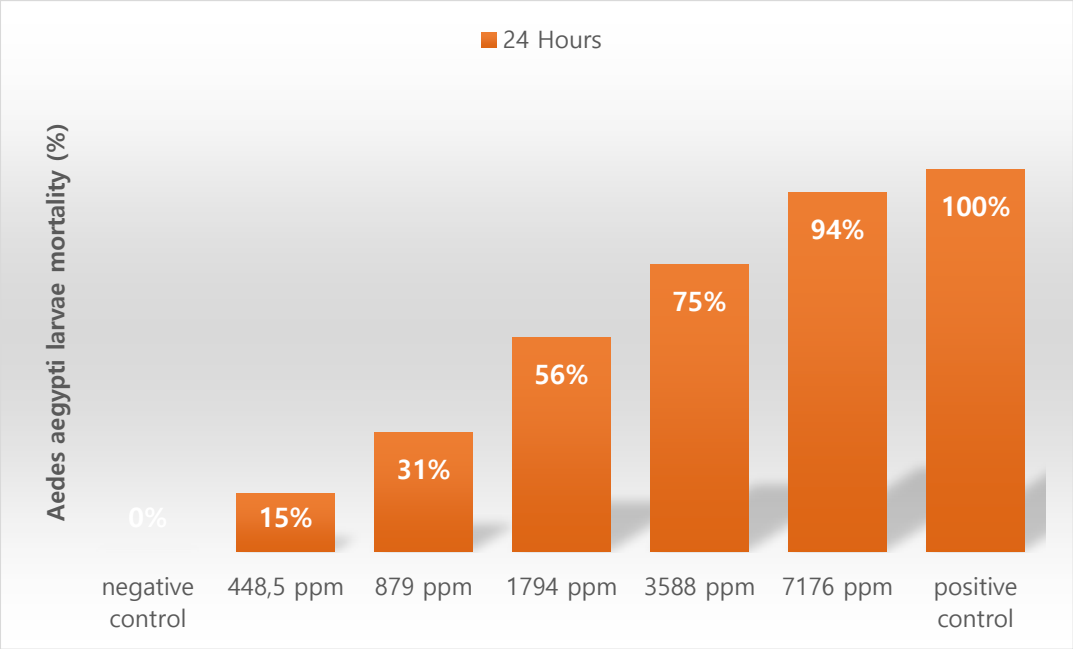

Figure 1. Effectiveness of Srigading Leaf Extract (Nyctanthes arbor-tristis) against fourth instar Aedes aegypti larvae for 24 hours of exposure

Figure 1 shows a graphic depiction of the percentage of mortality of fourth instar Aedes aegypti larvae after 24 hours of exposure to Srigading leaf extract (Nyctanthes arbor-tristis). In the negative control, there was no larval death so there was no need to correct the calculation of larval mortality using the Abbot formula. In the positive control using 1% temephos there was larval mortality with an average percentage of 100%. There was an increase in the mortality of Aedes aegypti larvae instar IV along with the increasing concentration of Srigading leaf extract (Nyctanthes arbor-tristis). The average larval mortality at a concentration of 448.5 ppm was 15%, at a concentration of 879 ppm was 31%, at a concentration of 3588 ppm was 56%, at a concentration of 1794 ppm was 75% and at a concentration of 7176 ppm, Aedes aegypti larvae mortality was 94%.

Table 1. ANOVA test results

	Sum of Squares	df	Mean Square	F	Sig.

Between Groups	1937.333	5	387.467	149.987	**.000**
Within Groups	46.500	18	2.583		
Total	1983.833	23			

Based on table 1, it can be seen that the mortality of Aedes aegypty larvae using the one way ANOVA test obtained a significance value or p-value of 0.0001 <0.05 which indicates that there is a significant difference between the number of mosquito larvae deaths and the concentration of Srigading extract given. Then a post-hoc Least Signification Difference (LSD) test was carried out to determine the location of the difference in the average larval mortality at each concentration and used as a reference in determining whether the average of the two treatment concentrations was statistically different or not, so that the concentration could be determined. which has larvicidal activity.

Table 2. LSD post-hoc test results

Consentration		***p-value***	**Meaning**
Negative Control	With 448,5 ppm	0,203	Not significant
	With 897 ppm	0,828	Not significant
	With 1794 ppm	1,000	Not significant
	With 3588 ppm	0,0001	Significant
	Positive Control	0,0001	Significant
448,5 ppm	Negative Control	0,203	Not significant
	With 897 ppm	0,286	Not significant
	With 1794 ppm	0,203	Not significant
	With 3588 pm	0,002	Significant
	Positive Control	0,0001	Significant
897 ppm	Negative Control	0,828	Not significant
	With 448,5 ppm	0,286	Not significant
	With 1794 ppm	0,828	Not significant
	With 3588 ppm	0,0001	Significant
	Positive Control	0,0001	Significant
1794 ppm	Negative Control	1,000	Not significant
	With 448,5 ppm	0,203	Not significant
	With 897 ppm	0,828	Not significant
	With 3588 ppm	0,0001	Significant
	Positive Control	0,0001	Significant
3588 ppm	Negative Control	0,0001	Significant
	With 448,5 ppm	0,002	Significant
	With 897 ppm	0,0001	Significant
	With 1794 ppm	0,0001	Significant
	Positive Control	0,0001	Significant
Positive Control	Negative Control	0,0001	Significant
	With 448,5 ppm	0,0001	Significant
	With 897 ppm	0,0001	Significant
	With 1794 ppm	0,0001	Significant
	With 3588 ppm	0,0001	Significant

Based on the results of the one way ANOVA test, a significance value or p-value of 0.0001 < 0.05 was obtained, which indicated that there was a significant difference between the number of mosquito larvae deaths and the concentration of Srigading extract given. Based on the results of the post-hoc test using LSD, it was found that there was a significant difference between the Srigading extract and the control group. The comparison found between the negative control and

the concentration was found at a concentration of 3588 ppm, at that concentration there was a significant difference, this can be seen from the p-value or the significance value of 0.0001 compared to the value of <0.05. From these results it can be concluded that there is one concentration of Srigading leaf extract (Nycthanthes arbor-tritis) which has larvicidal activity.

Then, for the comparison between the positive control with concentrations of 448.5 ppm, 897 ppm, 1794 ppm, and 3588 ppm had the same significance value or p-value, namely 0.0001 with a value of <0.05, which means there is a significant difference. So, it can be concluded that at concentrations of 448.5 ppm, 897 ppm, 1794 ppm, and 3588 ppm, it does not have the same effectiveness as 1% temephos. The average percentage of Aedes aegypti larvae mortality was not affected by increasing concentration, so the higher concentration of Srigading leaf extract did not affect the mortality of the test larvae.

Death of Aedes agypti larvae occurred after treatment in the form of Srigading (Nycthanthes arbor-tritis) leaf extract, this was due to the larvicidal effect of Srigading (Nycthanthes arbor-tritis) leaves which contain secondary metabolites. The secondary metabolites contained in Srigading consist of alkaloids, flavonoids, saponins, tannins, and triterpenoids (Bhalakiya & Modi, 2019). Alkaloid secondary metabolites are one of a group of compounds that can be found in most types of plants. The way alkaloids work as insecticides is by inhibiting the activity of the acetylcholinesterase enzyme (Zaynab et al., 2018). This compound works by stimulating the endocrine glands to secrete juvenile hormones, this increase can cause metamorphosis failure in mosquito larvae resulting in abnormal death in mosquito larvae. In addition, in inhibiting cell mitosis, alkaloids can synergize with triterpenoid compounds (Khameneh et al., 2019).

The secondary metabolites of flavonoids are compounds that can damage the cytoplasmic membrane and cause cell leakage and can turn off the enzyme system (Jogawat et al., 2021). This of course can result in phospholipids being unable to maintain the shape of the cytoplasmic membrane itself until it will eventually burst and the larvae themselves will die (Tlak Gajger & Dar, 2021). Saponin secondary metabolites are compounds that work by lowering the surface tension on the mucous membrane of the larval digestive tract. This will inhibit the rate of nutrient absorption by the larvae. In addition, saponins have another effect, namely destroying the chitin layer on the surface of the larvae so that the extract can easily enter the body of the larvae (Rohmah et al., 2020). The secondary metabolites of tannins are polyphenolic compounds that work by causing astringency in plant parts that can enter mosquito larvae through the body wall, causing disturbances in the larval muscles (Suryani et al., 2020). As a result, the larvae will experience weakness in the muscles of movement, so that the movement of the larvae will slow down. In addition to entering through the body wall, tannins can enter through the digestive tract of the larvae, this can cause interference with protein absorption in the larvae's intestines by reducing the activity of digestive enzymes and food absorption, so that the larvae will experience nutritional deficiencies and can cause death (Yusuf et al., 2020). Triterpenoid secondary metabolites are compounds that work by remembering free sterols in digestion where sterols act as precursors of the hormone ecdysone, so that a decrease in the amount of free sterols will disrupt the process of skin turnover in insects. In addition, these compounds can cause a decrease in the activity of digestive enzymes and affect the process of food absorption (Bhalakiya & Modi, 2019).

Determining the LC50 and LC90 values from the ethanol extract of Srigading leaves (Nyctanthes arbor-tristis) was performed using Probit Analysis with a 95% confidence level. Probit analysis for LC50 and LC90 can be seen in table 3 as follows

Table 3. LC50 and LC90 values from the results of probit analysis of Srigading leaf ethanol

extract (Nyctanthes arbor-tristis) against IV instar Aedes aegypti larvae after 24 hours

Mortality (%)	Estimate (%)	Confidence Level (%)	Confidence Interval	
			Lower Limit	Upper Limit
50	2.527	95	-9.649	3.507
90	5.460	95	4.389	20.775

Based on table 3, it can be seen that the results of the Probit analysis on the mortality rate of Aedes aegypti larvae obtained an LC50 value of 2.527%, this indicates that at a concentration of 2.527% within 24 hours it was able to kill 50% of the test larvae. While the obtained LC90 value of 5.460%, this shows that at a concentration of 5.460% within 24 hours it is able to kill 90% of the test larvae. Based on the results of the Probit analysis in table 3, the 24-hour LC50 value of Srigading leaf ethanol extract (Nyctanthes arbor-tristis) on the mortality of Aedes aegypti mosquito larvae was obtained at a concentration of 2.527%, which means that at a concentration of 2.527% ethanol extract of Srigading leaves (Nyctanthes arbor-tristis)) was able to kill 50% of Aedes aegypti larvae which were exposed for 24 hours, so it can be stated that the ethanol extract of Srigading leaves (Nyctanthes arbor-tristis) was effective against Aedes aegypti mosquito larvae with an LC50 value at 24 hours. While the 24-hour LC90 value of the ethanol extract of Srigading leaves (Nyctanthes arbor-tristis) on the mortality of Aedes aegypti mosquito larvae was obtained at a concentration of 5.460%, which means that at a concentration of 5.460% the ethanol extract of Srigading leaves (Nyctanthes arbor-tristis) was able to kill 90% of Aedes larvae. aegypti which was exposed for 24 hours, so it can be stated that the ethanol extract of Srigading leaves (Nyctanthes arbor-tristis) was effective against Aedes aegypti mosquito larvae with an LC90 value at 24 hours.

The results of this study showed that the ethanol extract of the leaves of Srigading (Nyctanthes arbor-tristis) had larvicidal activity against the larvae of the Aedes aegypti mosquito. However, according to previous studies, it is said that an extract of natural ingredients is effective as a pesticide if the LC50 is not more than 1000 ppm (0.1%). The LC50 value of the ethanol extract of Srigading leaves (Nyctanthes arbor-tristis) obtained was 2.527% or 25.270 ppm (conversion 1% = 10000 ppm), so it can be concluded that the ethanol extract of Srigading leaves (Nyctanthes arbor-tristis) still has high killing power, less against Aedes aegypti larvae. This may be caused by the form of the extract which is still in the form of crude extract or not in the form of pure compounds, so it is necessary to purify the compounds before they can be used as larvicides to obtain more optimal results.

The ethanol extract of Srigading leaves (Nyctanthes arbor-tristis) has larvicidal activity against Aedes aegypti mosquito larvae. Although the ability of Srigading leaf ethanol extract (Nyctanthes arbor-tristis) was still under positive control (temephos 1%), the ability of Srigading leaf ethanol extract (Nyctanthes arbor-tristis) could cause mortality of Aedes aegypti mosquito larvae, which was able to kill 50% of Aedes larvae. aegypti which was exposed for 24 hours with a concentration of 2.527%, this makes Srigading leaves (Nyctanthes arbor-tristis) have the potential to be used as biolarvicides and are relatively safer for the environment and not persistent in nature, in contrast to temephos 1% which has the potential to cause pollution and the occurrence of resistance in larvae if not using the appropriate dose.

Conclusion

Srigading leaf extract (Nyctanthes arbor-tristis) has the effect of larvacide Aedes aegypti instar IV at a concentration of 448.5 ppm, 879 ppm, 3588 ppm, 1794 ppm and at a concentration

of 7176 ppm it has the equivalent effectiveness of 1% temephos. With the discovery of natural ingredients from the Srigading plant (Nyctanthes arbor-tristis L) which functions as a natural larvicide, it will help the community to carry out 3M activities in overcoming the problem of DHF outbreak, especially for rural areas that are difficult to get access to. buy abate powder.

REFERENCES

Adnyana, I. M. D. M., Sudaryati, N. L. G., & Sitepu, I. (2021). Toxicity of Legiayu incense as Insecticide and Larvicide against Aedes aegypti Mosquitoes Mortality. *Indonesian Journal of Pharmacy*, *32*(4), 514–521.

Araujo, A. F. D. O., Ribeiro-Paes, J. T., Deus, J. T. D., Cavalcanti, S. C. D. H., Nunes, R. D. S., Alves, P. B., & Macoris, M. D. L. D. G. (2016). Larvicidal activity of Syzygium aromaticum (L.) Merr and Citrus sinensis (L.) Osbeck essential oils and their antagonistic effects with temephos in resistant populations of Aedes aegypti. *Memórias Do Instituto Oswaldo Cruz*, *111*(1), 443–449.

Bhalakiya, H., & Modi, N. R. (2019). Traditional medicinal uses, phytochemical profile and pharmacological activities of Nyctanthes arbortris. *RJLBPCS*, *5*(1), 1003–1023.

Bowman, L. R., Donegan, S., & McCall, P. J. (2016). Is dengue vector control deficient in effectiveness or evidence?: Systematic review and meta-analysis. *PLoS Neglected Tropical Diseases*, *10*(3), 1–24.

Dinas Kesehatan Provinsi Kalimantan Selatan. (2020). *Data Demam Berdarah Dengeu (DBD) Provinsi Kalimantan Selatan*. Dinas Kesehatan Provinsi Kalimantan Selatan.

Doughari, J. (2015). An overview of plant immunity. *J. Plant Pathol. Microbiol*, *6*(11), 4110–4172.

Espinal, M. A., Andrus, J. K., Jauregui, B., Waterman, S. H., Morens, D. M., Santos, J. I., & Olson, D. (2019). Emerging and reemerging Aedes-transmitted arbovirus infections in the region of the Americas: implications for health policy. *American Journal of Public Health*, *109*(3), 387–392.

Faisal, A. P. (2018). Identifikasi Metabolit Sekunder dan Aktivitas Larvasida dari Daun Matahari (Helianthus annuus L.) terhadap Larva Aedes aegypti Sp. *Mahakam Medical Laboratory Technology Journal*, *2*(2), 80–90.

Fatimah, G., & Hasmiwati, R. R. (2020). Lethal concentration (LC50, 90, and 98) and lethal time (LT50, 90, and 98) at various temephos concentrations of Aedes aegypti L. larvae. *International Journal of Mosquito Research*, *7*(1), 1–3.

Jogawat, A., Yadav, B., Lakra, N., Singh, A. K., & Narayan, O. P. (2021). Crosstalk between phytohormones and secondary metabolites in the drought stress tolerance of crop plants: a review. *Physiologia Plantarum*, *17*(2), 1106–1132.

Khameneh, B., Iranshahy, M., Soheili, V., & Fazly Bazzaz, B. S. (2019). Review on plant antimicrobials: a mechanistic viewpoint. *Antimicrobial Resistance & Infection Control*, *8*(1), 1–28.

Kurniawan, M. E., Mohamed, A. M. D., Siyam, N., Fatikha, N., & Fitriani, N. A. (2017). Relation Between Knowledge and Attitude Regarding DHF with PSN Behavior Among the Community Around the Campus. *KEMAS: Jurnal Kesehatan Masyarakat*, *13*(2), 145–151.

Lesmana, S. D., Maryanti, E., Susanty, E., Afandi, D., Harmas, W., Octaviani, D. N., & Mislindawati, M. (2022). Organophosphate Resistance in Aedes aegypti: Study from Dengue Hemorrhagic Fever Endemic Subdistrict in Riau, Indonesia. *Reports of Biochemistry & Molecular Biology*, *10*(4), 589–596.

Mandasari, N., Hazar, S., & Sadiyah, E. R. (2016). Uji Aktivitas Sitotoksik Ekstrak Metanol Daun

Srigading (Nyctanthes arbor-tristis L.). *Prosiding Farmasi*, 717–722.
Minarti, M., Anwar, C., Irfannuddin, I., & Irsan, C. (2021). Community Knowledge and Attitudes about the Transmission of Dengue Haemorrhagic Fever and Its Relationship to Prevention Behaviour in Palembang, South Sumatra, Indonesia. *Open Access Macedonian Journal of Medical Sciences*, *9*(5), 1534–1543.
Nisrina, H. (2022). Toxicity Assessment Of Avovo Leaf Extract (Persea Americana Miller) On Mortality Of Aedes Aegypti Larva. *International Journal of Health, Education & Social (IJHES)*, *5*(5), 1–7.
Patel, M. I., Patel, A., Patel, A., Patel, S., & Padsala, S. (2018). Study of haematological, biochemical profile and clinical presentation in dengue positive patients: 82 cases. *International Journal of Research in Medical Sciences*, *6*(6), 2099–2105.
Rohmah, E. A., Subekti, S., & Rudyanto, M. (2020). Larvicidal Activity and Histopathological Effect of Averrhoa bilimbi Fruit Extract on Aedes aegypti from Surabaya, Indonesia. *Journal of Parasitology Research*, 1–5.
Şengül Demirak, M. Ş., & Canpolat, E. (2022). Plant-based bioinsecticides for mosquito control: Impact on insecticide resistance and disease transmission. Insects. *Insects*, *13*(2), 1–24.
Suryani, A. I., Hariani, N., Majid, A. F., & Amalia, D. N. (2020). Histological changes in the midgut of Spodoptera litura larvae exposured by the extract of Mirabilis jalapa leaves. *In IOP Conference Series: Earth and Environmental Science. IOP Publishing*, *484*(1).
Tlak Gajger, I., & Dar, S. A. (2021). Plant allelochemicals as sources of insecticides. *Insects*, *12*(3), 1–21.
Wahyuni, E. (2021). Potensi Eksudat Daun Sirih Merah (Piper ornatum L.) sebagai Insektisida Herbal terhadap Mortalitas Semut Hitam. *Hydrogen: Jurnal Kependidikan Kimia*, *9*(2), 97–104.
Wang, W. H., Urbina, A. N., Chang, M. R., Assavalapsakul, W., Lu, P. L., Chen, Y. H., & Wang, S. F. (2020). Dengue hemorrhagic fever–A systemic literature review of current perspectives on pathogenesis, prevention and control. *Journal of Microbiology, Immunology and Infection*, *53*(6), 963–978.
Wanti, W., Sila, O., Irfan, I., & Sinaga, E. (2016). Transovarial transmission and dengue virus serotypes in Aedes aegypti in Kupang. *Kemas: Jurnal Kesehatan Masyarakat*, *12*(1), 131–138.
World Health Organization. (2020). *Dengue and Severe Dengue. Dengue Data Application*. World Health Organization Press. https://ntdhq.shinyapps.io/dengue5/
Yousaf, M. Z., Siddique, A., Ashfaq, U. A., & Ali, M. (2018). Scenario of dengue infection & its control in Pakistan: An up—date and way forward. *Asian Pacific Journal of Tropical Medicine*, *11*(1), 15–23.
Yusuf, Y., Efendi, K., & Diantasari, S. (2020). Larvicidal Activity Test of Ethanolic Extract of (Euphorbia tirucalli Linn) Stem on Aedes aegypti Larvae. *Systematic Reviews in Pharmacy*, *11*(3).
Zaynab, M., Fatima, M., Abbas, S., Sharif, Y., Umair, M., Zafar, M. H., & Bahadar, K. (2018). Role of secondary metabolites in plant defense against pathogens. *Microbial Pathogenesis*, *124*(1), 198–202.

The Potential of Srigading Plants (Nyctanthes arbor-tristis) as Aedes aegypti Larvicides

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ABSTRACT

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Dengue Hemorrhagic Fever (DHF) is an endemic disease that causes high morbidity and mortality rates. The prevalence of DHF in Indonesia (2018) reached 24.75 per 100,000 and in South Kalimantan (2019) it reached 56.83 per 100,000 population. One of the efforts to control dengue vectors is the use of larvicides. The purpose of this study was to determine the potential of Srigading Plants (*Nyctanthes arbor-tristis*) as Aedes aegypti larvae as vectors of DHF in Wetlands. This study used a true experimental research design using the post test only controlled group design to determine the effectiveness of Srigading extract (Nyctanthes arbor-tristis) against the death of Aedes aegypti instar IV mosquito larvae. Based on the results, a significance value or p-value of 0.0001 < 0.05 was obtained, which indicated that there was a significant difference between the number of mosquito larvae deaths and the concentration of Srigading extract given.

Keywords: Aedes Aegypti, Dengue Hemorrhagic Fever, Larvacides, Srigading plants, Vector

INTRODUCTION

DHF is a disease caused by a virus from the flaviviridae family which is transmitted through mosquito bites (arthropod borne viruses/arbovirus) namely Aedes aegypti and Aedes albopictus mosquitoes and can causing mortality if does not seriously taken care of (Kurniawan et al., 2017; Wang et al., 2020). DHF have clinical manifestations of fever, muscle or joint pain accompanied by leukopenia, rash, lymphodenopathy, and thrombocytopenia (Patel et al., 2018; Wang et al., 2020). The serotypes DEN-1, DEN-2, DEN-3 and DEN-4 are the cause of DHF which is transmitted by Aedes aegypti and Aedes albopictus mosquitoes that have been infected with the dengue virus (Yousaf et al., 2018). DHF is transmitted through the bite of female Aedes sp, where Aedes aegypti is the main vector and Aedes albopictus as the secondary vector in Indonesia, but for other countries such as Costa Rica Aedes albopictus is the main vector. DEN-1 virus can be found only in sporadic and endemic areas (Wanti et al., 2016). DEN-2 serotype is the highest serotype and is most often associated with severe cases (Faisal, 2018).

Globally, DHF is reported to range from 1-3 million cases while the number of deaths ranges from 2-4 thousand in the last decade (Espinal et al., 2019). According to data from the Ministry of Health of the Republic of Indonesia, the prevalence of DHF in 2016 was 78.85 per 100,000 population, in 2017 and 2018 it decreased to 26.1 per 100,000 population, and again decreased to 24.75 per 100,000 (Irma & Swaidatul, 2021). The morbidity rate of DHF according to data from the Health Office of South Kalimantan Province, namely in 2016 amounted to 106.21 per 100,000 population, then in 2017 there was a decrease to 13.49 per 100,000 population but increased again in 2018 by 47.91 and in 2019 by 56.83 per 100,000 population (Dinas Kesehatan Provinsi Kalimantan Selatan, 2020).

Vector control of DHF in Indonesia has not yet reached the program target. The target of the vector control program is 95%. The indicator of the DHF vector control program is the larva-free rate (ABJ). Nationally, ABJ in 2016 was 67.6%, in 2017 it decreased to 46.7% and then decreased again in 2018 which was 31.5% (Arisanti & Suryaningtyas, 2021; Kementerian Kesehatan RI, 2019). Based on data from the South Kalimantan Provincial Health Office, ABJ from 2015-2019 fluctuated with an increasing trend. The lowest ABJ in the five years was in 2016 at 83.85% and the highest was in 2017 at 89.08%. The lower the ABJ or below the program target, it means that the mosquito density will be higher. If the mosquito density is high, it will be a threat to increase the transmission of DHF in the future (Dinas Kesehatan Provinsi Kalimantan Selatan, 2020).

Vector control of DHF can be done in various ways, one of which is by using temephos (Fatimah & Hasmiwati, 2020). Temephos is still part of the government's program for eradicating mosquito nests, especially the Aedes aegypti mosquito with 3M plus (Minarti et al., 2021). The use of temephos for a long time will cause negative impacts such as environmental pollution and cause resistance (Araujo et al., 2016). On the other hand, this resistance results in ineffective vector control. Reports of resistance to Aedes aegypti larvae, where administration of temephos can harm humans by causing cancer in a number of body parts (Lesmana et al., 2022). Another chemical control that is also commonly used in the community is fumigation or fogging, which targets the adult Aedes aegypti mosquito. This method also has the same negative impact as the use of temephos, especially in the human lungs when inhaled directly (Bowman et al., 2016). To reduce this effect, then efforts to use natural larvicides for controlling Aedes Sp. larvae. In general, natural larvicides are defined as pesticides and the basic ingredients come from plants (Pratiwi, 2012).

The World Health Organization (WHO) recommends the development of natural vector control that is environmentally friendly because it will be safer for the environment and human health (World Health Organization, 2020). Therefore, natural larvicidation efforts continue to be developed from various plants that have potential as larvicides. Natural larvicides are obtained from secondary metabolites that function as a means of self-defense from attack and are known to be able to kill nuisance organisms (Wahyuni, 2021). Indonesia is a country that is rich in various types of plants. One of these plants is the Srigading plant (Nyctanthes arbor-tristis L). This plant usually thrives on the islands of Java and Kalimantan as an ornamental plant in tombs or graves. Apart from being an ornamental plant, Srigading plant is also used as a medicinal plant. Srigading contains high antioxidants. This Srigading plant contains secondary metabolites which include saponins, tannins, terpenoids and steroids (Mandasari et al., 2016).

Saponins can cause cell damage, interfere with metabolic processes and damage the outer protective barrier so that mosquito larvae will lose a lot of fluids (Tlak Gajger & Dar, 2021). Tannins can cause nutritional disturbances by reducing the activity of digestive enzymes in mosquito larvae (Nisrina, 2022). Terpenoids function as disruptors of cell membranes and tissues in mosquito larvae. Steroids function as growth hormones that work to inhibit growth by influencing skin turnover in mosquito larvae (Doughari, 2015). So far as literature searches have been carried out, there has been no research on the effect of Srigading extract (Nyctanthes arbor-tristis L) as a larvicide on Aedes aegypti mosquito larvae. Therefore, it is necessary to conduct research on Srigading extract (Nyctanthes arbor-tristis L) as a natural larvicide that can kill Aedes aegypti mosquito larvae as the basis for this research.

METHODS

This study used a true experimental research design using the post test only controlled group design to determine the effectiveness of Srigading extract (Nyctanthes arbor-tristis) against the death of Aedes aegypti instar IV mosquito larvae. In this study, five treatments were tested with concentrations of 448.5 ppm, 879 ppm, 1794 ppm, 3588 ppm, 7176 ppm and using two controls (positive and negative). In research, the selection of larval age is a very important activity. The age of mosquito larvae has a very influential factor on the resistance of the larvae to exposure to chemical substances (Adnyana et al., 2021). The age range of 2-5 days is the best age range and is in accordance with the Guidelines for Biological Insecticide Testing (Rahmawati et al., 2020). The longer the age of mosquito larvae, the higher their resistance and the more mature their physical condition (Şengül Demirak & Canpolat, 2022). Therefore, the subjects in this study were Aedes aegypti mosquito larvae instar IV 5 days old and had signs of life such as active movement. The fourth instar Aedes aegypti mosquito larvae were obtained from the Center for the Development and Research of Seasoned Soil Disease Vectors, South Kalimantan.

The research materials used were Srigading (Nyctanthes arbor-tristis), aquades, 70% ethanol, temephos, fish food and Aedes aegypti instar IV mosquito larvae which were colonized at the Entomology Laboratory of the Lokalitbang Center for the Development and Research of Spice Soil Disease Vector Reservoirs, South Kalimantan. The Srigading plant (Nyctanthes arbor-tristis) will be identified at the Laboratory of the Faculty of Medicine, University of Lambung Mangkurat Banjarbaru, this is done to avoid errors in the selection of plant species so that they can be accounted for. Extracts of Srigading (Nyctanthes arbor-tristis) were made by researchers Together with laboratory analysts of Agricultural Industrial Engineering (TIP) Faculty of Agriculture, University of Lambung Mangkurat Banjarbaru using the maceration

method. Preparation of stock solutions with One part per million (ppm) is a concentration of 1 mg of solute 1000 ml, then to make a stock solution of 13,903.5 ppm is by weighing the extract 13,903.5 mg of solute 1000 ml.

The results of the calculation of the number of deaths of Aedes aegypti mosquito larvae from seven treatments were tabulated in tabular form. The data were statistically analyzed using a computer program. Normality test was calculated using Shapiro-Wilk and homogeneity test was calculated using Levene's test. If the data is normally distributed and homogeneous, then the One Way ANOVA test is carried out with post Hoc Bonferroni. If the data is not normally distributed, the Kruskal-Wallis test is performed. If there is a difference in Kruskal-Wallis, then it is continued with the Mann-Whitney test. Determining the LC50 and LT50 values of Srigading Leaf extract was carried out using probit analysis with a 95% confidence level.

RESULTS AND DISCUSSION

The concentration of Srigading leaf extract (Nyctanthes arbor-tristis) in the larvicidal test was 448.5 ppm, 879 ppm, 1794 ppm, 3588 ppm, 7176 ppm, aquadest as a negative control and temephos as a positive control, which were exposed to Aedes aegypti larvae instar IV during 24 hours.

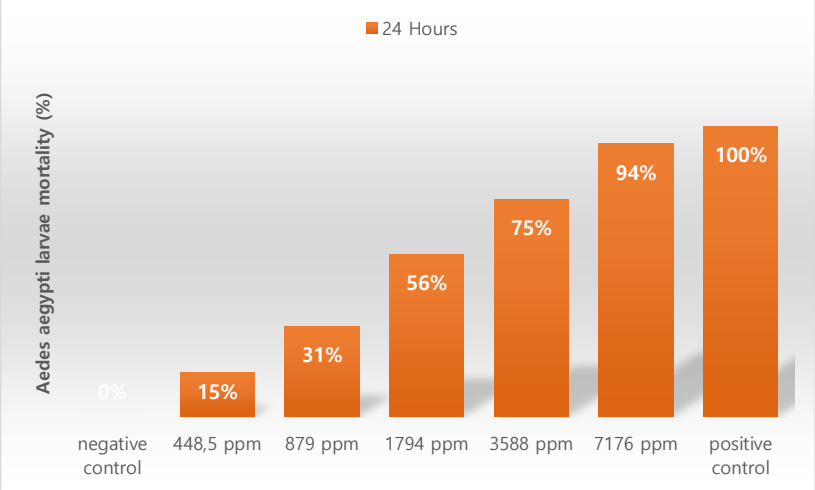

Figure 1. Effectiveness of Srigading Leaf Extract (Nyctanthes arbor-tristis) against fourth instar Aedes aegypti larvae for 24 hours of exposure

Figure 1 shows a graphic depiction of the percentage of mortality of fourth instar Aedes aegypti larvae after 24 hours of exposure to Srigading leaf extract (Nyctanthes arbor-tristis). In the negative control, there was no larval death so there was no need to correct the calculation of larval mortality using the Abbot formula. In the positive control using 1% temephos there was larval mortality with an average percentage of 100%. There was an increase in the mortality of Aedes aegypti larvae instar IV along with the increasing concentration of Srigading leaf extract (Nyctanthes arbor-tristis). The average larval mortality at a concentration of 448.5 ppm was 15%, at a concentration of 879 ppm was 31%, at a concentration of 3588 ppm was 56%, at a

concentration of 1794 ppm was 75% and at a concentration of 7176 ppm, Aedes aegypti larvae mortality was 94%.

Table 1. ANOVA test results

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1937.333	5	387.467	149.987	**.000**
Within Groups	46.500	18	2.583		
Total	1983.833	23			

Based on table 1, it can be seen that the mortality of Aedes aegypty larvae using the one way ANOVA test obtained a significance value or p-value of 0.0001 <0.05 which indicates that there is a significant difference between the number of mosquito larvae deaths and the concentration of Srigading extract given. Then a post-hoc Least Signification Difference (LSD) test was carried out to determine the location of the difference in the average larval mortality at each concentration and used as a reference in determining whether the average of the two treatment concentrations was statistically different or not, so that the concentration could be determined. which has larvicidal activity.

Table 2. LSD post-hoc test results

Consentration		*p-value*	Meaning
Negative Control	With 448,5 ppm	0,203	Not significant
	With 897 ppm	0,828	Not significant
	With 1794 ppm	1,000	Not significant
	With 3588 ppm	0,0001	Significant
	Positive Control	0,0001	Significant
448,5 ppm	Negative Control	0,203	Not significant
	With 897 ppm	0,286	Not significant
	With 1794 ppm	0,203	Not significant
	With 3588 pm	0,002	Significant
	Positive Control	0,0001	Significant
897 ppm	Negative Control	0,828	Not significant
	With 448,5 ppm	0,286	Not significant
	With 1794 ppm	0,828	Not significant
	With 3588 ppm	0,0001	Significant
	Positive Control	0,0001	Significant
1794 ppm	Negative Control	1,000	Not significant
	With 448,5 ppm	0,203	Not significant
	With 897 ppm	0,828	Not significant
	With 3588 ppm	0,0001	Significant
	Positive Control	0,0001	Significant
3588 ppm	Negative Control	0,0001	Significant
	With 448,5 ppm	0,002	Significant
	With 897 ppm	0,0001	Significant
	With 1794 ppm	0,0001	Significant
	Positive Control	0,0001	Significant
Positive Control	Negative Control	0,0001	Significant
	With 448,5 ppm	0,0001	Significant
	With 897 ppm	0,0001	Significant
	With 1794 ppm	0,0001	Significant
	With 3588 ppm	0,0001	Significant

Based on the results of the one way ANOVA test, a significance value or p-value of 0.0001 < 0.05 was obtained, which indicated that there was a significant difference between the number of mosquito larvae deaths and the concentration of Srigading extract given. Based on the results of the post-hoc test using LSD, it was found that there was a significant difference between the Srigading extract and the control group. The comparison found between the negative control and the concentration was found at a concentration of 3588 ppm, at that concentration there was a significant difference, this can be seen from the p-value or the significance value of 0.0001 compared to the value of <0.05. From these results it can be concluded that there is one concentration of Srigading leaf extract (Nycthanthes arbor-tritis) which has larvicidal activity.

Then, for the comparison between the positive control with concentrations of 448.5 ppm, 897 ppm, 1794 ppm, and 3588 ppm had the same significance value or p-value, namely 0.0001 with a value of <0.05, which means there is a significant difference. So, it can be concluded that at concentrations of 448.5 ppm, 897 ppm, 1794 ppm, and 3588 ppm, it does not have the same effectiveness as 1% temephos. The average percentage of Aedes aegypti larvae mortality was not affected by increasing concentration, so the higher concentration of Srigading leaf extract did not affect the mortality of the test larvae.

Death of Aedes agypti larvae occurred after treatment in the form of Srigading (Nycthanthes arbor-tritis) leaf extract, this was due to the larvicidal effect of Srigading (Nycthanthes arbor-tritis) leaves which contain secondary metabolites. The secondary metabolites contained in Srigading consist of alkaloids, flavonoids, saponins, tannins, and triterpenoids (Bhalakiya & Modi, 2019). Alkaloid secondary metabolites are one of a group of compounds that can be found in most types of plants. The way alkaloids work as insecticides is by inhibiting the activity of the acetylcholinesterase enzyme (Zaynab et al., 2018). This compound works by stimulating the endocrine glands to secrete juvenile hormones, this increase can cause metamorphosis failure in mosquito larvae resulting in abnormal death in mosquito larvae. In addition, in inhibiting cell mitosis, alkaloids can synergize with triterpenoid compounds (Khameneh et al., 2019).

The secondary metabolites of flavonoids are compounds that can damage the cytoplasmic membrane and cause cell leakage and can turn off the enzyme system (Jogawat et al., 2021). This of course can result in phospholipids being unable to maintain the shape of the cytoplasmic membrane itself until it will eventually burst and the larvae themselves will die (Turner, 2018). Saponin secondary metabolites are compounds that work by lowering the surface tension on the mucous membrane of the larval digestive tract. This will inhibit the rate of nutrient absorption by the larvae. In addition, saponins have another effect, namely destroying the chitin layer on the surface of the larvae so that the extract can easily enter the body of the larvae (Rohmah et al., 2020). The secondary metabolites of tannins are polyphenolic compounds that work by causing astringency in plant parts that can enter mosquito larvae through the body wall, causing disturbances in the larval muscles (Suryani et al., 2020). As a result, the larvae will experience weakness in the muscles of movement, so that the movement of the larvae will slow down. In addition to entering through the body wall, tannins can enter through the digestive tract of the larvae, this can cause interference with protein absorption in the larvae's intestines by reducing the activity of digestive enzymes and food absorption, so that the larvae will experience nutritional deficiencies and can cause death (Yusuf et al., 2020). Triterpenoid secondary metabolites are compounds that work by remembering free sterols in digestion where sterols act as precursors of the hormone ecdysone, so that a decrease in the amount of free sterols will disrupt the process of skin turnover in insects. In addition, these compounds can cause a decrease

in the activity of digestive enzymes and affect the process of food absorption (Arora & Sandhu, 2017).

Determining the LC50 and LC90 values from the ethanol extract of Srigading leaves (Nyctanthes arbor-tristis) was performed using Probit Analysis with a 95% confidence level. Probit analysis for LC50 and LC90 can be seen in table 3 as follows

Table 3. LC50 and LC90 values from the results of probit analysis of Srigading leaf ethanol extract (Nyctanthes arbor-tristis) against IV instar Aedes aegypti larvae after 24 hours

Mortality (%)	Estimate (%)	Confidence Level (%)	Confidence Interval	
			Lower Limit	Upper Limit
50	2.527	95	-9.649	3.507
90	5.460	95	4.389	20.775

Based on table 3, it can be seen that the results of the Probit analysis on the mortality rate of Aedes aegypti larvae obtained an LC50 value of 2.527%, this indicates that at a concentration of 2.527% within 24 hours it was able to kill 50% of the test larvae. While the obtained LC90 value of 5.460%, this shows that at a concentration of 5.460% within 24 hours it is able to kill 90% of the test larvae. Based on the results of the Probit analysis in table 3, the 24-hour LC50 value of Srigading leaf ethanol extract (Nyctanthes arbor-tristis) on the mortality of Aedes aegypti mosquito larvae was obtained at a concentration of 2.527%, which means that at a concentration of 2.527% ethanol extract of Srigading leaves (Nyctanthes arbor-tristis)) was able to kill 50% of Aedes aegypti larvae which were exposed for 24 hours, so it can be stated that the ethanol extract of Srigading leaves (Nyctanthes arbor-tristis) was effective against Aedes aegypti mosquito larvae with an LC50 value at 24 hours. While the 24-hour LC90 value of the ethanol extract of Srigading leaves (Nyctanthes arbor-tristis) on the mortality of Aedes aegypti mosquito larvae was obtained at a concentration of 5.460%, which means that at a concentration of 5.460% the ethanol extract of Srigading leaves (Nyctanthes arbor-tristis) was able to kill 90% of Aedes larvae. aegypti which was exposed for 24 hours, so it can be stated that the ethanol extract of Srigading leaves (Nyctanthes arbor-tristis) was effective against Aedes aegypti mosquito larvae with an LC90 value at 24 hours.

The results of this study showed that the ethanol extract of the leaves of Srigading (Nyctanthes arbor-tristis) had larvicidal activity against the larvae of the Aedes aegypti mosquito. However, according to previous studies, it is said that an extract of natural ingredients is effective as a pesticide if the LC50 is not more than 1000 ppm (0.1%). The LC50 value of the ethanol extract of Srigading leaves (Nyctanthes arbor-tristis) obtained was 2.527% or 25.270 ppm (conversion 1% = 10000 ppm), so it can be concluded that the ethanol extract of Srigading leaves (Nyctanthes arbor-tristis) still has high killing power, less against Aedes aegypti larvae. This may be caused by the form of the extract which is still in the form of crude extract or not in the form of pure compounds, so it is necessary to purify the compounds before they can be used as larvicides to obtain more optimal results.

The ethanol extract of Srigading leaves (Nyctanthes arbor-tristis) has larvicidal activity against Aedes aegypti mosquito larvae. Although the ability of Srigading leaf ethanol extract (Nyctanthes arbor-tristis) was still under positive control (temephos 1%), the ability of Srigading leaf ethanol extract (Nyctanthes arbor-tristis) could cause mortality of Aedes aegypti mosquito larvae, which was able to kill 50% of Aedes larvae. aegypti which was exposed for 24 hours with a concentration of 2.527%, this makes Srigading leaves (Nyctanthes arbor-tristis) have the

potential to be used as biolarvicides and are relatively safer for the environment and not persistent in nature, in contrast to temephos 1% which has the potential to cause pollution and the occurrence of resistance in larvae if not using the appropriate dose.

Conclusion

Srigading leaf extract (Nyctanthes arbor-tristis) has the effect of larvacide Aedes aegypti instar IV at a concentration of 448.5 ppm, 879 ppm, 3588 ppm, 1794 ppm and at a concentration of 7176 ppm it has the equivalent effectiveness of 1% temephos. With the discovery of natural ingredients from the Srigading plant (Nyctanthes arbor-tristis L) which functions as a natural larvicide, it will help the community to carry out 3M activities in overcoming the problem of DHF outbreak, especially for rural areas that are difficult to get access to. buy abate powder.

REFERENCES

Adnyana, I. M. D. M., Sudaryati, N. L. G., & Sitepu, I. (2021). Toxicity of Legiayu incense as Insecticide and Larvicide against Aedes aegypti Mosquitoes Mortality. *Indonesian Journal of Pharmacy*, *32*(4), 514–521.

Araujo, A. F. D. O., Ribeiro-Paes, J. T., Deus, J. T. D., Cavalcanti, S. C. D. H., Nunes, R. D. S., Alves, P. B., & Macoris, M. D. L. D. G. (2016). Larvicidal activity of Syzygium aromaticum (L.) Merr and Citrus sinensis (L.) Osbeck essential oils and their antagonistic effects with temephos in resistant populations of Aedes aegypti. *Memórias Do Instituto Oswaldo Cruz*, *111*(1), 443–449.

Arisanti, M., & Suryaningtyas, N. H. (2021). Kejadian demam berdarah dengue (DBD) di Indonesia tahun 2010-2019. *Spirakel*, *13*(1), 34–41.

Arora, R., & Sandhu, S. (2017). Insect-plant interrelationships. Breeding insect resistant crops for sustainable agriculture. *Springer*, 1–44.

Bhalakiya, H., & Modi, N. R. (2019). Traditional medicinal uses, phytochemical profile and pharmacological activities of Nyctanthes arbortris. *RJLBPCS*, *5*(1), 1003–1023.

Bowman, L. R., Donegan, S., & McCall, P. J. (2016). Is dengue vector control deficient in effectiveness or evidence?: Systematic review and meta-analysis. *PLoS Neglected Tropical Diseases*, *10*(3), 1–24.

Dinas Kesehatan Provinsi Kalimantan Selatan. (2020). *Data Demam Berdarah Dengeu (DBD) Provinsi Kalimantan Selatan*. Dinas Kesehatan Provinsi Kalimantan Selatan.

Doughari, J. (2015). An overview of plant immunity. *J. Plant Pathol. Microbiol*, *6*(11), 4110–4172.

Espinal, M. A., Andrus, J. K., Jauregui, B., Waterman, S. H., Morens, D. M., Santos, J. I., & Olson, D. (2019). Emerging and reemerging Aedes-transmitted arbovirus infections in the region of the Americas: implications for health policy. *American Journal of Public Health*, *109*(3), 387–392.

Faisal, A. P. (2018). Identifikasi Metabolit Sekunder dan Aktivitas Larvasida dari Daun Matahari (Helianthus annuus L.) terhadap Larva Aedes aegypti Sp. *Mahakam Medical Laboratory Technology Journal*, *2*(2), 80–90.

Fatimah, G., & Hasmiwati, R. R. (2020). Lethal concentration (LC50, 90, and 98) and lethal time (LT50, 90, and 98) at various temephos concentrations of Aedes aegypti L. larvae. *International Journal of Mosquito Research*, *7*(1), 1–3.

Irma, I., & Swaidatul, M. A. F. (2021). Trend Penyakit Demam Berdarah Dengue (DBD) di Sulawesi Tenggara Berbasis Ukuran Epidemiologi. *JUMANTIK (Jurnal Ilmiah Penelitian*

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Kesehatan), *6*(1), 70–78.
Jogawat, A., Yadav, B., Lakra, N., Singh, A. K., & Narayan, O. P. (2021). Crosstalk between phytohormones and secondary metabolites in the drought stress tolerance of crop plants: a review. *Physiologia Plantarum*, *17*(2), 1106–1132.
Kementerian Kesehatan RI. (2019). *Profil Kesehatan Indonesia Tahun 2018*. Kementerian Kesehatan RI.
Khameneh, B., Iranshahy, M., Soheili, V., & Fazly Bazzaz, B. S. (2019). Review on plant antimicrobials: a mechanistic viewpoint. *Antimicrobial Resistance & Infection Control*, *8*(1), 1–28.
Kurniawan, M. E., Mohamed, A. M. D., Siyam, N., Fatikha, N., & Fitriani, N. A. (2017). Relation Between Knowledge and Attitude Regarding DHF with PSN Behavior Among the Community Around the Campus. *KEMAS: Jurnal Kesehatan Masyarakat*, *13*(2), 145–151.
Lesmana, S. D., Maryanti, E., Susanty, E., Afandi, D., Harmas, W., Octaviani, D. N., & Mislindawati, M. (2022). Organophosphate Resistance in Aedes aegypti: Study from Dengue Hemorrhagic Fever Endemic Subdistrict in Riau, Indonesia. *Reports of Biochemistry & Molecular Biology*, *10*(4), 589–596.
Mandasari, N., Hazar, S., & Sadiyah, E. R. (2016). Uji Aktivitas Sitotoksik Ekstrak Metanol Daun Srigading (Nyctanthes arbor-tristis L.). *Prosiding Farmasi*, 717–722.
Minarti, M., Anwar, C., Irfannuddin, I., & Irsan, C. (2021). Community Knowledge and Attitudes about the Transmission of Dengue Haemorrhagic Fever and Its Relationship to Prevention Behaviour in Palembang, South Sumatra, Indonesia. *Open Access Macedonian Journal of Medical Sciences*, *9*(5), 1534–1543.
Nisrina, H. (2022). Toxicity Assessment Of Avovo Leaf Extract (Persea Americana Miller) On Mortality Of Aedes Aegypti Larva. *International Journal of Health, Education & Social (IJHES)*, *5*(5), 1–7.
Patel, M. I., Patel, A., Patel, A., Patel, S., & Padsala, S. (2018). Study of haematological, biochemical profile and clinical presentation in dengue positive patients: 82 cases. *International Journal of Research in Medical Sciences*, *6*(6), 2099–2105.
Pratiwi, A. (2012). Penerimaan masyarakat terhadap larvasida alami. *KEMAS: Jurnal Kesehatan*, *8*(1), 88–93.
Rahmawati, U., Gustina, M., & Mirza, R. (2020). Efektivitas Anti Nyamuk Alami Elektrik Mat Serai Wangi (Cymbopogon Nardus) Dalam Mematikan Nyamuk Aedes Aegypti. *Journal of Nursing and Public Health*, *8*(2), 100–107.
Rohmah, E. A., Subekti, S., & Rudyanto, M. (2020). Larvicidal Activity and Histopathological Effect of Averrhoa bilimbi Fruit Extract on Aedes aegypti from Surabaya, Indonesia. *Journal of Parasitology Research*, 1–5.
Şengül Demirak, M. Ş., & Canpolat, E. (2022). Plant-based bioinsecticides for mosquito control: Impact on insecticide resistance and disease transmission. Insects. *Insects*, *13*(2), 1–24.
Suryani, A. I., Hariani, N., Majid, A. F., & Amalia, D. N. (2020). Histological changes in the midgut of Spodoptera litura larvae exposured by the extract of Mirabilis jalapa leaves. *In IOP Conference Series: Earth and Environmental Science. IOP Publishing*, *484*(1).
Tlak Gajger, I., & Dar, S. A. (2021). Plant allelochemicals as sources of insecticides. *Insects*, *12*(3), 1–21.
Turner, R. (2018). *Essentials of microbiology. Scientific e-Resources.*
Wahyuni, E. (2021). Potensi Eksudat Daun Sirih Merah (Piper ornatum L.) sebagai Insektisida

Herbal terhadap Mortalitas Semut Hitam. *Hydrogen: Jurnal Kependidikan Kimia*, *9*(2), 97–104.

Wang, W. H., Urbina, A. N., Chang, M. R., Assavalapsakul, W., Lu, P. L., Chen, Y. H., & Wang, S. F. (2020). Dengue hemorrhagic fever–A systemic literature review of current perspectives on pathogenesis, prevention and control. *Journal of Microbiology, Immunology and Infection*, *53*(6), 963–978.

Wanti, W., Sila, O., Irfan, I., & Sinaga, E. (2016). Transovarial transmission and dengue virus serotypes in Aedes aegypti in Kupang. *Kemas: Jurnal Kesehatan Masyarakat*, *12*(1), 131–138.

World Health Organization. (2020). *Dengue and Severe Dengue. Dengue Data Application*. World Health Organization Press. https://ntdhq.shinyapps.io/dengue5/

Yousaf, M. Z., Siddique, A., Ashfaq, U. A., & Ali, M. (2018). Scenario of dengue infection & its control in Pakistan: An up—date and way forward. *Asian Pacific Journal of Tropical Medicine*, *11*(1), 15–23.

Yusuf, Y., Efendi, K., & Diantasari, S. (2020). Larvicidal Activity Test of Ethanolic Extract of (Euphorbia tirucalli Linn) Stem on Aedes aegypti Larvae. *Systematic Reviews in Pharmacy*, *11*(3).

Zaynab, M., Fatima, M., Abbas, S., Sharif, Y., Umair, M., Zafar, M. H., & Bahadar, K. (2018). Role of secondary metabolites in plant defense against pathogens. *Microbial Pathogenesis*, *124*(1), 198–202.

The Potential of Srigading Plants (Nyctanthes arbor-tristis) as Aedes aegypti Larvicides

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ABSTRACT

Dengue Hemorrhagic Fever (DHF) is an endemic disease that causes high morbidity and mortality rates. The prevalence of DHF in Indonesia (2018) reached 24.75 per 100,000 and in South Kalimantan (2019) it reached 56.83 per 100,000 population. One of the efforts to control dengue vectors is the use of larvicides. The purpose of this study was to determine the potential of Srigading Plants (*Nyctanthes arbor-tristis*) as Aedes aegypti larvae as vectors of DHF in Wetlands. This study used a true experimental research design using the post test only controlled group design to determine the effectiveness of Srigading extract (Nyctanthes arbor-tristis) against the death of Aedes aegypti instar IV mosquito larvae. Based on the results, a significance value or p-value of 0.0001 < 0.05 was obtained, which indicated that there was a significant difference between the number of mosquito larvae deaths and the concentration of Srigading extract given.

Keywords: Aedes Aegypti, Dengue Hemorrhagic Fever, Larvacides, Srigading plants, Vector

INTRODUCTION

DHF is a disease caused by a virus from the flaviviridae family which is transmitted through mosquito bites (arthropod borne viruses/arbovirus) namely Aedes aegypti and Aedes albopictus mosquitoes and can causing mortality if does not seriously taken care of (Kurniawan et al., 2017; Wang et al., 2020). DHF have clinical manifestations of fever, muscle or joint pain accompanied by leukopenia, rash, lymphodenopathy, and thrombocytopenia (Patel et al., 2018; Wang et al., 2020). The serotypes DEN-1, DEN-2, DEN-3 and DEN-4 are the cause of DHF which is transmitted by Aedes aegypti and Aedes albopictus mosquitoes that have been infected with the dengue virus (Yousaf et al., 2018). DHF is transmitted through the bite of female Aedes sp, where Aedes aegypti is the main vector and Aedes albopictus as the secondary vector in Indonesia, but for other countries such as Costa Rica Aedes albopictus is the main vector. DEN-1 virus can be found only in sporadic and endemic areas (Wanti et al., 2016). DEN-2 serotype is the highest serotype and is most often associated with severe cases (Faisal, 2018).

Globally, DHF is reported to range from 1-3 million cases while the number of deaths ranges from 2-4 thousand in the last decade (Espinal et al., 2019). According to data from the Ministry of Health of the Republic of Indonesia, the prevalence of DHF in 2016 was 78.85 per 100,000 population, in 2017 and 2018 it decreased to 26.1 per 100,000 population, and again decreased to 24.75 per 100,000 (Irma & Swaidatul, 2021). The morbidity rate of DHF according to data from the Health Office of South Kalimantan Province, namely in 2016 amounted to 106.21 per 100,000 population, then in 2017 there was a decrease to 13.49 per 100,000 population but increased again in 2018 by 47.91 and in 2019 by 56.83 per 100,000 population (Dinas Kesehatan Provinsi Kalimantan Selatan, 2020).

Vector control of DHF in Indonesia has not yet reached the program target. The target of the vector control program is 95%. The indicator of the DHF vector control program is the larva-free rate (ABJ). Nationally, ABJ in 2016 was 67.6%, in 2017 it decreased to 46.7% and then decreased again in 2018 which was 31.5% (Arisanti & Suryaningtyas, 2021; Kementerian Kesehatan RI, 2019). Based on data from the South Kalimantan Provincial Health Office, ABJ from 2015-2019 fluctuated with an increasing trend. The lowest ABJ in the five years was in 2016 at 83.85% and the highest was in 2017 at 89.08%. The lower the ABJ or below the program target, it means that the mosquito density will be higher. If the mosquito density is high, it will be a threat to increase the transmission of DHF in the future (Dinas Kesehatan Provinsi Kalimantan Selatan, 2020).

Vector control of DHF can be done in various ways, one of which is by using temephos (Fatimah & Hasmiwati, 2020). Temephos is still part of the government's program for eradicating mosquito nests, especially the Aedes aegypti mosquito with 3M plus (Minarti et al., 2021). The use of temephos for a long time will cause negative impacts such as environmental pollution and cause resistance (Araujo et al., 2016). On the other hand, this resistance results in ineffective vector control. Reports of resistance to Aedes aegypti larvae, where administration of temephos can harm humans by causing cancer in a number of body parts (Lesmana et al., 2022). Another chemical control that is also commonly used in the community is fumigation or fogging, which targets the adult Aedes aegypti mosquito. This method also has the same negative impact as the use of temephos, especially in the human lungs when inhaled directly. To reduce this effect, then efforts to use natural larvicides for controlling Aedes Sp. larvae. In general, natural larvicides are defined as pesticides and the basic ingredients come from plants (Bowman et al., 2016).

The World Health Organization (WHO) recommends the development of natural vector control that is environmentally friendly because it will be safer for the environment and human health (World Health Organization, 2020). Therefore, natural larvicidation efforts continue to be

developed from various plants that have potential as larvicides. Natural larvicides are obtained from secondary metabolites that function as a means of self-defense from attack and are known to be able to kill nuisance organisms (Wahyuni, 2021). Indonesia is a country that is rich in various types of plants. One of these plants is the Srigading plant (Nyctanthes arbor-tristis L). This plant usually thrives on the islands of Java and Kalimantan as an ornamental plant in tombs or graves. Apart from being an ornamental plant, Srigading plant is also used as a medicinal plant. Srigading contains high antioxidants. This Srigading plant contains secondary metabolites which include saponins, tannins, terpenoids and steroids (Mandasari et al., 2016).

Saponins can cause cell damage, interfere with metabolic processes and damage the outer protective barrier so that mosquito larvae will lose a lot of fluids (Tlak Gajger & Dar, 2021). Tannins can cause nutritional disturbances by reducing the activity of digestive enzymes in mosquito larvae (Nisrina, 2022). Terpenoids function as disruptors of cell membranes and tissues in mosquito larvae. Steroids function as growth hormones that work to inhibit growth by influencing skin turnover in mosquito larvae (Doughari, 2015). So far as literature searches have been carried out, there has been no research on the effect of Srigading extract (Nyctanthes arbor-tristis L) as a larvicide on Aedes aegypti mosquito larvae. Therefore, it is necessary to conduct research on Srigading extract (Nyctanthes arbor-tristis L) as a natural larvicide that can kill Aedes aegypti mosquito larvae as the basis for this research.

METHODS

This study used a true experimental research design using the post test only controlled group design to determine the effectiveness of Srigading extract (Nyctanthes arbor-tristis) against the death of Aedes aegypti instar IV mosquito larvae. In this study, five treatments were tested with concentrations of 448.5 ppm, 879 ppm, 1794 ppm, 3588 ppm, 7176 ppm and using two controls (positive and negative). In research, the selection of larval age is a very important activity. The age of mosquito larvae has a very influential factor on the resistance of the larvae to exposure to chemical substances (Adnyana et al., 2021). The age range of 2-5 days is the best age range and is in accordance with the Guidelines for Biological Insecticide Testing (Rahmawati et al., 2020). The longer the age of mosquito larvae, the higher their resistance and the more mature their physical condition (Şengül Demirak & Canpolat, 2022). Therefore, the subjects in this study were Aedes aegypti mosquito larvae instar IV 5 days old and had signs of life such as active movement. The fourth instar Aedes aegypti mosquito larvae were obtained from the Center for the Development and Research of Seasoned Soil Disease Vectors, South Kalimantan.

The research materials used were Srigading (Nyctanthes arbor-tristis), aquades, 70% ethanol, temephos, fish food and Aedes aegypti instar IV mosquito larvae which were colonized at the Entomology Laboratory of the Lokalitbang Center for the Development and Research of Spice Soil Disease Vector Reservoirs, South Kalimantan. The Srigading plant (Nyctanthes arbor-tristis) will be identified at the Laboratory of the Faculty of Medicine, University of Lambung Mangkurat Banjarbaru, this is done to avoid errors in the selection of plant species so that they can be accounted for. Extracts of Srigading (Nyctanthes arbor-tristis) were made by researchers Together with laboratory analysts of Agricultural Industrial Engineering (TIP) Faculty of Agriculture, University of Lambung Mangkurat Banjarbaru using the maceration method. Preparation of stock solutions with One part per million (ppm) is a concentration of 1 mg of solute 1000 ml, then to make a stock solution of 13,903.5 ppm is by weighing the extract 13,903.5 mg of solute 1000 ml.

The results of the calculation of the number of deaths of Aedes aegypti mosquito larvae from seven treatments were tabulated in tabular form. The data were statistically analyzed using a

computer program. Normality test was calculated using Shapiro-Wilk and homogeneity test was calculated using Levene's test. If the data is normally distributed and homogeneous, then the One Way ANOVA test is carried out with post Hoc Bonferroni. If the data is not normally distributed, the Kruskal-Wallis test is performed. If there is a difference in Kruskal-Wallis, then it is continued with the Mann-Whitney test. Determining the LC50 and LT50 values of Srigading Leaf extract was carried out using probit analysis with a 95% confidence level.

RESULTS AND DISCUSSION

The concentration of Srigading leaf extract (Nyctanthes arbor-tristis) in the larvicidal test was 448.5 ppm, 879 ppm, 1794 ppm, 3588 ppm, 7176 ppm, aquadest as a negative control and temephos as a positive control, which were exposed to Aedes aegypti larvae instar IV during 24 hours.

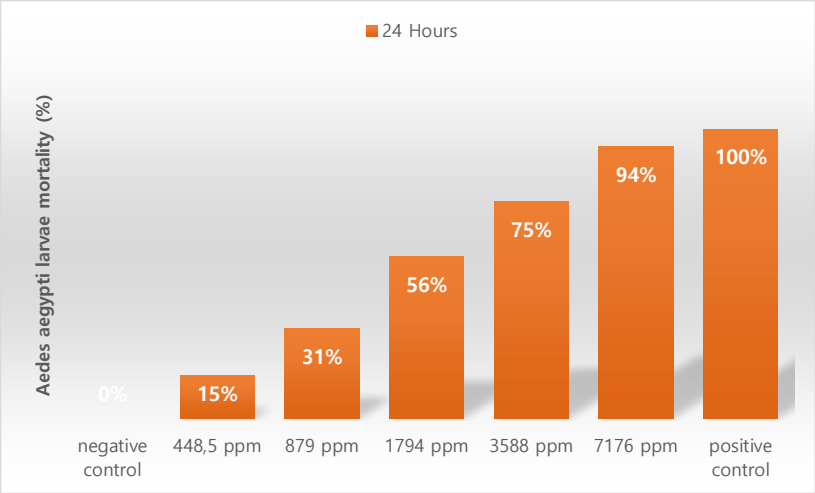

Figure 1. Effectiveness of Srigading Leaf Extract (Nyctanthes arbor-tristis) against fourth instar Aedes aegypti larvae for 24 hours of exposure

Figure 1 shows a graphic depiction of the percentage of mortality of fourth instar Aedes aegypti larvae after 24 hours of exposure to Srigading leaf extract (Nyctanthes arbor-tristis). In the negative control, there was no larval death so there was no need to correct the calculation of larval mortality using the Abbot formula. In the positive control using 1% temephos there was larval mortality with an average percentage of 100%. There was an increase in the mortality of Aedes aegypti larvae instar IV along with the increasing concentration of Srigading leaf extract (Nyctanthes arbor-tristis). The average larval mortality at a concentration of 448.5 ppm was 15%, at a concentration of 879 ppm was 31%, at a concentration of 3588 ppm was 56%, at a concentration of 1794 ppm was 75% and at a concentration of 7176 ppm, Aedes aegypti larvae mortality was 94%.

Table 1. ANOVA test results

	Sum of Squares	df	Mean Square	F	Sig.

Between Groups	1937.333	5	387.467	149.987	**.000**
Within Groups	46.500	18	2.583		
Total	1983.833	23			

Based on table 1, it can be seen that the mortality of Aedes aegypty larvae using the one way ANOVA test obtained a significance value or p-value of 0.0001 <0.05 which indicates that there is a significant difference between the number of mosquito larvae deaths and the concentration of Srigading extract given. Then a post-hoc Least Signification Difference (LSD) test was carried out to determine the location of the difference in the average larval mortality at each concentration and used as a reference in determining whether the average of the two treatment concentrations was statistically different or not, so that the concentration could be determined. which has larvicidal activity.

Table 2. LSD post-hoc test results

Consentration		***p-value***	**Meaning**
Negative Control	With 448,5 ppm	0,203	Not significant
	With 897 ppm	0,828	Not significant
	With 1794 ppm	1,000	Not significant
	With 3588 ppm	0,0001	Significant
	Positive Control	0,0001	Significant
448,5 ppm	Negative Control	0,203	Not significant
	With 897 ppm	0,286	Not significant
	With 1794 ppm	0,203	Not significant
	With 3588 pm	0,002	Significant
	Positive Control	0,0001	Significant
897 ppm	Negative Control	0,828	Not significant
	With 448,5 ppm	0,286	Not significant
	With 1794 ppm	0,828	Not significant
	With 3588 ppm	0,0001	Significant
	Positive Control	0,0001	Significant
1794 ppm	Negative Control	1,000	Not significant
	With 448,5 ppm	0,203	Not significant
	With 897 ppm	0,828	Not significant
	With 3588 ppm	0,0001	Significant
	Positive Control	0,0001	Significant
3588 ppm	Negative Control	0,0001	Significant
	With 448,5 ppm	0,002	Significant
	With 897 ppm	0,0001	Significant
	With 1794 ppm	0,0001	Significant
	Positive Control	0,0001	Significant
Positive Control	Negative Control	0,0001	Significant
	With 448,5 ppm	0,0001	Significant
	With 897 ppm	0,0001	Significant
	With 1794 ppm	0,0001	Significant
	With 3588 ppm	0,0001	Significant

Based on the results of the one way ANOVA test, a significance value or p-value of 0.0001 < 0.05 was obtained, which indicated that there was a significant difference between the number of mosquito larvae deaths and the concentration of Srigading extract given. Based on the results of the post-hoc test using LSD, it was found that there was a significant difference between the Srigading extract and the control group. The comparison found between the negative control and

the concentration was found at a concentration of 3588 ppm, at that concentration there was a significant difference, this can be seen from the p-value or the significance value of 0.0001 compared to the value of <0.05. From these results it can be concluded that there is one concentration of Srigading leaf extract (Nycthanthes arbor-tritis) which has larvicidal activity.

Then, for the comparison between the positive control with concentrations of 448.5 ppm, 897 ppm, 1794 ppm, and 3588 ppm had the same significance value or p-value, namely 0.0001 with a value of <0.05, which means there is a significant difference. So, it can be concluded that at concentrations of 448.5 ppm, 897 ppm, 1794 ppm, and 3588 ppm, it does not have the same effectiveness as 1% temephos. The average percentage of Aedes aegypti larvae mortality was not affected by increasing concentration, so the higher concentration of Srigading leaf extract did not affect the mortality of the test larvae.

Death of Aedes agypti larvae occurred after treatment in the form of Srigading (Nycthanthes arbor-tritis) leaf extract, this was due to the larvicidal effect of Srigading (Nycthanthes arbor-tritis) leaves which contain secondary metabolites. The secondary metabolites contained in Srigading consist of alkaloids, flavonoids, saponins, tannins, and triterpenoids (Bhalakiya & Modi, 2019). Alkaloid secondary metabolites are one of a group of compounds that can be found in most types of plants. The way alkaloids work as insecticides is by inhibiting the activity of the acetylcholinesterase enzyme (Zaynab et al., 2018). This compound works by stimulating the endocrine glands to secrete juvenile hormones, this increase can cause metamorphosis failure in mosquito larvae resulting in abnormal death in mosquito larvae. In addition, in inhibiting cell mitosis, alkaloids can synergize with triterpenoid compounds (Khameneh et al., 2019).

The secondary metabolites of flavonoids are compounds that can damage the cytoplasmic membrane and cause cell leakage and can turn off the enzyme system (Jogawat et al., 2021). This of course can result in phospholipids being unable to maintain the shape of the cytoplasmic membrane itself until it will eventually burst and the larvae themselves will die (Turner, 2018). Saponin secondary metabolites are compounds that work by lowering the surface tension on the mucous membrane of the larval digestive tract. This will inhibit the rate of nutrient absorption by the larvae. In addition, saponins have another effect, namely destroying the chitin layer on the surface of the larvae so that the extract can easily enter the body of the larvae (Rohmah et al., 2020). The secondary metabolites of tannins are polyphenolic compounds that work by causing astringency in plant parts that can enter mosquito larvae through the body wall, causing disturbances in the larval muscles (Suryani et al., 2020). As a result, the larvae will experience weakness in the muscles of movement, so that the movement of the larvae will slow down. In addition to entering through the body wall, tannins can enter through the digestive tract of the larvae, this can cause interference with protein absorption in the larvae's intestines by reducing the activity of digestive enzymes and food absorption, so that the larvae will experience nutritional deficiencies and can cause death (Yusuf et al., 2020). Triterpenoid secondary metabolites are compounds that work by remembering free sterols in digestion where sterols act as precursors of the hormone ecdysone, so that a decrease in the amount of free sterols will disrupt the process of skin turnover in insects. In addition, these compounds can cause a decrease in the activity of digestive enzymes and affect the process of food absorption (Arora & Sandhu, 2017).

Determining the LC50 and LC90 values from the ethanol extract of Srigading leaves (Nyctanthes arbor-tristis) was performed using Probit Analysis with a 95% confidence level. Probit analysis for LC50 and LC90 can be seen in table 3 as follows

Table 3. LC50 and LC90 values from the results of probit analysis of Srigading leaf ethanol

extract (Nyctanthes arbor-tristis) against IV instar Aedes aegypti larvae after 24 hours

Mortality (%)	Estimate (%)	Confidence Level (%)	Confidence Interval	
			Lower Limit	Upper Limit
50	2.527	95	-9.649	3.507
90	5.460	95	4.389	20.775

Based on table 3, it can be seen that the results of the Probit analysis on the mortality rate of Aedes aegypti larvae obtained an LC50 value of 2.527%, this indicates that at a concentration of 2.527% within 24 hours it was able to kill 50% of the test larvae. While the obtained LC90 value of 5.460%, this shows that at a concentration of 5.460% within 24 hours it is able to kill 90% of the test larvae. Based on the results of the Probit analysis in table 3, the 24-hour LC50 value of Srigading leaf ethanol extract (Nyctanthes arbor-tristis) on the mortality of Aedes aegypti mosquito larvae was obtained at a concentration of 2.527%, which means that at a concentration of 2.527% ethanol extract of Srigading leaves (Nyctanthes arbor-tristis)) was able to kill 50% of Aedes aegypti larvae which were exposed for 24 hours, so it can be stated that the ethanol extract of Srigading leaves (Nyctanthes arbor-tristis) was effective against Aedes aegypti mosquito larvae with an LC50 value at 24 hours. While the 24-hour LC90 value of the ethanol extract of Srigading leaves (Nyctanthes arbor-tristis) on the mortality of Aedes aegypti mosquito larvae was obtained at a concentration of 5.460%, which means that at a concentration of 5.460% the ethanol extract of Srigading leaves (Nyctanthes arbor-tristis) was able to kill 90% of Aedes larvae. aegypti which was exposed for 24 hours, so it can be stated that the ethanol extract of Srigading leaves (Nyctanthes arbor-tristis) was effective against Aedes aegypti mosquito larvae with an LC90 value at 24 hours.

The results of this study showed that the ethanol extract of the leaves of Srigading (Nyctanthes arbor-tristis) had larvicidal activity against the larvae of the Aedes aegypti mosquito. However, according to previous studies, it is said that an extract of natural ingredients is effective as a pesticide if the LC50 is not more than 1000 ppm (0.1%). The LC50 value of the ethanol extract of Srigading leaves (Nyctanthes arbor-tristis) obtained was 2.527% or 25.270 ppm (conversion 1% = 10000 ppm), so it can be concluded that the ethanol extract of Srigading leaves (Nyctanthes arbor-tristis) still has high killing power, less against Aedes aegypti larvae. This may be caused by the form of the extract which is still in the form of crude extract or not in the form of pure compounds, so it is necessary to purify the compounds before they can be used as larvicides to obtain more optimal results.

The ethanol extract of Srigading leaves (Nyctanthes arbor-tristis) has larvicidal activity against Aedes aegypti mosquito larvae. Although the ability of Srigading leaf ethanol extract (Nyctanthes arbor-tristis) was still under positive control (temephos 1%), the ability of Srigading leaf ethanol extract (Nyctanthes arbor-tristis) could cause mortality of Aedes aegypti mosquito larvae, which was able to kill 50% of Aedes larvae. aegypti which was exposed for 24 hours with a concentration of 2.527%, this makes Srigading leaves (Nyctanthes arbor-tristis) have the potential to be used as biolarvicides and are relatively safer for the environment and not persistent in nature, in contrast to temephos 1% which has the potential to cause pollution and the occurrence of resistance in larvae if not using the appropriate dose.

Conclusion

Srigading leaf extract (Nyctanthes arbor-tristis) has the effect of larvacide Aedes aegypti instar IV at a concentration of 448.5 ppm, 879 ppm, 3588 ppm, 1794 ppm and at a concentration

of 7176 ppm it has the equivalent effectiveness of 1% temephos. With the discovery of natural ingredients from the Srigading plant (Nyctanthes arbor-tristis L) which functions as a natural larvicide, it will help the community to carry out 3M activities in overcoming the problem of DHF outbreak, especially for rural areas that are difficult to get access to. buy abate powder.

REFERENCES

Adnyana, I. M. D. M., Sudaryati, N. L. G., & Sitepu, I. (2021). Toxicity of Legiayu incense as Insecticide and Larvicide against Aedes aegypti Mosquitoes Mortality. *Indonesian Journal of Pharmacy*, *32*(4), 514–521.

Araujo, A. F. D. O., Ribeiro-Paes, J. T., Deus, J. T. D., Cavalcanti, S. C. D. H., Nunes, R. D. S., Alves, P. B., & Macoris, M. D. L. D. G. (2016). Larvicidal activity of Syzygium aromaticum (L.) Merr and Citrus sinensis (L.) Osbeck essential oils and their antagonistic effects with temephos in resistant populations of Aedes aegypti. *Memórias Do Instituto Oswaldo Cruz*, *111*(1), 443–449.

Arisanti, M., & Suryaningtyas, N. H. (2021). Kejadian demam berdarah dengue (DBD) di Indonesia tahun 2010-2019. *Spirakel*, *13*(1), 34–41.

Arora, R., & Sandhu, S. (2017). Insect-plant interrelationships. Breeding insect resistant crops for sustainable agriculture. *Springer*, 1–44.

Bhalakiya, H., & Modi, N. R. (2019). Traditional medicinal uses, phytochemical profile and pharmacological activities of Nyctanthes arbortris. *RJLBPCS*, *5*(1), 1003–1023.

Bowman, L. R., Donegan, S., & McCall, P. J. (2016). Is dengue vector control deficient in effectiveness or evidence?: Systematic review and meta-analysis. *PLoS Neglected Tropical Diseases*, *10*(3), 1–24.

Dinas Kesehatan Provinsi Kalimantan Selatan. (2020). *Data Demam Berdarah Dengeu (DBD) Provinsi Kalimantan Selatan*. Dinas Kesehatan Provinsi Kalimantan Selatan.

Doughari, J. (2015). An overview of plant immunity. *J. Plant Pathol. Microbiol*, *6*(11), 4110–4172.

Espinal, M. A., Andrus, J. K., Jauregui, B., Waterman, S. H., Morens, D. M., Santos, J. I., & Olson, D. (2019). Emerging and reemerging Aedes-transmitted arbovirus infections in the region of the Americas: implications for health policy. *American Journal of Public Health*, *109*(3), 387–392.

Faisal, A. P. (2018). Identifikasi Metabolit Sekunder dan Aktivitas Larvasida dari Daun Matahari (Helianthus annuus L.) terhadap Larva Aedes aegypti Sp. *Mahakam Medical Laboratory Technology Journal*, *2*(2), 80–90.

Fatimah, G., & Hasmiwati, R. R. (2020). Lethal concentration (LC50, 90, and 98) and lethal time (LT50, 90, and 98) at various temephos concentrations of Aedes aegypti L. larvae. *International Journal of Mosquito Research*, *7*(1), 1–3.

Irma, I., & Swaidatul, M. A. F. (2021). Trend Penyakit Demam Berdarah Dengue (DBD) di Sulawesi Tenggara Berbasis Ukuran Epidemiologi. *JUMANTIK (Jurnal Ilmiah Penelitian Kesehatan)*, *6*(1), 70–78.

Jogawat, A., Yadav, B., Lakra, N., Singh, A. K., & Narayan, O. P. (2021). Crosstalk between phytohormones and secondary metabolites in the drought stress tolerance of crop plants: a review. *Physiologia Plantarum*, *17*(2), 1106–1132.

Kementerian Kesehatan RI. (2019). *Profil Kesehatan Indonesia Tahun 2018*. Kementerian Kesehatan RI.

Khameneh, B., Iranshahy, M., Soheili, V., & Fazly Bazzaz, B. S. (2019). Review on plant antimicrobials: a mechanistic viewpoint. *Antimicrobial Resistance & Infection Control*,

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Author: We decided to delete Pratiwi, A. (2012). Penerimaan masyarakat terhadap larvasida alami. *KEMAS: Jurnal Kesehatan*, *8*(1), 88–93.

8(1), 1–28.
Kurniawan, M. E., Mohamed, A. M. D., Siyam, N., Fatikha, N., & Fitriani, N. A. (2017). Relation Between Knowledge and Attitude Regarding DHF with PSN Behavior Among the Community Around the Campus. *KEMAS: Jurnal Kesehatan Masyarakat*, *13*(2), 145–151.
Lesmana, S. D., Maryanti, E., Susanty, E., Afandi, D., Harmas, W., Octaviani, D. N., & Mislindawati, M. (2022). Organophosphate Resistance in Aedes aegypti: Study from Dengue Hemorrhagic Fever Endemic Subdistrict in Riau, Indonesia. *Reports of Biochemistry & Molecular Biology*, *10*(4), 589–596.
Mandasari, N., Hazar, S., & Sadiyah, E. R. (2016). Uji Aktivitas Sitotoksik Ekstrak Metanol Daun Srigading (Nyctanthes arbor-tristis L.). *Prosiding Farmasi*, 717–722.
Minarti, M., Anwar, C., Irfannuddin, I., & Irsan, C. (2021). Community Knowledge and Attitudes about the Transmission of Dengue Haemorrhagic Fever and Its Relationship to Prevention Behaviour in Palembang, South Sumatra, Indonesia. *Open Access Macedonian Journal of Medical Sciences*, *9*(5), 1534–1543.
Nisrina, H. (2022). Toxicity Assessment Of Avovo Leaf Extract (Persea Americana Miller) On Mortality Of Aedes Aegypti Larva. *International Journal of Health, Education & Social (IJHES)*, *5*(5), 1–7.
Patel, M. I., Patel, A., Patel, A., Patel, S., & Padsala, S. (2018). Study of haematological, biochemical profile and clinical presentation in dengue positive patients: 82 cases. *International Journal of Research in Medical Sciences*, *6*(6), 2099–2105.
Rahmawati, U., Gustina, M., & Mirza, R. (2020). Efektivitas Anti Nyamuk Alami Elektrik Mat Serai Wangi (Cymbopogon Nardus) Dalam Mematikan Nyamuk Aedes Aegypti. *Journal of Nursing and Public Health*, *8*(2), 100–107.
Rohmah, E. A., Subekti, S., & Rudyanto, M. (2020). Larvicidal Activity and Histopathological Effect of Averrhoa bilimbi Fruit Extract on Aedes aegypti from Surabaya, Indonesia. *Journal of Parasitology Research*, 1–5.
Şengül Demirak, M. Ş., & Canpolat, E. (2022). Plant-based bioinsecticides for mosquito control: Impact on insecticide resistance and disease transmission. Insects. *Insects*, *13*(2), 1–24.
Suryani, A. I., Hariani, N., Majid, A. F., & Amalia, D. N. (2020). Histological changes in the midgut of Spodoptera litura larvae exposured by the extract of Mirabilis jalapa leaves. *In IOP Conference Series: Earth and Environmental Science. IOP Publishing*, *484*(1).
Tlak Gajger, I., & Dar, S. A. (2021). Plant allelochemicals as sources of insecticides. *Insects*, *12*(3), 1–21.
Turner, R. (2018). *Essentials of microbiology. Scientific e-Resources.*
Wahyuni, E. (2021). Potensi Eksudat Daun Sirih Merah (Piper ornatum L.) sebagai Insektisida Herbal terhadap Mortalitas Semut Hitam. *Hydrogen: Jurnal Kependidikan Kimia*, *9*(2), 97–104.
Wang, W. H., Urbina, A. N., Chang, M. R., Assavalapsakul, W., Lu, P. L., Chen, Y. H., & Wang, S. F. (2020). Dengue hemorrhagic fever–A systemic literature review of current perspectives on pathogenesis, prevention and control. *Journal of Microbiology, Immunology and Infection*, *53*(6), 963–978.
Wanti, W., Sila, O., Irfan, I., & Sinaga, E. (2016). Transovarial transmission and dengue virus serotypes in Aedes aegypti in Kupang. *Kemas: Jurnal Kesehatan Masyarakat*, *12*(1), 131–138.
World Health Organization. (2020). *Dengue and Severe Dengue. Dengue Data Application*. World Health Organization Press. https://ntdhq.shinyapps.io/dengue5/

Yousaf, M. Z., Siddique, A., Ashfaq, U. A., & Ali, M. (2018). Scenario of dengue infection & its control in Pakistan: An up—date and way forward. *Asian Pacific Journal of Tropical Medicine*, *11*(1), 15–23.

Yusuf, Y., Efendi, K., & Diantasari, S. (2020). Larvicidal Activity Test of Ethanolic Extract of (Euphorbia tirucalli Linn) Stem on Aedes aegypti Larvae. *Systematic Reviews in Pharmacy*, *11*(3).

Zaynab, M., Fatima, M., Abbas, S., Sharif, Y., Umair, M., Zafar, M. H., & Bahadar, K. (2018). Role of secondary metabolites in plant defense against pathogens. *Microbial Pathogenesis*, *124*(1), 198–202.

The Potential of Srigading Plants (Nyctanthes arbor-tristis) as Aedes aegypti Larvicides as Dengue Hemorrhagic Fever Vectors in Wetlands

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ABSTRACT

Dengue Hemorrhagic Fever (DHF) is an endemic disease that causes high morbidity and mortality rates. Globally, dengue fever is reported to range from 1-3 million cases while the number of deaths ranges from 2-4 thousand in the last decade. The prevalence of DHF in Indonesia in 2018 reached 24.75 per 100,000 and in South Kalimantan in 2019 it reached 56.83 per 100,000 population. One of the efforts to control dengue vectors is the use of larvicides. The purpose of this study was to determine the potential of Srigading Plants (*Nyctanthes arbor-tristis*) as Aedes aegypti larvae as vectors of DHF in Wetlands. This study used a true experimental research design using the post test only controlled group design to determine the effectiveness of Srigading extract (Nyctanthes arbor-tristis) against the death of Aedes aegypti instar IV mosquito larvae. Based on the results of the one way ANOVA test, a significance value or p-value of 0.0001 < 0.05 was obtained, which indicated that there was a significant difference between the number of mosquito larvae deaths and the concentration of Srigading extract given.

Based on the results of the post-hoc test using LSD, it was found that there was a significant difference between the Srigading extract and the control group. The comparison found between the negative control and the concentration was found at a concentration of 3588 ppm, at that concentration there was a significant difference. There is one concentration of Srigading leaf extract (Nycthanthes arbor-tritis) which has larvicidal activity, that is a concentration of 3588 ppm.

Keywords: Aedes Aegypti, Dengue Hemorrhagic Fever, Larvacides, Srigading plants, Vector

INTRODUCTION

Dengue Hemorrhagic Fever (DHF) is a disease caused by a virus from the flaviviridae family which is transmitted through mosquito bites (arthropod borne viruses/arbovirus) namely Aedes aegypti and Aedes albopictus mosquitoes with clinical manifestations of fever, muscle or joint pain accompanied by leukopenia, rash, lymphodenopathy, and thrombocytopenia. The serotypes DEN-1, DEN-2, DEN-3 and DEN-4 are the cause of Dengue Hemorrhagic Fever which is transmitted by Aedes aegypti and Aedes albopictus mosquitoes that have been infected with the dengue virus. The four serotypes can be found in various regions in Indonesia. Each percentage of infection incidence of the four serotypes, namely DEN-1 by 8%, DEN-2 by 65%, DEN-3 by 15% and DEN-4 by 12%. DEN-2 serotype is the highest serotype and is most often associated with severe cases (1, 2).

Globally, Dengue Hemorrhagic Fever is reported to range from 1-3 million cases while the number of deaths ranges from 2-4 thousand in the last decade. According to data from the Ministry of Health of the Republic of Indonesia, the prevalence of Dengue Hemorrhagic Fever in 2016 was 78.85 per 100,000 population, in 2017 and 2018 it decreased to 26.1 per 100,000 population, and again decreased to 24.75 per 100,000. Ministry of Health, 2019). The morbidity rate of Dengue Hemorrhagic Fever according to data from the Health Office of South Kalimantan Province, namely in 2016 amounted to 106.21 per 100,000 population, then in 2017 there was a decrease to 13.49 per 100,000 population but increased again in 2018 by 47.91 and in 2019 by 56.83 per 100,000 population (3, 4).

Vector control of Dengue Hemorrhagic Fever in Indonesia has not yet reached the program target. The target of the vector control program is 95%. The indicator of the dengue hemorrhagic fever vector control program is the larva-free rate (ABJ). Nationally, ABJ in 2016 was 67.6%, in 2017 it decreased to 46.7% and then decreased again in 2018 which was 31.5%. Based on data from the South Kalimantan Provincial Health Office, ABJ from 2015-2019 fluctuated with an increasing trend. The lowest ABJ in the five years was in 2016 at 83.85% and the highest was in 2017 at 89.08%. The lower the ABJ or below the program target, it means that the mosquito density will be higher. If the mosquito density is high, it will be a threat to increase the transmission of Dengue Hemorrhagic Fever in the future (5, 6).

Vector control of Dengue Hemorrhagic Fever can be done in various ways, one of which is by using temephos. Temephos is still part of the government's program for eradicating mosquito nests, especially the Aedes aegypti mosquito with 3M plus. The use of temephos for a long time will cause negative impacts such as environmental pollution and cause resistance. On the other hand, this resistance results in ineffective vector control. Reports of resistance to Aedes aegypti larvae, where administration of temephos can harm humans by causing cancer in a number of body parts. Another chemical control that is also commonly used in the community is fumigation or fogging, which targets the adult Aedes aegypti mosquito. This method also has the same negative impact as the use of temephos, especially in the human lungs when inhaled directly (7, 8, 9).

The World Health Organization (WHO) recommends the development of natural vector control that is environmentally friendly because it will be safer for the environment and human health. Therefore, natural larvicidation efforts continue to be developed from various plants that have potential as larvicides. Natural larvicides are obtained from secondary metabolites that function as a means of self-defense from attack and are known to be able to kill nuisance organisms. Indonesia is a country that is rich in various types of plants. One of these plants is the Srigading plant (Nyctanthes arbor-tristis L). This plant usually thrives on the islands of Java and

Kalimantan as an ornamental plant in tombs or graves. Apart from being an ornamental plant, Srigading plant is also used as a medicinal plant. Srigading contains high antioxidants. This Srigading plant contains secondary metabolites which include saponins, tannins, terpenoids and steroids (10, 11, 12).

Saponins can cause cell damage, interfere with metabolic processes and damage the outer protective barrier so that mosquito larvae will lose a lot of fluids. Tannins can cause nutritional disturbances by reducing the activity of digestive enzymes in mosquito larvae. Terpenoids function as disruptors of cell membranes and tissues in mosquito larvae. Steroids function as growth hormones that work to inhibit growth by influencing skin turnover in mosquito larvae (13). So far as literature searches have been carried out, there has been no research on the effect of Srigading extract (Nyctanthes arbor-tristis L) as a larvicide on Aedes aegypti mosquito larvae. Therefore, it is necessary to conduct research on Srigading extract (Nyctanthes arbor-tristis L) as a natural larvicide that can kill Aedes aegypti mosquito larvae as the basis for this research.

METHODS

This study used a true experimental research design using the post test only controlled group design to determine the effectiveness of Srigading extract (Nyctanthes arbor-tristis) against the death of Aedes aegypti instar IV mosquito larvae. In this study, five treatments were tested with concentrations of 448.5 ppm, 879 ppm, 1794 ppm, 3588 ppm, 7176 ppm and using two controls (positive and negative). In research, the selection of larval age is a very important activity. The age of mosquito larvae has a very influential factor on the resistance of the larvae to exposure to chemical substances. The age range of 2-5 days is the best age range and is in accordance with the Guidelines for Biological Insecticide Testing. The longer the age of mosquito larvae, the higher their resistance and the more mature their physical condition. Therefore, the subjects in this study were Aedes aegypti mosquito larvae instar IV 5 days old and had signs of life such as active movement. The fourth instar Aedes aegypti mosquito larvae were obtained from the Center for the Development and Research of Seasoned Soil Disease Vectors, South Kalimantan (14, 15).

The research materials used were Srigading (Nyctanthes arbor-tristis), aquades, 70% ethanol, temephos, fish food and Aedes aegypti instar IV mosquito larvae which were colonized at the Entomology Laboratory of the Lokalitbang Center for the Development and Research of Spice Soil Disease Vector Reservoirs, South Kalimantan. The Srigading plant (Nyctanthes arbor-tristis) will be identified at the Laboratory of the Faculty of Medicine, University of Lambung Mangkurat Banjarbaru, this is done to avoid errors in the selection of plant species so that they can be accounted for. Extracts of Srigading (Nyctanthes arbor-tristis) were made by researchers Together with laboratory analysts of Agricultural Industrial Engineering (TIP) Faculty of Agriculture, University of Lambung Mangkurat Banjarbaru using the maceration method. Preparation of stock solutions with One part per million (ppm) is a concentration of 1 mg of solute 1000 ml . then to make a stock solution of 13,903.5 ppm is by weighing the extract 13,903.5 mg of solute 1000 ml.

The results of the calculation of the number of deaths of Aedes aegypti mosquito larvae from seven treatments were tabulated in tabular form. The data were statistically analyzed using a computer program. Normality test was calculated using Shapiro-Wilk and homogeneity test was calculated using Levene's test. If the data is normally distributed and homogeneous, then the One Way ANOVA test is carried out with post Hoc Bonferroni. If the data is not normally distributed, the Kruskal-Wallis test is performed. If there is a difference in Kruskal-Wallis, then

it is continued with the Mann-Whitney test. Determining the LC50 and LT50 values of Srigading Leaf extract was carried out using probit analysis with a 95% confidence level (16, 17).

RESULTS AND DISCUSSION

The concentration of Srigading leaf extract (Nyctanthes arbor-tristis) in the larvicidal test was 448.5 ppm, 879 ppm, 1794 ppm, 3588 ppm, 7176 ppm, aquadest as a negative control and temephos as a positive control, which were exposed to Aedes aegypti larvae instar IV during 24 hours.

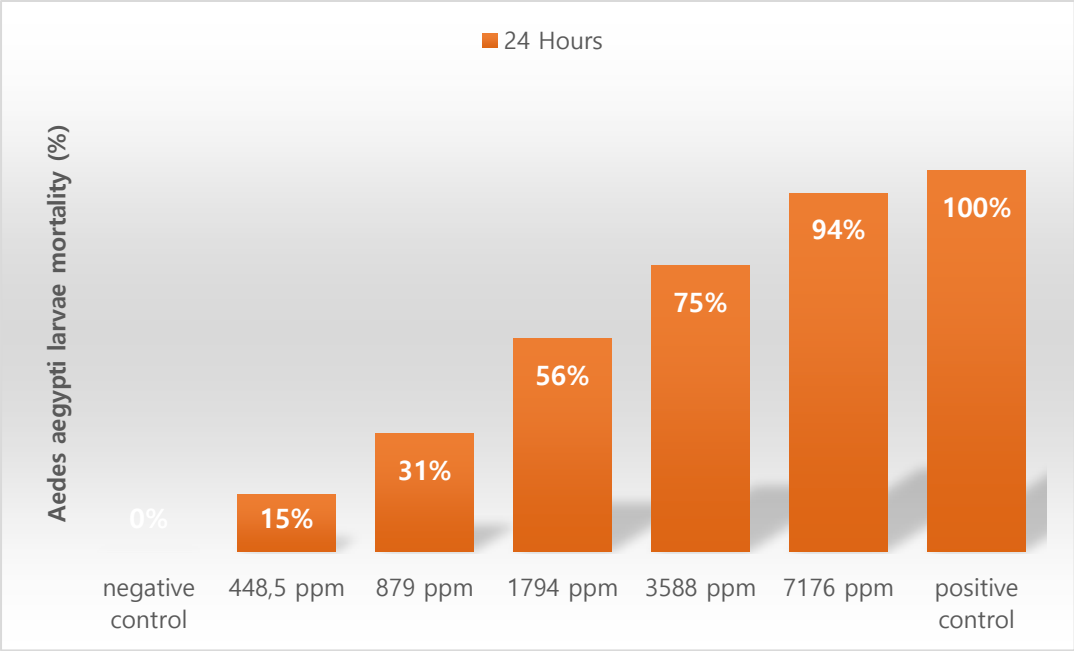

Figure 1. Effectiveness of Srigading Leaf Extract (Nyctanthes arbor-tristis) against fourth instar Aedes aegypti larvae for 24 hours of exposure

Figure 1 shows a graphic depiction of the percentage of mortality of fourth instar Aedes aegypti larvae after 24 hours of exposure to Srigading leaf extract (Nyctanthes arbor-tristis). In the negative control, there was no larval death so there was no need to correct the calculation of larval mortality using the Abbot formula. In the positive control using 1% temephos there was larval mortality with an average percentage of 100%. There was an increase in the mortality of Aedes aegypti larvae instar IV along with the increasing concentration of Srigading leaf extract (Nyctanthes arbor-tristis). The average larval mortality at a concentration of 448.5 ppm was 15%, at a concentration of 879 ppm was 31%, at a concentration of 3588 ppm was 56%, at a concentration of 1794 ppm was 75% and at a concentration of 7176 ppm, Aedes aegypti larvae mortality was 94%.

Table 1. ANOVA test results

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1937.333	5	387.467	149.987	**.000**
Within Groups	46.500	18	2.583		
Total	1983.833	23			

Based on table 1, it can be seen that the mortality of Aedes aegypty larvae using the one way ANOVA test obtained a significance value or p-value of 0.0001 <0.05 which indicates that there is a significant difference between the number of mosquito larvae deaths and the concentration of Srigading extract given. Then a post-hoc Least Signification Difference (LSD) test was carried out to determine the location of the difference in the average larval mortality at each concentration and used as a reference in determining whether the average of the two treatment concentrations was statistically different or not, so that the concentration could be determined. which has larvicidal activity.

Table 2. LSD post-hoc test results

Consentration		*p-value*	Meaning
Negative Control	With 448,5 ppm	0,203	Not significant
	With 897 ppm	0,828	Not significant
	With 1794 ppm	1,000	Not significant
	With 3588 ppm	0,0001	Significant
	Positive Control	0,0001	Significant
448,5 ppm	Negative Control	0,203	Not significant
	With 897 ppm	0,286	Not significant
	With 1794 ppm	0,203	Not significant
	With 3588 pm	0,002	Significant
	Positive Control	0,0001	Significant
897 ppm	Negative Control	0,828	Not significant
	With 448,5 ppm	0,286	Not significant
	With 1794 ppm	0,828	Not significant
	With 3588 ppm	0,0001	Significant
	Positive Control	0,0001	Significant
1794 ppm	Negative Control	1,000	Not significant
	With 448,5 ppm	0,203	Not significant
	With 897 ppm	0,828	Not significant
	With 3588 ppm	0,0001	Significant
	Positive Control	0,0001	Significant
3588 ppm	Negative Control	0,0001	Significant
	With 448,5 ppm	0,002	Significant
	With 897 ppm	0,0001	Significant
	With 1794 ppm	0,0001	Significant
	Positive Control	0,0001	Significant
Positive Control	Negative Control	0,0001	Significant
	With 448,5 ppm	0,0001	Significant
	With 897 ppm	0,0001	Significant
	With 1794 ppm	0,0001	Significant
	With 3588 ppm	0,0001	Significant

Based on the results of the one way ANOVA test, a significance value or p-value of 0.0001 < 0.05 was obtained, which indicated that there was a significant difference between the number of mosquito larvae deaths and the concentration of Srigading extract given. Based on the results

of the post-hoc test using LSD, it was found that there was a significant difference between the Srigading extract and the control group. The comparison found between the negative control and the concentration was found at a concentration of 3588 ppm, at that concentration there was a significant difference, this can be seen from the p-value or the significance value of 0.0001 compared to the value of <0.05. From these results it can be concluded that there is one concentration of Srigading leaf extract (Nycthanthes arbor-tritis) which has larvicidal activity.

Then, for the comparison between the positive control with concentrations of 448.5 ppm, 897 ppm, 1794 ppm, and 3588 ppm had the same significance value or p-value, namely 0.0001 with a value of <0.05, which means there is a significant difference. . So, it can be concluded that at concentrations of 448.5 ppm, 897 ppm, 1794 ppm, and 3588 ppm, it does not have the same effectiveness as 1% temephos. The average percentage of Aedes aegypti larvae mortality was not affected by increasing concentration, so the higher concentration of Srigading leaf extract did not affect the mortality of the test larvae.

Death of Aedes agypti larvae occurred after treatment in the form of Srigading (Nycthanthes arbor-tritis) leaf extract, this was due to the larvicidal effect of Srigading (Nycthanthes arbor-tritis) leaves which contain secondary metabolites. The secondary metabolites contained in Srigading consist of alkaloids, flavonoids, saponins, tannins, and triterpenoids. Alkaloid secondary metabolites are one of a group of compounds that can be found in most types of plants. The way alkaloids work as insecticides is by inhibiting the activity of the acetylcholinesterase enzyme. This compound works by stimulating the endocrine glands to secrete juvenile hormones, this increase can cause metamorphosis failure in mosquito larvae resulting in abnormal death in mosquito larvae. In addition, in inhibiting cell mitosis, alkaloids can synergize with triterpenoid compounds (27).

The secondary metabolites of flavonoids are compounds that can damage the cytoplasmic membrane and cause cell leakage and can turn off the enzyme system. This of course can result in phospholipids being unable to maintain the shape of the cytoplasmic membrane itself until it will eventually burst and the larvae themselves will die (27). Saponin secondary metabolites are compounds that work by lowering the surface tension on the mucous membrane of the larval digestive tract. This will inhibit the rate of nutrient absorption by the larvae. In addition, saponins have another effect, namely destroying the chitin layer on the surface of the larvae so that the extract can easily enter the body of the larvae (27). The secondary metabolites of tannins are polyphenolic compounds that work by causing astringency in plant parts that can enter mosquito larvae through the body wall, causing disturbances in the larval muscles. As a result, the larvae will experience weakness in the muscles of movement, so that the movement of the larvae will slow down. In addition to entering through the body wall, tannins can enter through the digestive tract of the larvae, this can cause interference with protein absorption in the larvae's intestines by reducing the activity of digestive enzymes and food absorption, so that the larvae will experience nutritional deficiencies and can cause death (28). Triterpenoid secondary metabolites are compounds that work by remembering free sterols in digestion where sterols act as precursors of the hormone ecdysone, so that a decrease in the amount of free sterols will disrupt the process of skin turnover in insects. In addition, these compounds can cause a decrease in the activity of digestive enzymes and affect the process of food absorption (28).

Determining the LC50 and LC90 values from the ethanol extract of Srigading leaves (Nyctanthes arbor-tristis) was performed using Probit Analysis with a 95% confidence level. Probit analysis for LC50 and LC90 can be seen in table 3 as follows

Table 3. LC50 and LC90 values from the results of probit analysis of Srigading leaf ethanol

extract (Nyctanthes arbor-tristis) against IV instar Aedes aegypti larvae after 24 hours

Mortality (%)	Estimate (%)	Confidence Level (%)	Confidence Interval	
			Lower Limit	Upper Limit
50	2.527	95	-9.649	3.507
90	5.460	95	4.389	20.775

Based on table 3, it can be seen that the results of the Probit analysis on the mortality rate of Aedes aegypti larvae obtained an LC50 value of 2.527%, this indicates that at a concentration of 2.527% within 24 hours it was able to kill 50% of the test larvae. While the obtained LC90 value of 5.460%, this shows that at a concentration of 5.460% within 24 hours it is able to kill 90% of the test larvae. Based on the results of the Probit analysis in table 3, the 24-hour LC50 value of Srigading leaf ethanol extract (Nyctanthes arbor-tristis) on the mortality of Aedes aegypti mosquito larvae was obtained at a concentration of 2.527%, which means that at a concentration of 2.527% ethanol extract of Srigading leaves (Nyctanthes arbor-tristis)) was able to kill 50% of Aedes aegypti larvae which were exposed for 24 hours, so it can be stated that the ethanol extract of Srigading leaves (Nyctanthes arbor-tristis) was effective against Aedes aegypti mosquito larvae with an LC50 value at 24 hours. While the 24-hour LC90 value of the ethanol extract of Srigading leaves (Nyctanthes arbor-tristis) on the mortality of Aedes aegypti mosquito larvae was obtained at a concentration of 5.460%, which means that at a concentration of 5.460% the ethanol extract of Srigading leaves (Nyctanthes arbor-tristis) was able to kill 90% of Aedes larvae. aegypti which was exposed for 24 hours, so it can be stated that the ethanol extract of Srigading leaves (Nyctanthes arbor-tristis) was effective against Aedes aegypti mosquito larvae with an LC90 value at 24 hours.

The results of this study showed that the ethanol extract of the leaves of Srigading (Nyctanthes arbor-tristis) had larvicidal activity against the larvae of the Aedes aegypti mosquito. However, according to previous studies, it is said that an extract of natural ingredients is effective as a pesticide if the LC50 is not more than 1000 ppm (0.1%). The LC50 value of the ethanol extract of Srigading leaves (Nyctanthes arbor-tristis) obtained was 2.527% or 25.270 ppm (conversion 1% = 10000 ppm), so it can be concluded that the ethanol extract of Srigading leaves (Nyctanthes arbor-tristis) still has high killing power. less against Aedes aegypti larvae. This may be caused by the form of the extract which is still in the form of crude extract or not in the form of pure compounds, so it is necessary to purify the compounds before they can be used as larvicides to obtain more optimal results (29).

The ethanol extract of Srigading leaves (Nyctanthes arbor-tristis) has larvicidal activity against Aedes aegypti mosquito larvae. Although the ability of Srigading leaf ethanol extract (Nyctanthes arbor-tristis) was still under positive control (temephos 1%), the ability of Srigading leaf ethanol extract (Nyctanthes arbor-tristis) could cause mortality of Aedes aegypti mosquito larvae, which was able to kill 50% of Aedes larvae. aegypti which was exposed for 24 hours with a concentration of 2.527%, this makes Srigading leaves (Nyctanthes arbor-tristis) have the potential to be used as biolarvicides and are relatively safer for the environment and not persistent in nature, in contrast to temephos 1% which has the potential to cause pollution and the occurrence of resistance in larvae if not using the appropriate dose (30).

Conclusion

Srigading leaf extract (Nyctanthes arbor-tristis) has the effect of larvacide Aedes aegypti instar IV at a concentration of 448.5 ppm, 879 ppm, 3588 ppm, 1794 ppm and at a concentration of 7176 ppm it has the equivalent effectiveness of 1% temephos. With the discovery of natural ingredients from the Srigading plant (Nyctanthes arbor-tristis L) which functions as a natural larvicide, it will help the community to carry out 3M activities in overcoming the problem of Dengue Hemorrhagic Fever (DHF) outbreak, especially for rural areas that are difficult to get access to. buy abate powder.

REFERENCES

1. Cahyati, Hary W, Siyam N. Perilaku Masyarakat dalam Penggunaan *Temephos. Higeia Journal Of Public Health Research and Development*. 2019;3(1):155–63.
2. Faizal AP. Identifikasi Metabolit Sekunder dan Aktivitas Larvasida dari Daun Matahari (*Helianthus annuus* L.) terhadap Larva *Aedes aegypti* Sp. *Mahakam Medical Laboratory Technology Journal*. 2017;2(2):80–90.
3. Firdhayani IN, Alamsjah MA, Subekti S. Eksplorasi Bahan Aktif Rumput Laut Coklat (*Phaeophyceae*) sebagai Biolarvasida *Aedes aegypti.* Jurnal Ilmiah Perikanan dan Kelautan. 2014;6(2):187–92.
4. Handayani SW, Prastowo D, Boesri H, Oktsariyanti A, Joharina AS. Efektivitas Ekstrak Daun Tembakau (*Nicotiana tabacum* L) dari Semarang, Temanggung, dan Kendal sebagai Larvasida *Aedes aegypti* L. Jurnal BALABA. 2018;14(1):23–30.
5. Hasbullah ML, Nurdian Y, Abrori C. Potensi Ekstrak Etanol Daun Meniran (*Phyllantus niruri* L.) sebagai Larvasidal Nyamuk *Aedes aegypti*. *Journal of Agromedicine and Medical Sciences*. 2019;5(1):1–6.
6. Henrique MO, Neto LS, Assis JB, Barros MS, Capurro ML, Lepique AP, *et al*. *Evaluation of Inflammatory Skin Infiltrate Following Aedes aegypti Bites in Sensitized and Non-Sensitized Mice Reveals Saliva-Dependent and Immune-Dependent Phenotypes. Immunology.* 2019;158(1):47–59.
7. Kartikasari D, Novitasari M. Uji Aktivitas Larvasida Perasan Herba Seledri (*Apium Graveolensl*.) terhadap Larva *Aedes aegypti*. Jurnal As-Syifaa. 2018;10(2):152–60.
8. Kementerian Kesehatan RI. Profil Kesehatan Indonesia Tahun 2018. Jakarta: Kementrian Kesehatan RI; 2019.
9. Khusna AM. Toksisitas Campuran Ekstrak Buah Srikaya (*Annona squamosa* L.) dan Ekstrak Buah Ketapang (*Terminalia catappa* L.) terhadap mortalitas Larva Nyamuk *Aedes aegypti* serta Pemanfaatannya. Universitas Jember; 2017
10. Koneri R, Pontororing HH. Uji Ekstrak Biji Mahoni (*Swietenia macrophylla*) terhadap Larva *Aedes aegypti* Vektor Penyakit Deman Berdarah. Jurnal Media Kesehatan Masyarakat Indonesia. 2016;12(4):216–24.
11. Loren I. Toksisitas Campuran Ekstrak Daun Sirih (*Piper betle* L.) dan Ekstrak Biji Srikaya (*Annona squamosa* L.) terhadap Mortalitas Larva Nyamuk *Aedes aegypti* L. Universitas Jember; 2016.
12. Merisia. Uji Ekstrak Batang Sereh (*Cymbpogon nardus* (L.) Rendle) dalam Membunuh Larva *Aedes aegypti*. STIKes Insan Cendekia Medika; 2018.
13. Nyctanthes arbor-tristis in Flora of Pakistan @ efloras.org". *www.efloras.org*. Diakses tanggal 2019-08-30.

14. Pambudi BC, Martini, Tarwotjo A, Hestiningsih R. Efektivitas *Temephos* sebagai Larvasida pada Stadium Pupa *Aedes aegypti*. Jurnal Kesehatan Masyarakat (*e-Journal*). 2018;6(1):381–8.
15. Puri, A., Saxena, R., Saxena, R. P., Saxena, K. C., Srivastava, V., & Tandon, J. S. Immunostimulant activity of Nyctanthes arbor-tristis L. *J. Ethnopharmacol*. 42 (1): 31-37.
16. Putranta NR, Musyabiq S. Efektifitas Ekstrak Kulit Duku (Lansium domesticum corr) sebagai Larvasida *Aedes aegypti*. Jurnal Medusa. 2018;7(5):165–70.
17. Rawani A, Pal S, Chandra G. *Evaluation of Antimicrobial Properties of Four Plant Extracts Againts Human Pathogen. Asian Pacific Journal Of Tropical Biomedicine.* 2011;1(1):S71–5.
18. Ramadhan MRF. Toksisitas Campuran Ekstrak Biji Pepaya (*Carica papaya* L.) dan Biji Srikaya (*Annona squamosa* L.) terhadap Mortalitas Larva Nyamuk *Aedes aegypti*. Universitas Jember; 2016.
19. Rosyid A, Santoso A, Naila IU. Analisis Efektivitas Biaya Terapi Suportif Imunomodulator dan Kapsul Ekstrak Daun Jambu Biji Demam Berdarah Dengue. Jurnal Farmasi Sains dan Praktis. 2019;5(1):1–6.
20. Sandra T, Sofro MA, Suhartono, Martini, Hadisaputro S. Faktor-Faktor yang Berpengaruh terhadap Kejadian Demam Berdarah Dengue Pada Anak Usia 6-12 Tahun Di Kecamatan Tembalang. Jurnal Epidemiologi Kesehatan Komunitas. 2019;4(1):1–10.
21. Seksi Surveilans dan Imunisasi. Data Penyakit Demam Berdarah Dengue. Banjarmasin; 2020.
22. Syadid R. Hu n Pengetahuan, Sikap dan Pendidikan dengan Perilaku Pencegahan Demam Berdarah Dengue. Universitas Lambung Mangkurat; 2017.
23. Tina L, Misnawati, Nirmala F. Uji Perbandingan Efektivitas Ekstrak Daun Sirih Hijau (*Piper betle* Linn) dengan Ekstrak Daun Pandan Wangi (*Pandanus amaryllifolius* Roxb) terhadap Kematian Larva Nyamuk *Aedes aegypti* Tahun 2018. Jurnal Ilmiah Praktisi Kesehatan Masyarakat. 2018;3(1):1–11.
24. World Health Organization. *Dengue and Severe Dengue* [Internet]. *Dengue Data Application*. 2020 [cited 2020 Feb 29]. *Available from: https://ntdhq.shinyapps.io/dengue5/*
25. Widyastuti DA, Rahayu P, Dewi LR. Potensi Ekstrak Sirsak (*Annona muricata*) sebagai Larvasida Pengendali Populasi *Aedes albopictus*. Jurnal Bioeksperimen. 2019;5(1):48–54.
26. Wahyuni D. Toksisitas Ekstrak Tanaman sebagai Bahan Dasar Biopestisida Baru Pembasmi Larva Nyamuk *Aedes aegypti* (Ekstrak Daun Sirih, Ekstrak Biji Pepaya, dan Ekstrak Biji Srikaya) Berdasarkan Hasil Penelitian. Malang: Media Nusa Creative; 2016.
27. Sogandi S, Gunarto F. Efek Larvasida Fraksi Etil Asetat Daun Bangun-bangun (Plectranthus amboinicus) terhadap Mortalitas Larva Aedes aegypti. ASPIRATOR - J Vector-borne Dis Stud. 2020;12(1):27–36.
28. Nadila I, Istiana I, Wydiamala E. AKTIVITAS LARVASIDA EKSTRAK ETANOL DAUN BINJAI (Mangifera caesia) TERHADAP LARVA Aedes aegypti. Berk Kedokt. 2017;13(1):61–8.

29. Maulana H, Wydiamala E, Biworo A. Uji Aktivitas Larvasida Ekstrak Etanol Daun Pucuk Merah (Syzygium myrtifolium Walp.) terhadap Nyamuk Aedes aegypti. Homeostasis. 2021 Dec 29;4(3):567-74.
30. Rodriguez, M.M., *et al.* Detection of insecticide resistence in *Aedes aegypti* (Diptera: Culicidae) from Cuba and Venezuela. Journal of Medical Entomology 2001. 38: 623-628.

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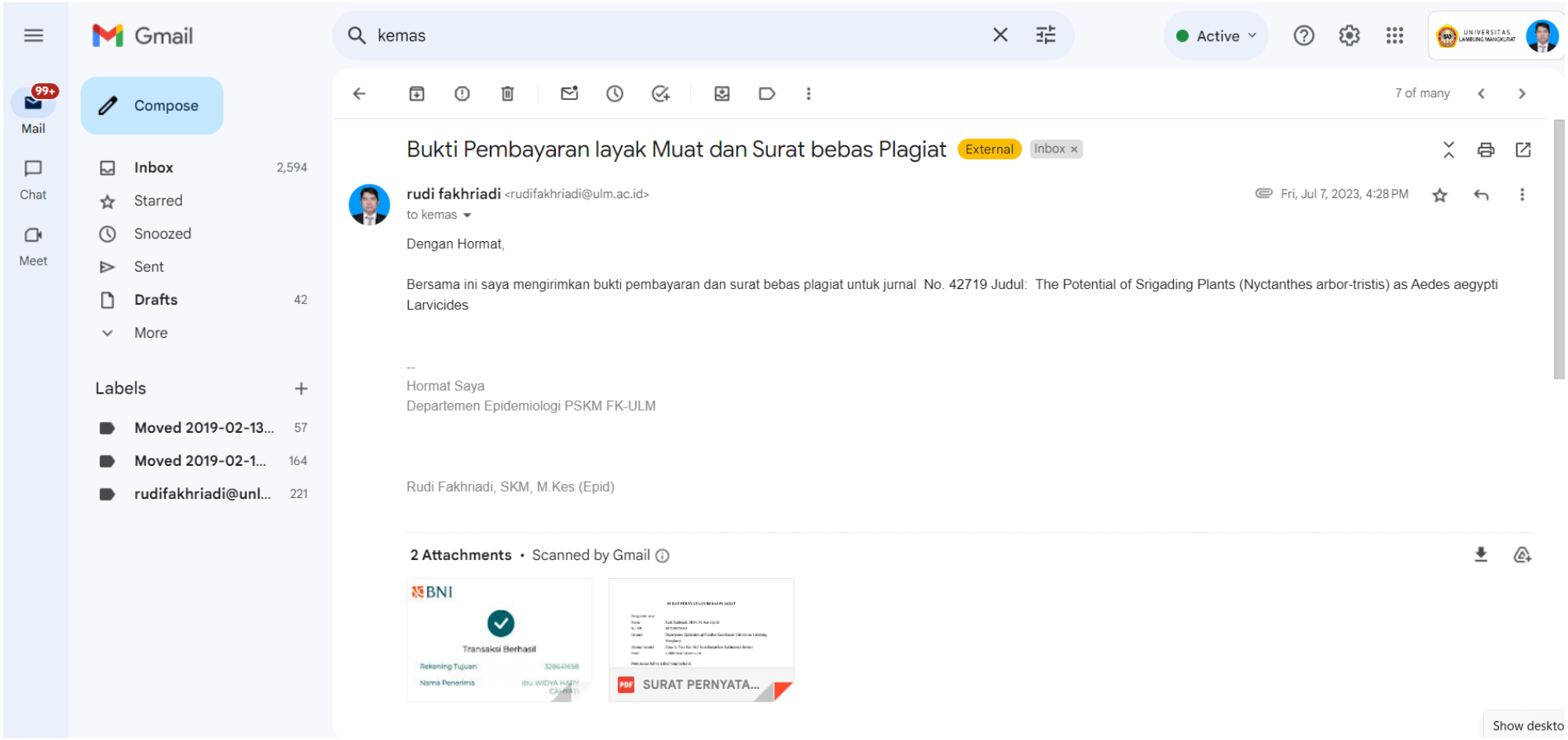

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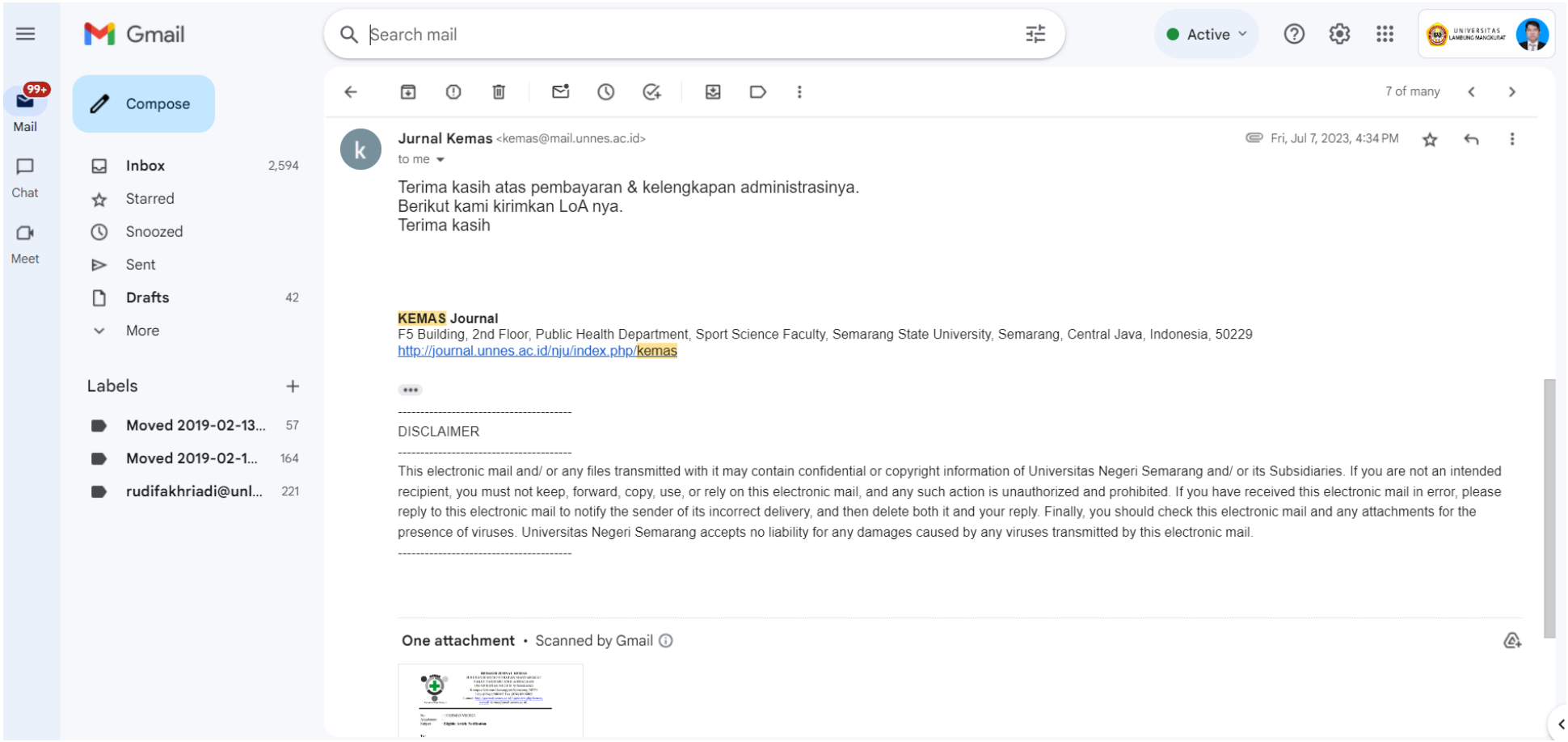
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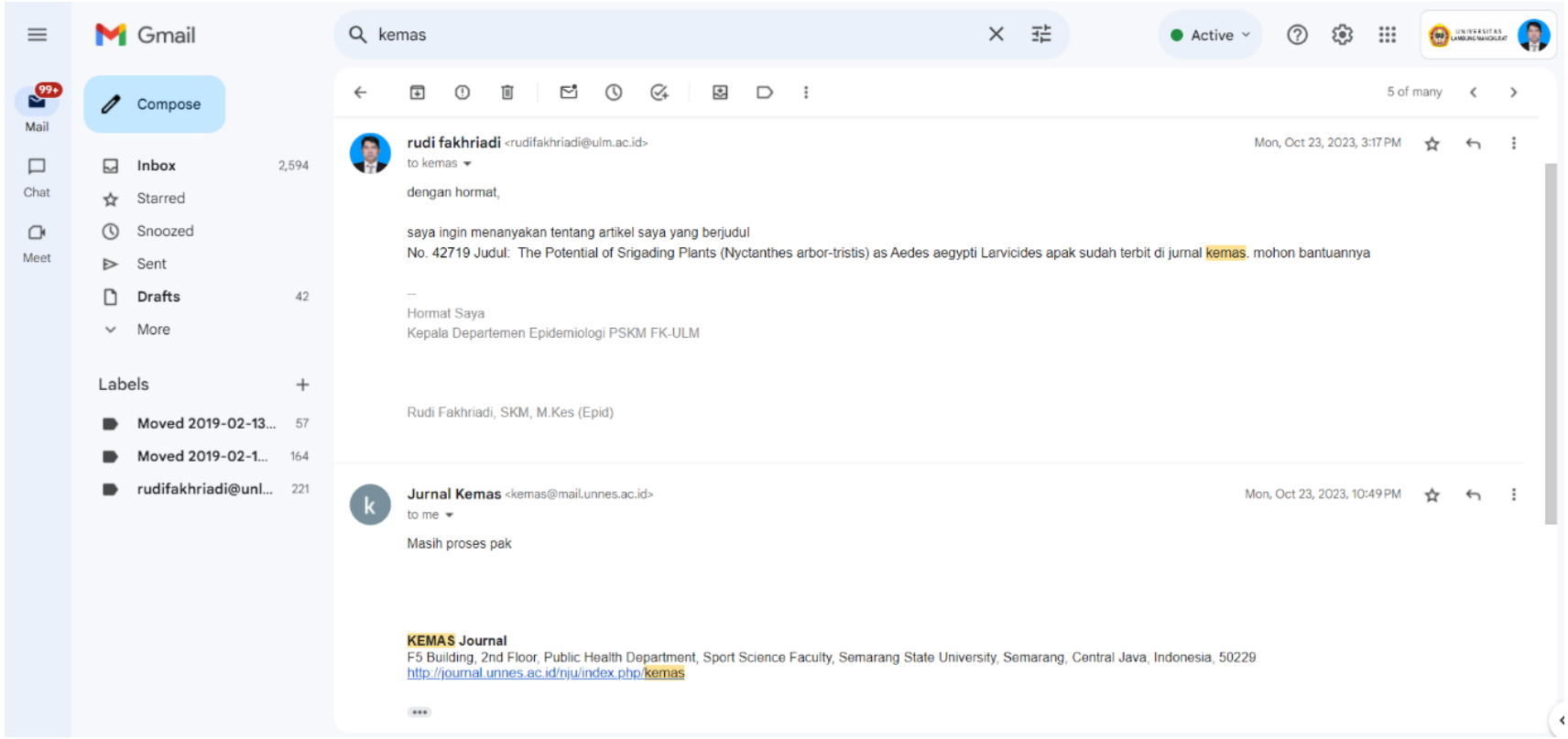

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