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Potential Extract Ethanol Citrus Amblycarpa as a Bioinsecticide Against Aedes Aegypti Larvae

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ABSTRACT

Introduction: Utilization of compounds active plant into an alternative control of mosquito when it and a front for a more hospitable environment and do not cause interference health. Kuit Limau Plant (Citrus amblycarpa) is local citrus from South Kalimantan which has bioinsecticide potential which can be used in mosquito control.

Objective: This study aims to determine the effectiveness of the ethanol extract of the Peel and Citrus amblycarpa leaves as natural larvicides of Aedes aegypti larvae.

Methods: An experimental study with a completely randomized design of Citrus amblycarpa ethanol extract (500, 1000, 2000, 3000, and 4000 ppm). The positive control used abate and the negative control used distilled water and tween 80. The number of repetitions of each concentration was three times with 25 larvae in each test glass. Larval mortality was analyzed using probit analysis at a 95% confidence level.

Results: Extract Peel requires a time that is shorter (5.3 hours) to kill the larvae of Ae. Aegypti compared with leaf extract (42.8 hours). Is not there a difference significant mortality of larvae in the group leather 4000 ppm with a group of control.

Conclusion: The ethanol extract of Citrus amblycarpa rind has potential as a larvicide for Aedes aegypti.

Keywords: Ethanol extract, Bioinsecticide, Citrus amblycarpa, Aedes aegypti

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INTRODUCTION

Dengue hemorrhagic fever is a tropical and subtropical disease (1) (2) (3) which is transmitted through the bite of the mosquito Aedes aegypti or Aedes albopictus and is caused by the Dengue virus (4)(5) DHF cases in Indonesia that were reported in 2019 were 138,127 cases. This number increased compared to 2018 of 65,602 cases. Deaths due to dengue fever in 2019 also increased compared to 2018, from 467 to 919 deaths. The incidence rate of dengue fever in 2019 is 51.53 per 100,000 population. This figure shows an increase compared to the previous two years, namely in 2017 and 2018 when the Incidence Rate of DHF was 26.1 and 24.75 per 100,000 population (6). The condition of the occurrence of dengue fever which is evident encourages the need to control the Aedes aegypti mosquito. One method that can be done to break the cycle and kill mosquito larvae is to use insecticides (Susilowati, Darmanto, and Aminah, 2018). The continuous use of synthetic insecticides (temephos (abate), malathion, cypermethrin, lambdasihalothrin, and deltamethrin) against the mosquito vector Aedes aegypti causes resistance to mosquitoes (8) (9), bioactive properties that are difficult to degrade in nature can pollute the environment, the toxic substances in synthetic insecticides will also have an impact on human health problems (10) (Susilowati, Darmanto, and Aminah, 2018). Natural larvae are an alternative in controlling mosquitoes by utilizing plants that are more environmentally friendly so that they can reduce the use of synthetic insecticides and anticipate negative impacts on health (11).

There are 2,400 species of bioinsecticide-producing plants in Indonesia that are toxic to insects (12). One of the local and abundant plants in the South Kalimantan Region, Indonesia with potential as a bioinsecticide against Aedes aegypti is the Citrus amblycarpa plant. This plant has active secondary metabolites such as flavonoids, tannins, saponins, alkaloids (13) (14). The fresh extract of Citrus amblycarpa fruit Peel has been shown to kill Aedes aegypti instar III larvae in just 7 (seven) hours in all concentrations (15). This study aims to determine the effectiveness of the ethanol extract of Citrus amblycarpa in killing Aedes aegypti larvae.

MATERIALS AND METHODS

This research is experimental research with a completely randomized design. In this study, various concentrations of the ethanol extract of the Peel and Citrus amblycarpa leaves were used, namely 500 ppm, 1000 ppm, 2000 ppm, 3000 ppm, and 4000 ppm. The positive control used abate and negative control used distilled water and Tween 80. The number of repetitions of each concentration was three times. The number of larvae in each test glass was 25 according to WHO standards.

Provision of Citrus amblycarpa samples

A sampling of Citrus amblycarpa, which are local oranges typical of South Kalimantan, were taken in several places in South Kalimantan. Samples are taken and picked directly from the farmers' plantations to maintain its freshness. The leaves and peel are sorted then cleaned of dirt by washing with clean water, drained and spread on a newspaper so that the water is absorbed, weighed as much as 2.5 kg wet weight, aerated for 7 (seven) days by placing it in a place protected from the sun. This activity will be carried out at the Basic Laboratory of the Islamic University of Kalimantan, Banjarmasin, Indonesia.

Ethanol extract preparation

The ethanol extract was made at the Laboratory of Basic Medicine, Faculty of Veterinary Medicine, Airlangga University. The dried leaves and Peel of the Citrus amblycarpa are then mashed to form a powder. One kg of leaf and Peel Simplicia was macerated with ethyl alcohol p.a solvent for three days, filtered every day and the

filtrate was collected and evaporated using a rotary evaporator with a decrease in pressure until a dry extract was obtained.

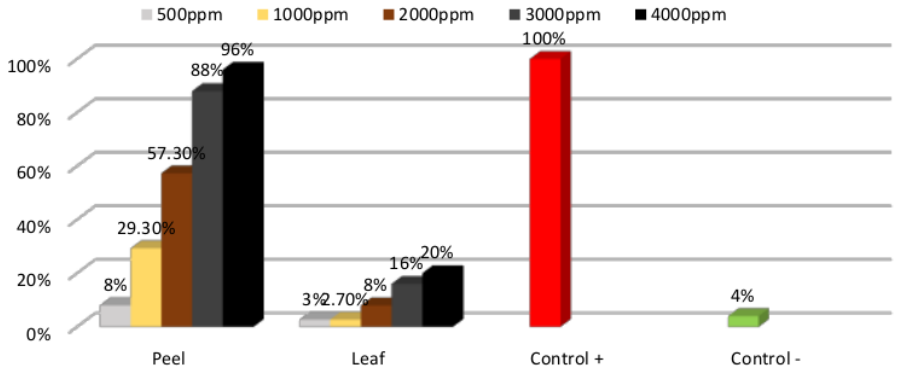
Larvicide Testing

The experimental animal used was the third instar larvae of the Ae. aegypti results from rearing in the Entomology Laboratory of the Indonesian Tanah Bumbu Research Institute for 900 instars III larvae. The count of the dead larvae was carried out every hour for 24 hours of immersion, recorded in tabular form. The data obtained were analyzed statistically using the ANOVA test to determine the difference in larva mortality between each group and Probit analysis to determine the killing power of Peel extract and Citrus amblycarpa Leaf against Aedes aegypti instar III larvae expressed in Lethal Time (LT_{50} and LT_{90}).

RESULTS AND DISCUSSION

Comparison of the Effectiveness of Citrus amblycarpa Peel and Leaf Extract

Graph 1 shows the comparison of mortality of Aedes aegypti larvae after 24 hours of treatment. The mortality of larvae with ethanol extract of Citrus amblycarpa leaves was higher than that of larvae with ethanol extract of Citrus amblycarpa leaves. Larvae mortality in the control group (+) showed 100% larval mortality while in the control group (-) there was 4% larvae mortality.



Graph 1. Comparison of Larvae Mortality

Probit Analysis

Table 1 shows the results of the probit analysis to calculate the Lethal Time of the ethanol extract of Citrus amblycarpa leaves. LT_{50} shows the time taken to kill 50% of larvae. At a concentration of 4000 ppm it takes 5.3 hours to kill 50% of larvae and it takes 13.2 hours to kill 90% of larvae. In the control + group, it took 3.2 to kill 50% of larvae, and it took 5 hours to kill 90% of larvae.

Table 1. Value of LT_{50} and LT_{90} Mortality of Aedes aegypti Larvae Instar III Treatment Group Ethanol Extract Citrus amblycarpa Peel.

Concentration	LT_{50} (hour)	95% CI		LT_{90} (hour)	95% CI	
		Min	Maks		Min	Maks
500 ppm	402.8	127.4	$96x10^{4}$	5,775	598.1	$40x10^{9}$
1000 ppm	46.8	36.5	65.3	337.7	189.1	917.5
2000 ppm	18.3	15.8	20.8	78.0	63.1	112.1
3000 ppm	8.3	7.0	9.3	25.8	23.5	28.9
4000 ppm	5.3	4.4	6.1	13.2	11.8	14.7
Kontrol +	3.2	2.1	3.8	5.0	4.3	5.8
Kontrol -	$44x10^{3}$	$1x10^{3}$	$58x10^{15}$	$15x10^{6}$	$3x10^{4}$	$8x10^{27}$

Table 2 shows the results of the probit analysis to calculate the Lethal Time of the ethanol extract of Citrus amblycarpa leaves. LT_{50} shows the time taken to kill 50% larvae. At a concentration of 4000 ppm, it takes 42.81 hours to kill 50% of larvae and 136.49 hours to kill 90% of larvae. In the control + group, it took 3.19 hours to kill 50% of larvae, and it took 4.98 hours to kill 90% of larvae.

Table 2. Value of LT_{50} and LT_{90} Mortality of Aedes aegypti Larvae Instar III Treatment Group of Citrus amblycarpa Leaves Ethanol Extract

Concentration	LT_{50} (hour)	95% CI		LT_{90} (hour)	95% CI	
		Min	Maks		Min	Maks
500 ppm	1.692	253	$2x10^{9}$	35.966	1.529	$1x10^{15}$
1000 ppm	1.695	229	$2x10^{8}$	29.532	1.226	$4x10^{12}$

2000 ppm	74,1	46,8	408,8	229,9	96,4	6.216
3000 ppm	43,9	36,2	60,5	119,9	80,7	241,7
4000 ppm	42,8	35,4	56,6	136,5	92,2	261,3
Kontrol +	3,2	2,1	3,8	4,9	4,3	5,8
Kontrol -	$44x10^{3}$	$1x10^{3}$	$58x10^{15}$	$15x10^{6}$	$3x10^{4}$	$8x10^{27}$

Prevention of the spread of DHF can be done in various ways, but until now the most effective way is to break the chain of transmission through vector control. Control that has been done a lot is by carrying out chemical control, namely by using synthetic insecticides. Synthetic insecticides work more effectively and quickly than biological and physical controls. However, repeated and long-term use can cause poisoning to humans and livestock, pollute the environment, and cause insect resistance.

Another alternative that can be used to control the dengue mosquito vector with a more environmentally friendly method is to use vegetable insecticides. Vegetable insecticides can kill or interfere with pests and diseases through a unique way of working, namely through a combination of various methods or singly. (16). Secondary metabolite compounds from plants are an alternative to insecticides that have a toxic effect on insects but have no side effects and are relatively safe for the environment and human health because they have a small risk. (17) (18). The use of natural insecticides from plants is an alternative to current insecticides, as well as vector control in the nature (19).

This study aims to determine the effectiveness of the ethanol extract of the skin and leaves of Citrus amblycarpa in killing Aedes aegypti larvae. The larvicidal activity test was carried out using 25 larvae with their respective concentrations in the ethanol extract of the skin and Citrus amblycarpa leaves starting from a concentration of 500 ppm, 1000 ppm, 2000 ppm, 3000 ppm, and 4000 ppm. The solvent media used were aquadest and tween 80. The addition of Tween 80, in this case, served to increase the solubility of the ethanol extract of the skin and Citrus amblycarpa leaves in water. Extracts and fractions were tested against larvae of the Aedes aegypti mosquito, instar III, the selection of the third instar as the test phase because the larvae are active foraging and their morphological shape is more perfect than instar I and II larvae. (20). The positive control used in this test was temephos 0.01gr / 100ml, while the negative control used in this test was aquadest and tween 80. The results of the larvicidal activity test can be seen in Figure 1.

This study used various concentrations of the ethanol extract of the skin and Citrus amblycarpa leaves that were tested in each group of larvae with 3 times the observed repetition for 24 hours of observation. The mortality of larvae increased with increasing concentration. This proves that the higher the concentration and the longer the exposure time, the higher the mortality of larvae. This research is in line with the results of research (21) that the increasing the concentration of the ethanol extract of kirinyuh leaves, the toxic substances contained in the larva's living media also increase so that the number of toxic substances that enter the larva's body also increases so that the larvae it's easier to experience death. The high mortality rate of test larvae can be caused by the presence of chemical compounds in this extract that play a role in biological activity in larval growth and development. The results of the study (15), also stated that at a concentration of 4.0 ml/100ml and 5.0 ml/100ml had 100% death after 6 hours of measurement, with an LT_{50} value of 5.0% concentration was 2.58 hours and an LT_{99} value. is 5.86 hours. So, from the results of the probit lethal time and lethal concentration tests, it was concluded that the greater the concentration is given and the longer the treatment time, it could increase the number of Aedes aegypti instar III larvae that died with the condition that the larvae were seen experiencing convulsions and bending the body.

The ability of the ethanol extract of Citrus amblycarpa peel to affect the mortality of Aedes aegypti larvae is due to the presence of certain active ingredients such as terpenoid / free steroid compounds, polyphenols, and saponins (22). Saponins are compounds that are included in terpenoid compounds. The activity of these saponins in the insect's body is to bind free sterols in the digestive tract of food where the sterol itself is a substance that functions as a precursor to the hormone ecdysone so that by decreasing the number of free sterols in the insect's body it will disrupt the process of skin change (molting) in insects. (23). Saponin compounds have the ability to damage cell membranes so that they can cause corrosion on the walls of the larvae digestive tract as stomach poison (24). The results of GC-MS shows the main components of the ethanol extract of citron biscuits are compounds 2H-1-Benzopyran-2-one, known as coumarin compound (25). Coumarin is a secondary metabolic compound from the phenolic compound group which generally comes from higher plants and is rarely found in microorganisms. Coumarin can be found in almost all parts of plants from roots, stems, leaves, flowers, and fruit (26). Coumarin compounds are known for several pharmacological properties including insecticidal activity. The results of research (27), coumarin with a content of 27.7% is a class of compounds contained in the essential oil of lime peel (Citrus aurantifolia). This compound has the potential as a mosquito larvicide (28) (29). One of the chemical components contained in the Citrus sinensis plant is coumarin which has biological activity as an insecticide (30) is highly toxic to insects (31). This compound is very toxic to M. persicae aphids with a value ($LC_{50(90)}$ values of 1.3(1.9) mg L^{-1}) (32). One of the compound components of soursop (A. muricata) leaf and seed extract is that coumarin has the potential to be used as a vegetable pesticide to control cheap and environmentally friendly pest attacks. (33).

The ability of the ethanol extract of Citrus amblycarpa leaves to affect the mortality of Aedes aegypti larvae is due to the presence of certain active ingredients such as terpenoids / free steroids, flavonoids, polyphenols, and saponins. (14). Flavonoid compounds are one of the toxic compounds for insecticides because of their ability to enter the larva's body through the respiratory system and cause weakness in the nerves. (34). The body position of the larvae that changes from normal can also be caused by flavonoid compounds due to how they enter through the siphon, causing damage so that the larvae have to align their position with the water surface to make it easier to take oxygen (35). Flavonoids have an antifeedant action by inhibiting the taste receptors in the mouth area of the larvae, which will result in the larvae failing to get a taste stimulus, so the larvae are unable to recognize the food

around them. The low feeding activity of larvae causes the energy for larval development to decrease so that the growth process is also inhibited.

The results of the study (36) show that the ethanol extract of cassava leaves (Manihot utilissima Pohl) with the chemical compounds of saponins and flavonoids has Aedes aegypti larvicidal activity with an LC_{90} value of 2,613% and larvicidal activity of ethanol extract of cassava leaves at a concentration of 3% and 3.5% There was no significant difference in Aedes aegypti larvae compared to temefos. The GC-MS results showed that the main component of the Citrus amblycarpa leaf ethanol extract was Maragenin I compound (14). Maragenin I is a triterpenoid compound derivative (37) (38). Maragenin I compounds can control insect growth and have potential as an insecticide (39). This compound is a derivative of the triterpenoid/steroid class, so it can be concluded that this compound has the potential as a biopesticide, is toxic to the Aedes aegypti mosquito. (40).

The results of this study also showed a comparison of the effectiveness of an ethanol extract from the Citrus amblycarpa plant on its ability as a vegetable larvicide to kill Aedes aegypti larvae. The difference is seen from the number of larvae deaths, with the largest concentration of treatment, namely 4000 ppm, the ethanol extract of Citrus amblycarpa skin is more effective in killing larvae with a mortality percentage of 96%, while the ethanol extract of Citrus amblycarpa leaves only kills larvae with a mortality percentage of 17.3%. This is in line with Jubaedah's research (2020), based on a literature study of several types of citrus plants based on chemical compounds that have the potential to be natural larvicides of Aedes aegypti, with the highest potential found in the skin of sweet orange (Citrus aurantium) fruit compared to other parts.

The use of the ethanol extract of the skin and Citrus amblycarpa leaves has the potential to become a natural larvicide and in the future, it can be applied to the community as a substitute for the use of chemical larvicides in killing Aedes aegypti larvae, but before application, it is necessary to do more in-depth studies and further research related to other plant extract formulations that can synergize with Citrus amblycarpa plant is like from the leaves which are indeed very small percentage in killing Aedes aegypti larvae.

CONCLUSION

Peel Extract can kill Aedes ae Larvae. Up to 96%. Bark extract requires less time and concentration to kill Ae larvae. Aegypti compared to Leaf Extract. There was no significant difference in larval mortality in the 4000 ppm skin group and the control group. The ethanol extract of Citrus amblycarpa skin has potential as a larvicide for Aedes aegypti.

CONFLICT OF INTEREST STATEMENT

We declare that we have no conflicts of interest.

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