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Moringa leaf (*Moringa oleifera L*) flavonoids utilization in suppressing growth *of Aedes aegypti larvae*

Pemanfaatan Flavonoid Daun Kelor (*Moringa oleifera* L) dalam Menekan Pertumbuhan Larva Nyamuk *Aedes aegypti*

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ABSTRACT

The study was conducted to determine the potential use of Moringa leaf flavonoids as biolarvicides in suppressing the growth of Aedes aegypti mosquito larvae. The design used was a completely randomized design (CRD) with six flavonoid treatments including P0 (0 ppm flavonoids as a negative control), P1 (12.5 ppm flavonoids), P2 (25 ppm flavonoids), P3 (50 ppm flavonoids), P4 (flavonoids 75 ppm), and P5 (flavonoids 100 ppm). Each treatment was repeated three times. The results of the analysis of diversity (Anova) revealed that Moringa leaf flavonoids had a very significant effect (P = 0.000) on the mortality of A. aegypti mosquito larvae. The administration of 50 ppm flavonoids of Moringa leaf extract caused the mortality of A. aegypti larvae to reach 95%, and at a concentration of 75 ppm, it increased the mortality percentage to 100%. The results of the probit analysis showed that the lethal concentration 50 (LC50) was achieved at a concentration of 7.96 ppm with exposure for 24 hours, while the lethal concentration-time 50 (LT50) at a concentration of 75 ppm was achieved in a shorter time (2.08 hours).

Keywords: flavonoids, moringa leaf, Aedes aegypti, biolarvacide

ABSTRAK

Penelitian dilakukan untuk mengetahui potensi pemanfaatan flavonoid daun kelor sebagai biolarvasida dalam menekan pertumbuhan larva nyamuk Aedes aegypti. Rancangan yang digunakan adalah Rancangan Acak Lengkap (RAL) dengan enam perlakuan flavonoid meliputi P0 (flavonoid 0 ppm sebagai kontrol negatif), P1 (flavonoid 12,5 ppm), P2 (flavonoid 25 ppm), P3 (flavonoid 50 ppm), P4 (flavonoid 75 ppm), dan P5 (flavonoid 100 ppm). Setiap perlakuan diulang sebanyak tiga kali. Hasil analisis keragaman (Anova) menunjukkan bahwa flavonoid daun kelor berpengaruh sangat nyata (P = 0,000) terhadap mortalitas larva nyamuk A. aegypti. Pemberian 50 ppm flavonoid ekstrak daun kelor menyebabkan kematian larva A. aegypti mencapai 95% dan pada konsentrasi 75 ppm meningkatkan persentase kematian hingga 100%. Hasil analisis probit menunjukkan bahwa lethal concentration 50 (LC_{50}) dicapai pada konsentrasi 7,96 ppm dengan pemaparan selama 24 jam sedangkan lethal concentration time 50 (LT_{50}) pada konsentrasi 75 ppm dicapai dalam waktu lebih singkat (2,08 jam).

Kata Kunci: flavonoid; daun kelor; Aedes aegypti; biolarvasida

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1. INTRODUCTION

The *Aedes aegypti* mosquito is a biological vector that aids in spreading dengue fever. The dengue fever cases in East Nusa Tenggara (NTT) as of April 1, 2020, were 4,518 cases, with 48 deaths. The four regencies/cities in NTT with the highest cases were Sikka 1,548 cases, Kupang City 578 cases, Belu Regency 569 cases, and Alor Regency 401 cases (Bere, 2020). Data from the Indonesian Ministry of Health shows that cases of DHF between January to July 2020 in Indonesia reached 71,633 cases, with the number of deaths reaching 459 (Maulana et al., 2021).

Djojosumarto concluded that eradication attempts involving the killing of adult mosquitoes were ineffective compared to the use of larvicides to destroy mosquito larvae (Yasi et al., 2018). Synthetic larvicides have always been the preferred method of preventing the spread of the *Aedes aegypti* mosquito due to their ease of use, availability, and effectiveness. However, the use of conventional insecticides to control mosquitoes, such as organochlorine, carbamates, pyrethroids, malathion, DDT, and organophosphorus, has raised concerns about environmental pollution, residual effects, and resistance of certain mosquito populations, as well as adverse effects on human health (Gautam et al. 2013; Ferreira et al., 2009). The investigation of alternative controllers that are safer and derived from plants is motivated by the adverse effects of chemical pesticides on non-target creatures, human health concerns, and the environment. The use of ecologically acceptable bioactive compounds that are readily biodegradable and have specific targets that are poisonous to nuisance insects is a viable alternative.

Plants contain bioactive organic chemicals, which are superior to synthetic pesticides because they are less toxic, less susceptible to the development of resistance, and are easily biodegradable (Prabhu et al., 2011). Research and development of natural larvicides derived from plants have been widely carried out. Nwankwo et al. (2015) showed that the seed oil extracts of *M. oleifera* and *Anona muricata* were beneficial as larvacides to control *A. aegypti* in eradicating yellow fever outbreaks and reducing the impact of conventional insecticides on the environment.

Nature provides a variety of medicinal plants that are useful in controlling infectious disease vectors (Prabhu et al., 2011). Moringa leaves are widely known as medicinal plants. Moringa leaf extract can be used directly on mosquito habitats for effective control (Sharma et al., 2013).

Moringa oleifera, according to Coppin et al. (2013), is a multipurpose tropical plant that is underutilized as a medicinal and nutritional ingredient. However, this plant contains high concentrations of flavonoids, which could be a new bioactive source in developing natural commercial products. Flavonoids are polyphenolic compounds that are abundant in nature. Flavonoids consist of several hydroxyl groups attached to an aromatic ring structure (Mohammed and Manan, 2015) and are generally soluble in water (Lin et al., 2018). Its antioxidant properties are determined through the mechanism of chelation formation or scavenging (Astuti et al., 2021). Gautam et al. (2013) proved that the flavonoid extracts of *Vitex negundo* and *Andrographis paniculata* had larvicidal activity against *Anopheles stephensi* and *A. aegypti* mosquitoes. Phytochemicals derived from plant materials can act as larvicides, insect growth regulators, repellants, and oviposition attractants (Prabhu et al., 2011).

Moringa leaf extract from the Giri Banyuwangi area can *kill A. aegypti* mosquito larvae with an LC50 of 3953.17 ppm and an LT50 of 18.98 hours (Yasi and Harsanti, 2018). Hikmah and Ardiansyah (2018) showed that Moringa leaf extract (*M. oleifera*) combined with fig leaf (*Ficus carica*) at a ratio of 75%: 25% was effective in triggering the mortality of *A. aegypti* larvae. With this combination, larval

mortality reached 62% after 10 hours of exposure.

Moringa plants contain various bioactive chemicals, such as alkaloids, saponins, tannins, and phenols, that have the potential as larvicides (Putra et al., 2016). Meye et al. (2021) proved that the alkaloid and tannin compounds of Moringa leaf extract are toxic to *A. aegypti* larvae, indicating that they have the potential to be developed as natural pesticides or biolarvicides. However, little data show the application of flavonoid compounds from Moringa leaf extract directly to *A. aegypti* larvae. This research was conducted to study the potential use of Moringa leaf flavonoids as biolarvicides in suppressing the growth of *A. aegypti* mosquito larvae.

2. MATERIALS AND METHODS

2.1. Materials

The materials used were Moringa leaves (*Moringa oleifera* L) obtained from Baumata Timur Village, Kupang Regency (semi-arid region), *A. aegypti* eggs (Waikabubak Health Research and Development Center NTT), aquadest, 70% ethanol, n-hexane (Sigma), ethyl acetate (Merck), chloroform (Sigma), magnesium powder, concentrated HCL (Sigma), 10% NaOH, acetic acid (Sigma), n-butanol (Merck), 1% $AlCl_3$ detector, and methanol (Merck). The tools used were graduated cylinder, Erlenmeyer, test tube racks, tongs, beakers, blenders (National), UV-Vis spectrophotometer (Genesys 10S UV-Vis Spectrophotometer), filter paper, oven, medium-sized plastic containers, sifter trays, filter cloth, rotary vacuum evaporator (Buchi Rotavapor R-210), stirring rod, analytical balance (Digital SF400), glass plate, Thin Layer Chromatography (TLC) plate, water bath, silica Gel G60F254, spray bottle, ultraviolet lamp, coverslip, syringe, funnel, dropper, sieve, knife, glass jar, static, test tube, volume pipette, loop, and digital camera.

2.2. Maceration

The Moringa leaves were used from stalks number 2-5. The leaves were dried, mashed with a blender, and filtered. A total of 400 grams of Moringa leaf powder was macerated with 800 mL of 70% ethanol and stirred twice a day. After 24 hours, it was filtered and separated between the dregs and the filtrate. The dregs were macerated again with new ethanol and repeated until the clear filtrate was obtained. The filtrate obtained was concentrated with an evaporator at 40ºC (Yasi and Harsanti, 2018). The yield was calculated using the following formula:

$$\%Yield = \frac{weight\ of\ extract}{\text{weight of moringa leaf powder use}} \times 100\%$$

2.3. Extract fractination

A total of 5 g of Moringa leaf extract was dissolved in 100 mL of water, stirred until it was runny and homogeneous, and then put into a separating funnel. Fractionation was carried out with 10 mL of n-hexane solvent. The solvent was put into a separating funnel and then shaken 3 times to form 2 layers, namely n-hexane and a layer of water. The n-hexane layer and the aqueous layer were further separated. The water layer was fractionated with 10 mL of chloroform to form 2 layers: the chloroform and the water. The chloroform layer and the aqueous layer were separated. The aqueous layer was fractionated with 10 mL of ethyl acetate to obtain an ethyl acetate and a water layer. The ethyl acetate layer and the aqueous layer were separated. The above process was repeated 5 times. This process produced n-hexane fraction, chloroform fraction, and ethyl acetate fraction. Furthermore, the three fractions were evaporated by heating using a water bath for 15 minutes at 100˚C (Simanjuntak, 2008).

2.4. Flavonoid phytochemical test

As much as 1 mL of the three fractions were put into 3 test tubes. The first tube was reacted with magnesium powder and 2-4 drops of concentrated HCl and shaken. The formation of red color indicates the presence of flavonoids from the flavonol and flavanone

groups. The second tube was dropped with concentrated HCl and heated for 15 minutes on a bath. The orange color formed indicates the presence of anthocyanidin group flavonoids. The third tube was dripped with 10% NaOH solution, and the color change indicated the presence of phenolic flavonoids (Zirconia et al., 2015).

2.5. Separation of flavonoid compounds with TLC

The stationary phase used 4 G60F254 silica plates measuring 7 x 1 cm, which were marked with a line 1 cm from the bottom edge of the plate to indicate the initial position of the spots and 1 cm from the top edge of the plate to indicate the boundaries of the elution process. The TLC plate was activated by heating in an oven at 105ºC for 15 minutes to remove moisture, then removed and cooled.

The mobile phase (eluent) used several mixed eluents, including n-hexane: ethyl acetate (16: 4), acetic acid: H2O: concentrated HCl (15: 5: 1.5), n-butanol: acetic acid: water (8: 2: 10), methanol: ethyl acetate (16: 4). Each eluent was put into a beaker. The positive ethyl acetate fraction containing flavonoids was taken as much as 5 mL using a syringe, and then it was spotted on a plate that had been marked and put into a beaker containing the eluent. The elution process was stopped when a predetermined limit was reached. The plate was removed, dried, and then wrapped in aluminum foil (Rahmawati, 2015; Zirconia et al., 2015). The silica plate was then observed under UV light at a wavelength of 366 nm. The visible spots were marked with a pencil, and the distance traveled by each spot and the Rf (Retardation factor) value were calculated.

$$RF = \frac{\text{the distance traveled by the substance}}{\text{distance traveled by the mobile phase}}$$

(Sari, 2011).

2.6. Larva preparation

A. aegypti eggs were incubated in water media. Larvae were reared for 3-5 days to reach instar III and IV larvae with complete and clear anatomical characteristics, there were parts of the head (chepal), chest (thorax), abdomen, spines on the chest begin to be clearly visible, and breathing funnel (siphon) which was blackish brown. Larvae were fed with dog food (Buni, 2013).

2.7. Research design

This study used a completely randomized design (CRD) consisting of 6 treatments with 3 replications. The treatments applied were variations in flavonoid concentrations (0 ppm (P0), 12.5 ppm (P1), 25 ppm (P2), 50 ppm (P3), 75 ppm (P4), and 100 ppm (P5). Into each container, flavonoid compounds were added according to the predetermined concentration and added water until it reached 100 mL. The solution was then stirred until it was runny and homogeneous. 20 *A. aegypti* larvae were placed into each container, and the larval mortality was observed every 2 hours for 24 hours (Imanta and Hidajati, 2017) using the formula:

$$M = \frac{Mt}{M0} \text{ x } 100\%$$

Where M is the percentage of larval mortality (%), Mt is the number of dead larvae, and M0 is the number of early larvae.

In addition, the Lethal Concentration 50 (LC50) was determined, which is the concentration of the extract that can cause 50% larval death, and the Lethal Concentration Time 50 (LT50) is the time needed to kill up to 50% of the larvae.

The percentage of larval mortality was analyzed using analysis of variance (ANOVA) to determine the effectiveness of flavonoid compounds in *A. aegypti* larvae. Significant differences between treatments were tested using Duncan MRT (Multiple Range Test), while LC50 and LT50 were analyzed using the Minitab version 16 program.

3. RESULTS AND DISCUSSION

3.1. Phytochemical test

The phytochemical test proved that the ethyl acetate fraction gave positive results of containing flavonoid compounds, whereas the chloroform and n-hexane fractions gave negative results (Fig.1). The first flavonoid test used magnesium powder and added with concentrated HCl. The polyhydroxy flavonols will be reduced by magnesium metal in hydrochloric acid, resulting in red benzopyrylium or flavilium salts and foaming. The second flavonoid test used concentrated HCl then heated. HCl hydrolyzes and breaks down anthocyanins into anthocyanidins, heating accelerates the reaction until an orange color was formed. The third flavonoid test with 10% NaOH produced a red to brown color because flavonoid compounds formed acetophenone which reacted with NaOH. Phytochemical tests conducted by Yasi and Harsanti (2018) proved that Moringa leaf extract contains flavonoid compounds. In this study, the ethyl acetate fraction was positive for flavonoids, so it was continued with Thin Layer Chromatography (TLC) (Zirconia, 2015).

The eluent mixture of n-butanol: acetic acid: water (8:2:10) in TLC provided the best separation by producing two stains with Rf values of 0.42 and 0.9, respectively (Fig. 1). In contrast, a mixture of n-hexane: ethyl acetate (16:4) eluent, concentrated acetic acid:water: HCl (15:5:1,5), and methanol: ethyl acetate (16:4) resulted in only one stain. The separation occurs because the eluent used is polar so that it can separate flavonoid compounds which are also polar. According to Latif et al. (2018), a good eluent is an eluent that can separate compounds in large quantities marked by the appearance of stains. Yuda (2017) used the mobile phase of n-butanol: acetic acid: water (1:4:5) for the flavonoid TLC test and produced six spots.

The appearance of a light blue color on the two TLC stains observed under UV light proved that the Moringa leaf extract was positive for flavonoids which were thought to be from the isoflavone flavonoid compound group. Yuda (2017) proved that the blue fluorescence at UV 366 nm with a length of Rf 0.76 is a type of isoflavone flavonoid containing a free 5-OH group.

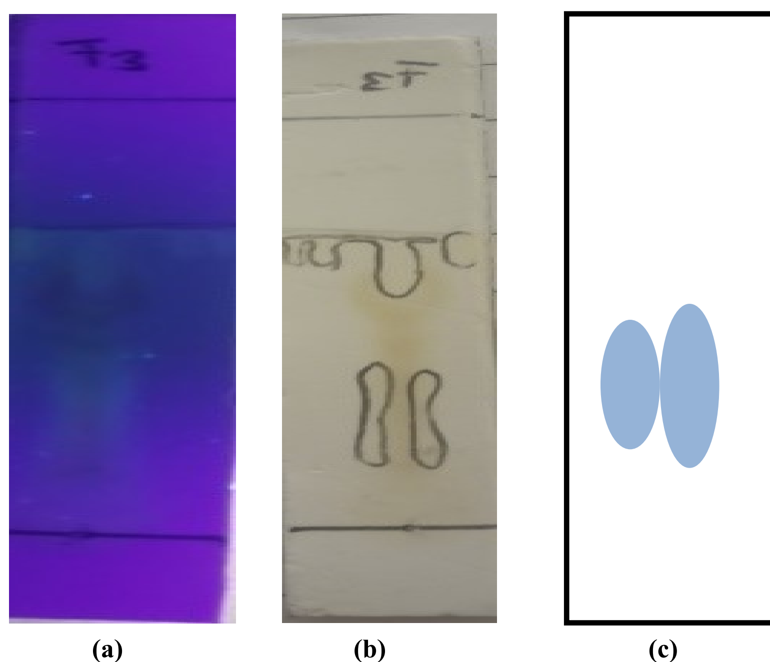

Figure 1. TLC results of the ethyl acetate fraction of Moringa leaf extract with n-butanol as eluent: acetic acid: water (8:2:10). Observations under UV light (λ 366 nm) (a), without UV light (b), and picture illustrations (c).

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Table 1. Mortality percentage of *A. aegypti* larvae after 24 hours of treatment

Treatment (ppm)	Repetition			Total Deaths	Average	Percentage
	1	2	3			
P0 (0)	0	0	0	0	0	0[a]
P1 (12.5)	14	14	15	43	14.3	72[b]
P2(25)	17	18	17	52	17.3	87[bc]
P3(50)	19	18	20	57	19.0	95[c]
P4 (75)	20	20	20	60	20.0	100[c]
P5 (100)	20	20	20	60	20.0	100[c]

[*] Numbers in columns with the same superscript are not significantly different

3.2. Effects of Moringa leaf extract flavonoids

Moringa leaf flavonoids are very potential as larvicides of *A. aegypti*. The results of the larvicidal test of Moringa leaf flavonoids are presented in Table 1. Table 1 shows that the higher the flavonoid concentration, the higher the mortality percentage of *A. aegypti* larvae. The analysis of variance (ANOVA) showed that the flavonoids of Moringa leaf extract had a very significant effect ($p = 0.000$) on the mortality of *A. aegypti* larvae. The use of flavonoids with concentrations of 75 ppm and 100 ppm can trigger larval death up to 100%. However, the two treatments were not statistically significant ($P>0.05$) in larval mortality compared to concentrations of 50 ppm and 25 ppm. This study showed that the application of Moringa leaf flavonoids at a dose of 25 ppm could initiate larval death, although it was 13% lower than the application of 75 ppm. Increasing the concentration of flavonoids to 50 ppm could initiate an increase in mortality 5% lower than the 75 ppm application. The toxic effects caused by larval mortality ranged from 87%-100%, proving that Moringa leaf flavonoids become toxic loads and are very potential as natural larvicides when applied at adequate concentrations.

A. aegypti larvae treated with flavonoids showed poisoning symptoms such as restlessness, up and down movements in water media (telescopic), and death marked by floated mosquito larvae on the surface of the media and did not respond when stimulated by touch. Flavonoids are plant secondary metabolites with various metabolic functions (Makita et al., 2016). Harith et al. (2018) stated that in plants, flavonoids act as attractants or excitants, repellents or feed deterrents, and also as oviposition inhibitors. The toxicity of flavonoids triggers larvicidal activity. In this study, flavonoids with a concentration of 75 ppm caused death up to 100% after exposure for 24 hours. In contrast, using moringa leaf extract tannins at a concentration of 75 ppm after 72 hours of exposure could only kill larvae with a mortality percentage of 67% (Meye et al., 2021).

Flavonoids work as respiratory toxins that enter the body through the respiratory system (Cania and Setyaningrum, 2013). Moringa leaf flavonoid compounds are thought to affect and damage the respiratory system and cause nervous wilting, resulting in larvae not being able to breathe and ending in death. Moringa leaf flavonoids stimulate cytoplasmic membrane damage at the cellular level, causing cells to leak and cause the enzyme system to inactivate. As a result, phospholipids cannot maintain the shape of the cytoplasmic membrane so that the membrane breaks and causes the death of *A. aegypti* larvae (Hebert and Hamidah, 2018).

3.3. Lethal concentration 50 (LC_{50}) of Moringa leaf flavonoids

LC_{50} was determined by probit analysis to determine the effective concentration that could kill 50% of the total larvae of the test. The relationship curve between flavonoid concentration and the number of larval mortality is presented in Fig. 2.

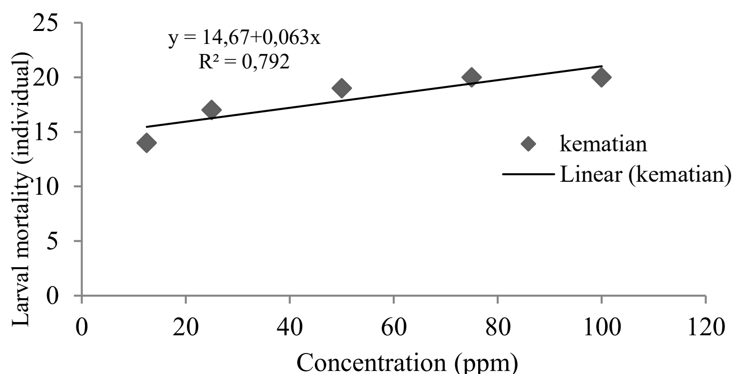

Figure 2. The relationship curve between flavonoid concentration and the number of deaths of A. aegypti mosquito larvae

Fig. 2 shows that the percentage of mortality of *A. aegypti* mosquito larvae and the concentration of Moringa leaf flavonoids follow the equation Y = 14.67 + 0.063x. Each increase in flavonoid concentration of Moringa leaf extract by one unit will increase the mortality of 0.063 individual larvae of *A. aegypti* with a coefficient of determination (R2) of 0.792. It means that 79.2% of mosquito larvae mortality was caused by the contribution of flavonoid compounds from Moringa leaf extract and the rest by other factors not observed in this study. Coelho et al. reported that sulfate flavonoids in the Sterculiaceae family showed strong larvicidal activity against *A. aegypti* larvae so that they have the potential to be developed as domestic larvicides in combating *A. aegypti*, which is an insect vector for several viral diseases such as dengue fever and Zika (Fernandes et al., 2018). According to Haditomo, flavonoids are stomach poisons that interfere with the digestive system of *A. aegypti* larvae and cause failure to grow and then die (Utami et al., 2016). Flavonoids such as furanoflavonoid karanjin, pyranoflavonoid karanjachromene, and oleic acid from Millettia pinnata were very effective against larvae of *A. aegypti* and *Culex pipiens* pallens. The flavonoids karanjachromene, pongamol, and pongarotene have strong inhibitory effects on mosquito larvae acetylcholinesterase but do not affect cAMP levels (Perumalsamy et al., 2015).

Probit analysis showed that the LC50 of Moringa leaf flavonoids in *A. aegypti* larvae was reached at a concentration of 7.96 ppm. It means that Moringa leaf flavonoids (Moringa oleifera L) could kill 50% of test larvae at 7.96 ppm application. The relatively small LC_{50} value proves that flavonoid compounds have the potential as biolarvicides in mosquito larvae (Rumengan, 2010). The LC_{50} value for the toxicity test is classified as very high if <1 mg/L, high if it ranges from 1-10 mg/L, moderate if >10-100 mg/L, and low if >100 mg/L (Hendri et al., 2010).

3.4. Lethal concentration time (LT_{50}) of Moringa leaf flavonoids

LT_{50} was determined by probit analysis to determine the effective time in killing 50% of the test larvae. The LT_{50} values for each concentration are shown in Table 2. Table 2 shows that the application of 75 ppm flavonoids gave a shorter LT_{50} value of 2.083 hours compared to other treatments. It means that after 2.08 hours of administration of 75 ppm flavonoids, 50% of *A. aegypti* larvae will die. Cania and Setyaningrum (2013), who used legundi leaf extract, found that the higher the concentration of the extract, the shorter the LT_{50}. The linear regression equation curve of the relationship between time and number of larval mortality after being treated with flavonoids from Moringa leaf extract is presented in Fig. 3.

Table 2. LT_{50} Value of Moringa Leaf Flavonoids

Treatment	LT_{50} Value
12.5 ppm	17.4357
25 ppm	6.32661
50 ppm	2.62772
75 ppm	2.08281
100 ppm	3.45742

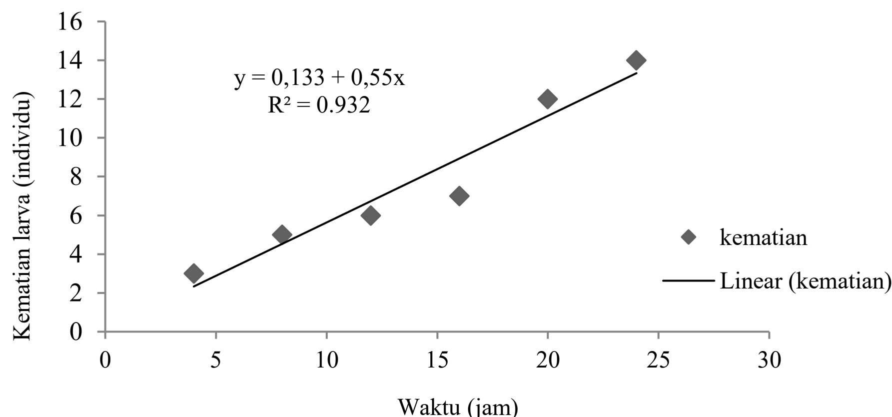

Figure 3. Relationship curve of time and number of larvae mortality treated with flavonoid

The pattern of the relationship between the time and the number of deaths of *A. aegypti* larvae given flavonoids as shown in Fig 3 shows that each time increase of one unit will increase the mortality of larvae by 0.55 individuals following the equation, Y = 0.133 + 0.55x with a coefficient of determination (R2) of 0.932. It indicates that 93.2% of larval mortality was triggered by the length of time the larvae were exposed to Moringa leaf flavonoids, while the rest was caused by other factors not observed in this study. This study confirms Gautam et al. (2013), who stated that the larvicidal activity of *Vitex negundo* and *Andrographis paniculata* against *Anopheles stephensi* and *A. aegypti* was a synergistic effect of phenolic compounds, including flavonoids. However, further research is needed before the commercial use of the flower bud flavonoid extract of *V. negundo*. Likewise, the flavonoids of Moringa leaf extract in this study need to be studied further before being applied commercially.

4. CONCLUSIONS

The application of 75 ppm Moringa leaf flavonoids could increase the mortality of *A. aegypti* larvae up to 100% with a shorter Lethal Concentration Time (LT_{50}) (2.08 hours) than other treatments. However, the administration of flavonoids with a concentration of 7.96 ppm could cause larval mortality up to 50% (LC_{50}).

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LIST OF REFERENCES

Astuti, M.D., M. Wulandari, K. Rosyidah, & R. Nurmasari. (2021). Analisis proksimat dan fitokimia buah pedada (*Sonneratia ovate* Back). *Jurnal Sains dan Terapan Kimia. 15*(2), 154-163.

Bere, S.M. (2020, April 3). *Jumlah penderita DBD di NTT bertambah jadi 4.518 orang, 48 meninggal.* [Halaman web]. Diakses dari https://regional.kompas.com/read/2020/04/03/07304521/jumlah-penderita-dbd-di-ntt-bertambah-jadi-4518-orang-48-meninggal?page=all.

Buni, V. A. (2013). Efektivitas daya bunuh ekstrak daun kirinyu (*Chromolaena odorata* L.) terhadap larva nyamuk *Aedes aegypti*. Skripsi. *Jurusan Biologi Fakultas Sains dan Teknik Universitas Nusa Cendana.*

Cania, E., & E.Setyaningrum. (2013). Uji efektifitas larvasida ekstrak daun legundi (*Vitex trifolia*) terhadap larva *Aedes aegypti*. *Medical Journal of Lampung University. 2*(4), 52-60.

Coppin, J.P., Yanping Xu, Hong Chen, Min-Hsiung Pan, Chi-Tang Ho, R. Juliani, J.E. Simon, & Qingli Wu. (2013). Determination of flavonoids by lc/ms and anti-inflamatory acitivity in *Moringa oleifera*. Journal of Functional Foods. *5*, 1892-1899.

Fernandes, D.A., M.S.R. Souza, Y.C.F. Teles, L.H.G. Oliveira, J.B. Lima, A.S.Conceicao, F.C. Nunes, T.M.S. Silva, & M.de Fatima V. de Souza. (2018). New sulphated flavonoids and larvicidal activity of *Helicteres velutina* K. Schum (Sterculiaceae). *Molecules*. *23*, 2784-2795.

Ferreira, P.M.P., A.E.U Carvalho, D.F. Farias, N.G. Cariolano, V.M.M. Melo, M.G.R. Queiroz, A.M.C. Martins dan J.G. Machado-Neto. (2009). Larvacidal acitivity of water extract of *Moringa oleifera* seeds against *Aedes aegypti* and its toxicity upon laboratory animals. *Anais da Academia Brasileira de Ciencias*. *81*(2), 207-216.

Gautam, K., P. Kumar dan S. Poonia. (2013). Larvacidal activity and gc-ms analysis of flavonoid of *Vitex negundo* and *Andrographis paniculata* against two vektor mosquitoes *Anopheles stephensi* and *Aedes aegypti*. *J Vector Borne*. *Dis 50 September*, 171-178.

Harith, S.S., S.N.A.S. Mohd, N.A. Aziz, M.M. Mydin, & N.T.S. Nasir. (2018). Phytochemical screening and larvacidal activity of *Murraya koenigii* leaves extracts against mosquito larvae. *Malaysian Journal of Analytical Science. 22*(3), 471-476.

Hebert, A. dan Hamidah. (2018). Evaluasi toksisitas ekstrak metanol daun jeruk nipis (Citrus aurantifolia) terhadap kematian larva nyamuk *Aedes aegypti. Aspirator. 10*(1), 57-64.

Hendri, M., G. Diansyah, & J.Tampubulon. (2010). Konsentrasi lethal (LC_{50}-48 jam) logam tembaga (Cu) dan logam kadmium (Cd) terhadap tingkat mortalitas juwana kudo laut (*Hippocampus* sp). Jurnal Penelitian *Sains. 13*(1D), 1310726 – 1310730.

Hikma, S.R. dan S. Ardiansyah. (2018). Kombinasi ekstrak daun kelor (*Moringa oleifera*) dengan ekstrak daun tin (*Ficus carica* L) sebagai larvasida terhadap larva *Aedes aegypti*. *Medica. 1*(2), 94-102.

Imanta, E., & N. Hidajati. (2017). Uji biolarvasida nyamuk *Aedes aegypti* dari hasil isolasi ekstrak metanol tanaman sambiloto *(Andrographis paniculata* NESS*). UNESA Journal of Chemistry. 6*(1), 36-41.

Latif, R.A., M.A. Mustapa, & S. Duengo. D. (2018). *Analisis kadar senyawa flavonoid ekstrak metanol kulit batang waru (Hibiscus tiliaceus L) dengan menggunakan metode spektrofotometri UV-VIS.* [Halaman web]. Diakses dari https://repository.ung.ac.id.

Lin, M., J. Zhang, & X. Chen. (2018). Bioactive flavonoids in *Moringa oleifera* and their healts-promoting properties. *Journal of Functional Foods. 47*, 469-479.

Makita, C., L. Chimuka, P. Steenkamp, E. Cukrowska, & E. Madala. (2016). Comparative analyses of flavonoid content in *Moringa oleifera* and *Moringan ovalifolia* with the aid of UHPLC-qTOF-MS Fingerprinting. South African *Journal of Botany. 105,* 116-122.

Maulana, S., F. Musthofa, A. Yamin, N. Juniarti, & A. Putri. (2021). Pengaruh biolarvasida daun tanaman sebagai kontrol vektor nyamuk *Aedes aegypti* penyebab demam berdarah: literature review. *Jurnal Medika Hutama. 2*(3), 978-989.

Meye, E.D., F. Kia Duan, S.R. Toly, V.M. Ati, F.M. Ike Septa, A.N. Momo, & T. Hermanus. (2021). Uji efektivitas senyawa alkaloid dan tanin ekstrak daun kelor (*Moringa oleifera*) terhadap mortalitas larva nyamuk (*Aedes aegypti*). *Jurnal Biotropikal Sains. 18*(1), 24-35.

Mohammed, S., & F. Abd Manan. (2015). Analysis of total phenolics, tannin and flavonoids from *Moringa oleifera* seed extract. *Journal of Chemical and Pharmaceutical Research. 7(*1), 132-135.

Nwankwo, E.N., N.J. Okonkwo, C.U. Ogbonna, C.J.O. Akpom, C.M. Egbuche, & B.C. Ukonze. (2015). *Moringan oleifera* and *Annona muricata s*eed oil extracts as biopesticides against the second and fourth larval instar of *Aedes Aegypti* L. (Diptera: Culicidae). *J.Biopest. 8*(1), 56-61.

Perumalsamy, H., M.J. Jang, J.R. Kim, M. Kadarkarai, & Y.J. Ahn. (2015). Larvicidal activity and possible mode of action of four flavonoids and two fatty acids identified in *Millettia pinnata* seed toward three mosquito species. *Parasites dan Vectors. 8,* 237-250.

Prabu, K., K. Murugan, A. Nareshkumar, N. Ramasubramanian, & S. Bragadeeswaran. (2011). Larvicidal and repellent potential of *Moringa oleifera* against malarial vector, *Anopheles stephensi* Liston (Insecta: Diptera: Culicidae). *Asian Pacisic Journal of Tropical Biomedicine. 1*(2), 124-129.

Putra, I.D, A.G. Dharmayudha, & L.M. Sudimatini. (2016). Identifikasi senyawa kimia ekstrak etanol daun kelor *(Moringa oleifera* L*)* di Bali. Medicus Veterinus Indonesia. 5 (5): 464-473.

Rahmawati, F. (2015). *Optimasi penggunaan kromatografi lapis tipis (KLT) pada pemisahan senyawa alakaloid daun pulai (Alstonia scholaris L.R.Br).* Skripsi. Universitas Islam Negeri Maulana Malik Ibrahim. Malang.

Rumengan, A.P. (2010). Uji larvasida nyamuk *Aedes aegypti* dari Ascidian *(Didemnum molle)*. *Jurnal Perikanan dan Kelautan. 6*(2), 83-86.

Sari, J.F. (2011). *Penerapan metode kromatografi lapis tipis (KLT) untuk membedakan Curcuma domestica Val, Curcuma xanthorrhiiza Roxb, Curcuma zedoria Rosc, Curcuma manga Val dan Zipj, Curcuma aeroginosa Roxb dalam campuran.* Departemen Farmakognosi Dan Fitokimia Fakultas Farmasi Universitas Arlangga. Surabaya.

Sharma, E., P Mathur, D. Kumar, M. Srivastava, & A. Prasad. (2013). *Larvicidal activity of Moringa oleifera leaves against human malaria vector Anopheles stephensi Liston (Insecta: Diptera: Culicidae)*. XII International Conference on Vector and Vector Borne Diseases Challenges in 21st Century: Their Global Impact and Strategic Management. 117-122.

Simanjuntak, M.R. (2008). *Ekstraksi dan fraksinasi komponen ekstrak daun tumbuhan senduduk (Melastoma malabathricum L) serta pengujian efek sediaan krim terhadap penyembuhan luka bakar.* Skripsi. Fakultas Farmasi Universitas Sumatera Utara. Medan.

Utami, W.W., A.R. Ahmad dan A. Malik. (2016). Uji aktivitas larvasida ekstrak daun jarak kepyar (*Ricinus communis* L) terhadap larva nyamuk *Aedes aegypti*. *Jurnal Fitofarmaka Indonesia. 3*(1), 141-145.

Yasi, M.R. dan R.S. Harsanti. 2018. *Uji* daya larvasida ekstrak daun kelor *(Moringa oleifera)* terhadap mortalitas larva *Aedes aegypti. Journal of Agromedicine and Medical Sciences. 4*(3), 159-164.

Yuda, P.E.S.K., E.C. Ningsih, N.L.P.Y. Winariyanthi. (2017). Skrinning fitokimia dan analisis kromatografi lapis tipis ekstrak tanaman patikan kebo (*euphorbia hirta* L). *Jurnal Ilmiah Medicamento. 3* (2), 61-70.

Zirconia, A., N. Kurniasih dan V. Amali. (2015). Identifikasi senyawa flavonoid dari daun kembang bulan *(Tithonia diversifolia)* dengan metode pereaksi geser. *Al Kimia. 2*(1), 9-17.