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# Bioactivity Test of Bitter Melon (*Momordica charantia* L.) Ethanol Extract As Larvacide on *Aedes* sp and *Culex* sp.

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**Abstract**

*Aedes aegypti and Culex sp. is a vector of dengue, filariasis, and malaria in Indonesia. One of the alternative efforts to control the spread of mosquitoes that are safe and harmless to the environment and humans can be done by giving natural ingredients that have the potential as larvicides, namely bitter melon. The purpose of this study was to determine the effect and effectiveness of bitter melon as a natural larvicide for Aedes aegypti and Culex sp. The design of this study was experimental using samples of bitter melon and fourth instar larvae of Aedes aegypti and Culex sp. The treatments in this study included ethanol extract of bitter melon with concentrations of 250 ppm, 500 ppm, 750 ppm and 1000 ppm, abate 1% as a positive control and aquades as a negative control. All treatments were given to the fourth instar larvae of Aedes aegypti Culex sp. each containing 20 larvae. Data analysis in this study was carried out by One-Way ANNOVA test, Independent sample t-test, and probit analysis. The results showed that the lowest-highest percentage of Aedes aegypti larvae mortality ranged from 26% - 85%, while Culex sp. ranged from 17% - 65%. The results of the ANOVA test showed significant differences between the treatment groups (P<0.05) in both Aedes aegypti and Culex sp. but the results of the Independent sample t-test showed no significant difference (P>0.05) between the Aedes aegypti and Culex sp. The results of the probit analysis showed that the LC50 value for Aedes aegypti was 728,789 ppm while Culex sp was 732,272 ppm. The conclusion of this study was that the administration of ethanol extract of bitter melon was more effective on the fourth instar larvae of Aedes aegypti than Culex sp.*



## Introduction

Mosquitoes are one of the vectors that cause various disease problems in Indonesia. The dominant of diseases in Indonesia that are spread by mosquitoes include Dengue Hemorrhagic Fever (DHF), malaria, and filariasis. There are two genera of mosquitoes that are generally vectors of malaria and filariasis, namely *Culex* sp and DHF, namely *Aedes* sp with the potential species being *Aedes aegypti*. The behavior of the two mosquitoes needs to be eradicated because they play a role in causing problems in the spread of disease and increasing malaria, filariasis, and dengue-endemic areas (Wasilah, 2019).

Based on data from the Indonesian Ministry of Health (2020) reported that malaria caused death in 12 provinces in Indonesia with the highest cases found in the province of Papua, while filariasis or

elephantiasis disease 2019 was widely spread in 34 provinces with the most chronic case sufferers, namely the provinces of Papua, Nusa East Southeast (NTT) and West Papua which cause lifelong disability and social stigma for sufferers and their families. DHF patients were reported in 2020 as 81,328 cases (the highest number of cases in West Java province while the lowest cases in Maluku province) with the highest number of death cases in West Java province as many as 121 deaths. The of these various diseases, dengue is still a dangerous disease commonly found in Indonesia.

Some previous studies that have confirmed the effectiveness of bitter melon fruit have been carried out by Syam and Pawenrusi (2017) ; Susilawati and Hermansyah (2015); Prakoso *et al*. (2016) ; Dheasabel and Azinar (2018) ; Mituiassu *et al*. (2022) who reported that bitter melon extract was able to kill the larvae of *Aedes aegypti* and *Culex* sp. with a percentage interval between 65% - 90% with an LC50 value in the category of low toxicity. From several previous research results, this study tries to bring up a novelty level that focuses on choosing a new concentration of bitter melon and comparing the effectiveness of bitter melon between *Aedes aegypti* and *Culex* sp. to complete the database from previous studies.

The purpose of this study was to determine the effect and effectiveness of the ethanol extract of bitter melon on the larvae of Instar IV *Aedes aegypti* and *Culex* sp. The results of this study are expected to be a source of information on the bioprospection of bitter melon as a natural larvicide that has been scientifically tested to be developed into a larvicidal product either in powder or liquid form.

## Methods

This type of research is quantitative with an experimental research design that examines the effect of bitter melon extract (independent variable) on the number of deaths of *Aedes* sp. and *Culex* sp. The research was conducted in February-April 2021 at the parasitology laboratory of STIKes Mitra Keluarga. The tools used in this study were stainless steel containers, filters, rearing containers, beakers (Pyrex, USA), measuring cups (Pyrex, USA), pipettes (Pyrex, USA), and volumetric flasks (Pyrex, USA), dropper pipettes, Buchner funnel (Pyrex, USA), Whatman filter paper no. 1 (Merck) 6 Hole water bath (HH, China), digital analytical balance (Acuplus, China), micropipette (Socorex, Switzerland) with white tip, yellow tips, and blue tip, hot plate and stirrer (IKA C-MAG HS7, Germany ), Erlenmeyer 2000 mL (Schoot), rotary evaporator (IKA-RV-3 V, Germany), The materials used in this study were dried bitter melon, *Aedes aegypti* larvae eggs and *Culex* sp. instar IV, 70% ethanol, and fish pellets.

### Research Sample

The samples in this study were 20 larvae of *Aedes aegypti* instar IV and 25 larvae of *Culex* sp. instar IV (WHO, 2005). All larvae were given 6 treatments consisting of positive control (abate), negative control (aquades) and ethanol extract of bitter melon with concentrations of 250 ppm, 500 ppm, 750 ppm, and 1000 ppm. Each treatment was repeated 3 times (replication) and the formula for calculating the total number of samples = number of samples of larvae (WHO standard) x number of treatments x number of repetitions was obtained. From this formula, the number of samples was 360 *Aedes aegypti* larvae and 450 *Culex* sp larvae.

### Preparation of larvae samples of *Aedes aegypti* and *Culex* sp.

*Aedes aegypti* eggs used in this study was obtained in dry form from the Bogor Agricultural Institute (IPB) Faculty of Veterinary Medicine, Bogor. Hatching starts from soaking *Aedes aegypti* eggs in plastic containers until the eggs hatch into larvae. The eggs will hatch within 1-2 days. Larvae will develop from stage I to IV for 5-7 days. During development, the larvae are fed with fish pellets. *Aedes aegypti* larvae used in this study was fourth instar larvae. As for the sample of *Culex* sp larvae, it was obtained using a filter from the breeding place (ditches and puddles), then the captured larvae were transferred to the sample container using a plastic pipette. All fourth instar larvae, both *Aedes aegypti* and *Culex* sp. identified microscopically using the ATLAS of medical parasitology.

### Identification of Larvae *Aedes* sp. and *Culex* sp.

Identification of the fourth instar of *Aedes aegypti* larvae was carried out macroscopically by looking at the characteristics, including having a slender body, moving agile, negative phototaxis, and at rest forming an almost perpendicular angle to the surface of the water, while microscopic identification was done by placing the larvae on an object-glass. and covered with a cover slip to see the morphology with the characteristics; the body consists of the head, thorax, abdomen, siphon, and anal segments, the abdomen consists of 10 segments, and in the abdominal 8 segment there are coomb teeth with large median spine and side spines (subapical spine), the comb teeth are only one row with 8-16 pieces, in the 10 segment it has a fat and short siphon, on the siphon, there is a pair of siphon hairs (hair tuft), and in the 10 segment

with a ventral brush, it has 5 put setae. The macroscopic identification of *Culex* sp. instar IV larvae was carried out by looking at the larvae size of 5-6 mm and forming an almost perpendicular angle to the surface of the water, while microscopic identification was carried out by placing the larvae on an object glass and covered with a cover glass and then observing the characteristics. morphological characteristics, such as the body consists of a head, thorax (3 segments), abdomen (10 segments), siphon, and anal segment, in the VIII abdominal segment there are comb teeth which are more than two rows, the siphon is conical, slender, long with siphon feathers present. more than one pair. At the end of the siphon, there is a spiracle (Pusarawati *et al*., 2013; Natadisastra and Agus, 2013).

**Preparation of bitter melon samples**

The bitter melon used in this study was obtained from Cimanggu Market, Bogor, which was selected fresh, dark green and whole as much as 10 kg. The bitter melon is then washed with running water. The flesh of the bitter melon is then seeded. The bitter melon fruit that has been separated from the seeds is then cut into small pieces with a thickness of ± 0.2 cm and dried by aerating and then mashed into a fine powder and sieved through a 60-100 mess sieve (Susilawati and Hermansyah, 2015).

**Extraction by maceration method**

Extraction by maceration method was carried out by inserting bitter melon powder into a 1000 mL beaker glass of as much as 800 gram which had been filled with 500 mL ethanol 70% pro-analyst. The sample was then soaked for 3x24 hours where every day it was stirred 1x until the solvent was completely mixed. Bitter melon powder that has been soaked in 70% ethanol for 3 days, then filtered using a Buchner funnel with Whatman filter paper No. 1. The liquid extract obtained from maceration was then concentrated using a rotary evaporator at a temperature of 60°C and a speed of 93 rpm for 3 hours. After that, it was continued with concentration using a water bath at 60°C until a thick extract was produced and then stored in a desiccator (Hanani, 2015 ; Annisa dkk. 2017). The extraction results were then carried out with phytochemical screening to see the presence of alkaloid compounds.

**Preparation of test solution**

The test solution in this study consisted of negative control in the form of aquadest, a positive control in the form of 1% abate larvicide (1 gram of abate in 100 mL aquadest), and an ethanol extract of bitter melon with a concentration of 250 ppm, 500 ppm, 750 ppm, and 1000 ppm made with the method of dissolving 10,000 ppm stock solution (1 g of the bitter melon extract is dissolved with 100 ml of distilled water (w/v) each as much as 2.5 ml, 5 ml, 7.5 ml, and 10 ml in 100 ml aquadest.

Testing ethanol extract of bitter melon in this study was carried out using 5 plastic containers measuring 100 mL. Each container contains variations in concentrations of 250 ppm, 500 ppm, 750 ppm, and 1000 ppm, negative control and positive control. Two series of containers were made for each larva of *Aedes aegypti* and *Culex* sp. Each set of containers with various variations included 25 for *Aedes aegypti* larvae instar IV and 20 for *Culex* sp larvae. instar IV using a dropper and let stand for 24 hours. After 24 hours, the number of larvae of *Aedes aegypti* and *Culex* sp. dead instar IV.

**Data analysis**

All data were analyzed with SPSS 20.0 software. The effect of killing power of bitter melon extract on mosquito larvae was tested by way ANOVA and t-test, while Probit analysis was used to determine LC50. Probit analysis was used to determine the killing ability of bitter melon extract against larvae of *Aedes aegypti* and *Culex* sp. in the form of the value of Lethal Concentration-50 (LC50). The LC50 value is the concentration used to measure the extract solution capable of causing the death of the mosquito larvae population up to 50%, with a 95% confidence degree (Ramayanti and Febriani, 2016).

## Results and Discussion

The results of microscopic identification of the fourth instar larvae of *Aedes aegypti* and *Culex* sp. in this study are shown in Figures 1 and 2.

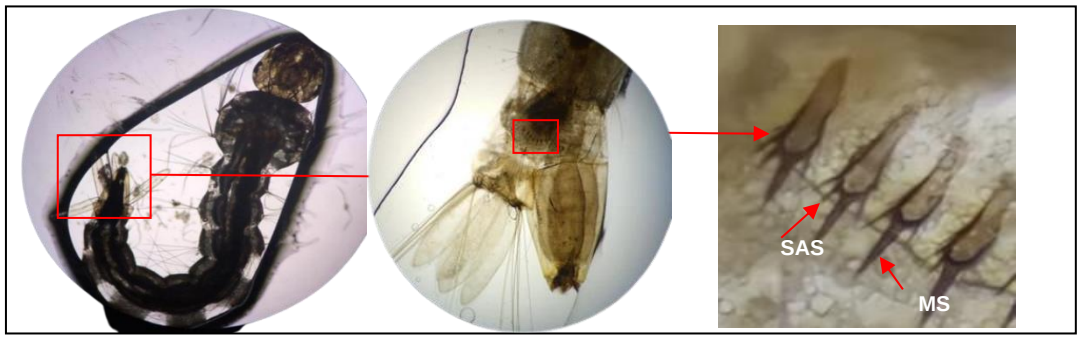


**Figure 1. The microscopic structure of the fourth instar larvae of *Aedes aegypti*. Caput (C), Thorax (T), Abdomen (A), Anal Segment (SA), Siphon (S), Comb Teeth (T), Median Spine (MS), Subapical Spine (SAS). Magnification 100x**

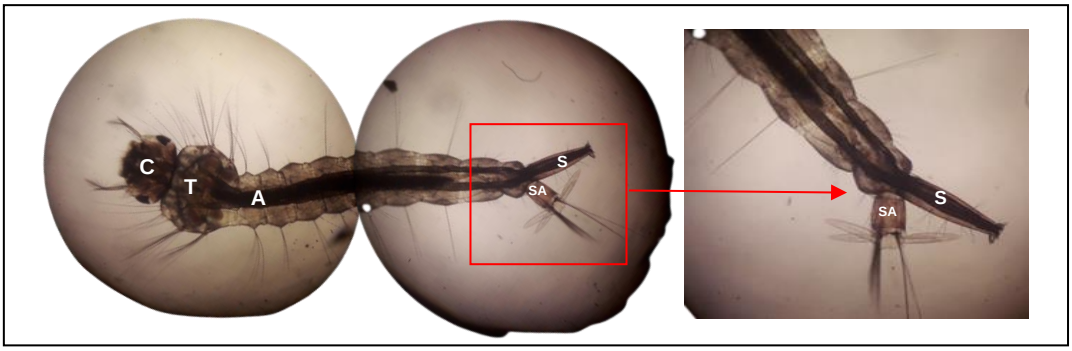


**Figure 2. Microscopic structure of the fourth instar larvae of *Culex* sp. Caput (C), Thorax (T), Abdomen (A), Anal Segment (SA), Siphon (S). 100x magnification.**

Figures 1 and 2 show the microscopic morphology of the fourth instar larvae of *Aedes aegypti* and *Culex* sp. consisting of caput, thorax, and abdomen. In the VIII abdominal segment of *Aedes aegypti*, there are characteristics of comb teeth (comb scale) with a large median spine and sub-apical spine. The difference between the two larvae was also seen that the fourth instar larvae of *Aedes aegypti* had wide and short siphon while *Culex* sp. has a conical siphon, long, and slender.

The test results of bitter melon ethanol extract with concentrations of 250 ppm, 500 ppm, 750 ppm, and 1000 ppm against *Aedes aegypti* and *Culex* sp which were observed for 24 hours showed that the lowest concentration of bitter melon ethanol extract in killing *Aedes aegypti* larvae was 250 ppm with a total average of 250 ppm. The average mortality was 5.25 larvae (26%) and the highest concentration was 1000 ppm with an average total mortality of 17 larvae (85%), while for *Culex* sp. larvae, the lowest concentration was 250 ppm with total average mortality of 4.25 larvae. (17%) and the highest concentration of 1000 ppm with total average mortality of 16.25 larvae (65%). The results of testing the ethanol extract of bitter melon on the mortality of *Aedes aegypti* and *Culex* sp. larvae can be seen in table 2.

**Table 1. Results of ethanol extract of bitter melon on mortality of *Aedes aegypti* and *Culex* sp larvae.**

| Treatment | *Aedes aegypti* | | | *Culex* sp. | | |
|---|---|---|---|---|---|---|
| | Number of samples | Number of larvae mortality | Larvae mortality percentage | Number of samples | Number of larvae mortality | Larvae mortality percentage |
| 250 ppm | 20 | $5,25^{a}$ | 26% | 20 | $4,25^{a}$ | 17% |
| 500 ppm | 20 | $7,25^{b}$ | 34% | 20 | $8,5^{b}$ | 36% |
| 750 ppm | 20 | $13,5^{c}$ | 68% | 20 | $12,25^{c}$ | 49% |
| 1000 ppm | 20 | $17^{d}$ | 85% | 20 | $16,25^{d}$ | 65% |
| Control (+) | 20 | $20^{e}$ | 100% | 20 | $20^{e}$ | 100% |
| Control (-) | 20 | $0^{f}$ | 0% | 20 | $0^{f}$ | 0% |

**Description: control (+): abate 1%; Control (-);**
**a,b,c,d,e,f,g : different superscripts showed significant differences (P<0.005)**

The results of statistical analysis using the One Way Anova test (Analysis of Variance) in table 1 showed significant difference (P<0.005) in the number of larvae mortality in both *Aedes aegypti* and *Culex* sp. treated with ethanol extract of bitter melon between concentrations of 250 ppm, 500 ppm, 750 ppm, and 1000 ppm. However, the results of the independent samples t-test analysis showed that there was no significant difference in the larvae mortality between the *Aedes aegypti* and *Culex* sp. The results of the comparison of the ethanol extract of bitter melon on larvae mortality between *Aedes aegypti* and *Culex* sp. can be seen in Figure 3.

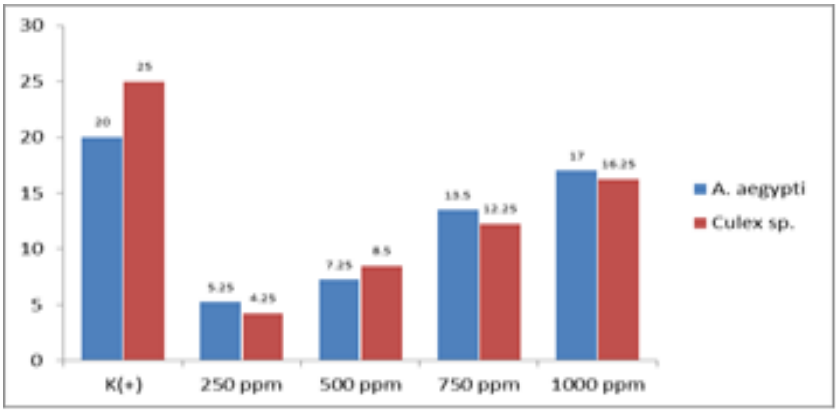


**Figure 3. Comparison of the number of deaths between larvae of *Aedes aegypti* and *Culex* sp. after being treated with ethanol extract of bitter melon**

The results were significantly different on table 1 and Figure 3 because the ethanolic extract of bitter melon contains phytochemical compounds (secondary metabolites) such as flavonoids, alkaloids, saponins, and steroids that have the potential as larvicides. The combination of phytochemical compounds can increase the killing effect of bitter melon extract against Aedes aegypti and Culex sp larvae (Rajashekara *et al.*, 2021). In this study, the results of phytochemical screening of the ethanolic extract of bitter melon were positive, indicating the presence of secondary metabolites, including alkaloids, tannins, flavonoids, saponins, polyphenols, and terpenoids. Overall secondary metabolites of the ethanol extract of bitter melon can be shown in table 2.

**Table 2. The content of phytochemical compounds in the ethanol extract of bitter melon**

| No | Phytochemical compound test | Result |
|---|---|---|
| 1 | Alkaloids | + |
| 2 | Tannins | + |
| 3 | Flavonoids | + |
| 4 | Saponins | + |
| 5 | Polyphenol | + |
| 6 | Terpenoids | + |

According to Aditama dan Sitepu (2019), flavonoids are phenolic compounds that function as antimicrobial, antiviral, antifungal, and larvicidal compounds. This larvicidal effect can inhibit the respiratory system of mosquito larvae. This mechanism occurs when flavonoids enter the larva's body through the respiratory system, causing damage to the nerves and respiratory system. This effect then results in respiratory problems, causing the death of *Aedes aegypti* and *Culex* sp larvae.

Syam dan Pawenrusi (2017) stated that another content in bitter melon is alkaloids with the ability to have multifunctional effects, including as an inhibitor of the acetylcholinesterase enzyme which causes a buildup of acetylcholine so that it has an impact on inhibiting the transmission of nerve impulses, causing the larva's body color to become transparent and larval body movement. becomes sluggish when stimulated by touch. The impact of the effect of alkaloids on mosquito larvae is evidenced in the results of this study (figure 4) which shows that the larvae of *Culex* sp. dead ones are transparent. In addition, the condition of two larvae looks immobile, do not respond to stimuli, and sink to the bottom of the container. Nurhaifah dan Sukesi (2015) added that if the secondary metabolite compounds entered the body of the larvae of *Aedes aegypti* and *Culex* sp. the larvae fail to metamorphose into pupae. This is due to a decrease in the metabolic rate so that energy for growth is inhibited.

The profiles of the two larvae that died after being treated with ethanol extract of bitter melon showed the same results, namely the larvae looked pale and transparent. The microscopic structure of the two larvae that died from the test material can be seen in Figure 4.

**Figure 4. Instar IV larvae that died after being treated with bitter melon extract**

The results of this study are in accordance with the research conducted by Prakoso *et al*. (2016); Annisa *et al*. (2017) ; Nursal and Yeanny (2019) who reported that the higher the concentration of bitter melon extract, the number and percentage of mortality of *Aedes aegypti* and *Culex* sp. more increasing. This is because the increase in the concentration of bitter melon extract indicates an increase in the active ingredients contained in the bitter melon extract so that the active ingredient content that enters the larvae body is also increasing. This increase then interferes with the physiological work of the larvae as evidenced by the increase in the number of larvae mortality along with the increase in the concentration of bitter melon extract.

Subramaniam *et al.* (2012); Lakshmi *et al*. (2018); Mituiassu *et al*. (2022) explained that the phytochemical compounds contained in the bitter melon extract could enter the larva's body through the digestive tract by entering through the mouth and then being absorbed by the intestinal wall and distributed to the blood circulation. If the concentration of these compounds in large quantities can interfere with the body's metabolic processes such as nervous and digestive disorders which have an impact on the death of *Aedes aegypti* and *Culex* sp larvae.

As for knowing the most effective dose of ethanol extract of bitter melon in killing the larvae of *Aedes aegypti* and *Culex* sp. This study was done by determining the $LC_{50}$ value using probit analysis. The results of the probit analysis in this study are shown in table 3.

**Table 3. Value of LC50 concentration of ethanol extract of bitter melon on *Aedes aegypti* and *Culex* sp . larvae**

| Larvae | Rated dose LC50 | Lower limit | Upper limit |
|---|---|---|---|
| *Aedes aegypti* | 728,789 ppm | 635.459 ppm | 865,793 ppm |
| *Culex* sp. | 732,272 ppm | 804,546 ppm | 611,817 ppm |

The results of the probit analysis in table 4. show that the $LC_{50}$ concentration of bitter melon extract in killing *Aedes aegypti* was 728.789 ppm while *Culex* sp. of 732.272. These results can be interpreted that the most effective concentration or the dose of ethanol extract of bitter melon which can kill 50% of the total larvae of *Aedes aegypti* and *Culex* sp in this study respectively was 728.789 ppm and 732.272 ppm.

The $LC_{50}$ concentration indicates the effectiveness of bitter melon extract in killing 50% of the total number of *Aedes aegypti* and *Culex* sp larvae. The $LC_{50}$ search in this study was used to assess the ability of the bitter melon extract to kill *Aedes aegypti* and *Culex* sp larvae within 24 hours. An extract is categorized as toxic if it has an $LC_{50}$ value of <1000 ppm or equivalent to 0.1%. In this study, the concentration that produced the $LC_{50}$ value for *Aedes aegypti* was 728.789 ppm while *Culex* sp. of 732.272 ppm, meaning that the toxicity of bitter melon extract is still in the very low category (Dheasabel dan Azinar, 2018). The lower the $LC_{50}$ value, the better the effectiveness of the larvicide because it only needs a small concentration to produce high larval killing power (Nurhaifah dan Sukesi, 2015). In this study, although the most effective concentration was obtained in killing 50% of mosquito larvae, the use of bitter melon extract was not effective as a vegetable insecticide for *Culex* sp. but effective on *Aedes aegypti* larvae, considering that according to WHO (2016) vegetable insecticides are said to be effective if they can kill 80%-90% of mosquito larvae. In the study, the bitter melon extract was able to cause maximum mortality of *Aedes aegypti* larvae by 86% while *Culex* sp. only 65%.

In this study, temefos in the form of 1% abate as a positive control had better killing power than the ethanol extract of bitter melon. However, continuous use of temefos can have a negative impact on human health, leaving residues that are not environmentally friendly and have the potential to trigger insect resistance. According to Ramayanti and Febriani (2016) abate insecticides are organophosphate insecticides that have the ability to poisons that affect the neurotransmitter system of mosquito larvae, but these insecticides trigger resistance by increasing detoxification (becoming non-toxic) against enzymes such as microsomal oxidase, glutathione transferase, hydrolases and esterases, decreased sensitivity of insecticide target points to acetylcholinesterase, and decreased penetration rate of insecticides on the skin (integument) of insects.

The advantages of this study are the use of extracts of natural ingredients using well-standardized methods, and the use of samples of instar IV mosquito larvae in accordance with the WHO standard (2005),but this study has limitations including the preparation of natural ingredients that have not been standardized, the temperature setting has not been carried out and Laboratory pH and selection of samples that have not tested in the pupa stage but only in the fourth instar larval stage.

## Conclusions and Recommendations

Conclusions are the answers to the research questions that are presented briefly and clearly. Conclusions can be written as descriptions in paragraphs or in points. Meanwhile, suggestions contain recommendations to the readers or researchers in related fields. Suggestions are written based on the results of research and discussion. If any, the limitations of research can also be one of the points of the authors' recommendations for further research to improve, complete, or perfect.

## References

Aditama, W., and Yosep Sitepu, F. (2019). Optimizing of Maseration With Ethanol and Water Solvents Against The Toxicity of Extract Of Wuluh Starfruit (*Averrhoa bilimbi* L.) in Controlling Larva of *Aedes aegypti. International Journal of Mosquito Research*, *6*(1), 109–113.

Annisa, R., Amir, M. and Kuncoro, H. (2017). Toxicity Study of Pare Leaf Extracts (M*omordica Charantia*) to *Culex Pipien*s Mosquito Larvae. *Journal of Islamic Pharmacy*, *2*(2), 13. https://doi.org/10.18860/jip.v2i2.4506

Dheasabel, G. dan Azinar, M. (2018). The Ability of Bitter Gourd Extract against Mosquito Death on *Aedes aegypti*. *HIGEIA (Journal of Public Health Research and Development)*, *2*(2), 331–341. https://doi.org/10.15294/higeia.v2i2.20866

Hanani, E. (2015). *Phytochemical Analysis.* Jakarta : EGC

Lakshmi, K. V., Sudhikumar, A. V. and Aneesh, E. M. (2018). Larvicidal Activity of Phytoextracts Against Dengue Fever Vector, *Aedes aegypti* - A Review. *Plant Science Today*, *5*(4), 167–174. https://doi.org/https://dx.doi.org/10.14719/pst.2018.5.4.407

Mituiassu, L. M. P., Serdeiro, M. T., Vieira, R. R. B. T., Oliveira, L. S. and Maleck, M. (2022). *Momordica charantia* L. Extracts against *Aedes aegypti* larvae. *Brazilian Journal of Biology*, *82*. https://doi.org/10.1590/1519-6984.236498

Natadisastra, D. and Agoes, R. 2005. *Medical Parasitology: Judging from the Body Organs Attacked.* Jakarta : EGC

Nurhaifah, D. and Sukesi, T. W. (2015). Effectiveness of Sweet Orange Peel Juice as Mosquito Larvicide *Aedes aegypti. Kesmas: National Public Health Journal*, *9*(3), 207. https://doi.org/10.21109/kesmas.v9i3.566

Nursal and Yeanny, M. S. (2019). The Egg Hatchability and the Development of *Aedes aegypti* Mosquitoes in Ethanol Extracts of the Leaves of Bitter Melon (*Momordica charantia* L.) and Basil (*Ocimum basilicum* L.). *IOP Conference Series: Earth and Environmental Science*, *305*(1). https://doi.org/10.1088/1755-1315/305/1/012048

Prakoso, G., Aulung, A. and Citrawati, M. (2016). Effectiveness Test of Bitter Gourd Extract (*Momordica*

*charantia*) on Larval Mortality *Aedes aegypti. Jurnal Profesi Medika*, *10*(1), 46–53.

Pusarawati, S., Ideham, B., Kusmartisnawati, Tantular, S.I, and Basuki, S. *Atlas of Medical Parasitology.* (2013). Jakarta : EGC.

Rajashekara, S., Kiran, R., Asha, R., Chaitra, P. N., Mahesh, M., Mala, N., Nandini, K. R., Hansda, N., Shashi, S., Suresh Kumar, M. and Venkatesha, M. G. (2021). Screening of Plant Extracts Against a Vector of Arboviruses, *Aedes aegypti* (Linnaeus) (Diptera: Culicidae). *Proceedings of the Zoological Society*, *74*(2), 205–210. https://doi.org/10.1007/s12595-021-00364-8

Ramayanti, I. dan Febriani, R. (2016). Larvicide Effectiveness Test of Papaya Leaf Extract (*Carica papaya* Linn.) Against *Aedes aegypti* larvae. *Syifa'MEDIKA*, *6*(2), 79–88.

Ministry of Health the Republic Indonesia. (2020). *Action Plan for Activities. 2020-2024*. 12–26.

Subramaniam, J., Murugan, K., Kovendan, K. and Kumar, P. M. (2012). Larvicidal and Pupcidal Efficacy of *Momordica charantia* Leaf Extract and Bacterial Insecticide, *Bacillus thuringiensis* against Malarial Vector, *Anopheles stephensi* Liston. (Diptera: Culicidae). *Journal of Biopesticides*, *5*(SUPPL.), 163–169.

Susilawati dan Hermansyah. (2015). Larvicidal Activity of Pare Fruit Methanol Extract (*Momordica charantia* L.) Againts Larvae *Aedes Aegypti. Molekul*, *10*(1), 33–37.

Syam, I. dan Pawenrusi, E. P. (2017). Larvicidal Activity of Pare Fruit (*Momordica Charantia*) in Killing Larvae *Aedes Aegypti. Jurnal Kesehatan Masyarakat Andalas*, *10*(1), 19. https://doi.org/10.24893/jkma.v10i1.158

Wasilah, Z. S. (2019). Larvicide Effectiveness of Sweet Starfruit Extract (*Averrhoa carambola* L) Against Mosquito Larvae *Aedes aegypti* and *Culex* sp. *Binawakya*, *14*(4), 2481–2492. http://ejurnal.binawakya.or.id/index.php/MBI

WHO. (2005). Guidelines for laboratory and field testing of mosquito larvicides. *World Health Organization*,1–41. http://whqlibdoc.who.int/hq/2005/WHO_CDS_WHOPES_GCDPP_2005.13.pdf?ua=1