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Effectiveness Test of Ketapang Leaf Ethanol Extract (*Terminalia catappa* L.) with TWEEN and PEG Diluents on the Mortality of *Aedes aegypti* Larvae

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Abstract: Diseases caused by *Aedes aegypti* mosquitoes can be controlled with vectors in the form of larvicide administration, but if it is excessive, it causes environmental pollution and causes larval resistance so that natural larvicides are needed. Ketapang leaves are tested to determine the effectiveness of larvicides against *Aedes aegypti* larvae. To determine the effectiveness of the ethanol extract of ketapang leaves (*Terminalia catappa* L.) with TWEEN dilution on the mortality of *Aedes aegypti* larvae. The research is experimental with the research design used is posttest only controlled group design. This design compares the experimental group with the control group. The groups used were groups K (+) and K (-), and the TWEEN/PEG treatment group which contained ketapang leaf extract with a concentration of 1,5% and 2%, respectively. The sample of *Aedes aegypti* larvae used was instar III IV. Normality, Kruskal-Wallis, Mann-Whitney and Probit tests are used for data processing. Mortality was highest with his PEG treatment at 1,5% and lowest with TWEEN at 2%. The effect of ethanol extract from ketapang leaves (*Terminalia catappa* L.) with TWEEN diluent was the same as PEG on the mortality of *Aedes aegypti* larvae. The ethanol extract of ketapang leaves (*Terminalia catappa* L.) with dilutions of TWEEN and PEG was less effective than abate.

Keywords: mortality of *Aedes aegypti* larvae, extract of ketapang leaves (*Terminalia catappa* L.), ethanol, TWEEN and PEG.

1. Introduction

Dengue hemorrhagic fever (DHF) is an infection caused by the dengue virus which is transmitted by *Aedes aegypti* and *Aedes albopictus* mosquitoes which are transmitted through the bite of infected mosquitoes, especially the female sex (Bestari et al., 2018). This virus is often found in tropical and subtropical regions such as Southeast Asia (RI Ministry of Health, 2019). Dengue fever is a dangerous viral disease, because it quickly causes the death of the affected patient (Lully, 2016).

DHF cases in Indonesia until September 2022 reached 87,501 cases and the number of deaths was 816 with an Incidence Rate (IR) of 31.38/100,000 population (RI Ministry of Health, 2022). Surakarta City is an endemic area of DHF. In 2020 there were 73 cases with an IR of 12.7/100,000 population, a decrease compared to 2019, that is 160 cases with an IR of 28.4/100,000 population

(Surakarta City Health Office, 2020). The high level of vulnerability of DHF makes Indonesia became a country that prioritizes DHF management (Husen *et al*., 2021).

There are four ways to prevent and deal with the DHF vector, one of that is vector control by administering larvicides (Sari & Khaira, 2020). If synthetic larvicides used excessively, it can cause several effects including environmental pollution, death of various types of living things and vector resistance (Nugraha *et al*., 2019). Natural larvicides can be used to reduce these effects and to control *Aedes aegypti* larvae. Natural larvicides can be defined as plant-based pesticides. If it used, it can not detrimental to the environment, health, and is safe in protecting living ecosystems (Prastiwi *et al*., 2019).

Indonesia is a country that has biodiversity. One of them that has the potential as a natural larvicidal is the ketapang plant (*Terminalia catappa* L.). Previous research by Bestari *et al*., (2020) about ketapang leaf extract with ethanol solvent on *Aedes aegypti* mortality. The results of this study showed 100% mortality of *Aedes aegypti* larvae at a concentration of 2%. According to another study, Redo *et al*., (2019) said that the larvicidal activity of the water-ethanol fraction of ketapang leaves at a concentration of 1800 ppm was comparable to temephos. Another study regarding ketapang leaf extract which was tested on Aedes aegypti larvae showed an LC50 value of 166.00 ppm (Unnikrishnan, 2014).

The results of ethanol extraction from ketapang leaves are in the form of a paste, other components are needed, so the ethanol extract from ketapang leaves is mixed evenly with water and spreads over the surface of the larvae's body. These substances are TWEEN (Polysorbate) and Polyethylene Glicol (PEG). TWEEN was chosen, because it has non-ionized properties in water and usually used to combine water solvents with oil-based materials (Wikantyasning & Indianie, 2021). While PEG is a substance that has stable solubility in water, it also has the benefit that is not irritating the skin and does not easily enter the skin. PEG can also be used to increase the water solubility of compounds that are difficult to decompose (Shah *et al*., 2021).

The researchers were encouraged to carry out this research by taking the title "Comparison of the Effectiveness Test of Ketapang Leaf Ethanol Extract (*Terminalia catappa* L.) with TWEEN and PEG Diluents on the Mortality of *Aedes aegypti* Larvae", because there has not been a similar study discussing the ethanol extract of ketapang leaves treated with TWEEN and PEG to kill *Aedes aegypti* larvae.

2. Materials and Methods

The tools that used in this study were trays, black cloth, jars, blenders, stirrers, filter paper, label paper, scales, analytical balances, cups, pipettes, beakers, hand counters, waterbaths, and rotatory evaporators.

The materials that used were *Aedes aegypti* larvae III - IV instars, 95% ethanol, distilled water, ketapang leaves (*Terminalia catappa* L.), abate, polysorbate (TWEEN), polyethylene glycol (PEG).

The preparatory step begins with determination with the aim of obtaining the truth that the plants used are suitable plants. The plant determination test was carried out at the Biology

Laboratory of FKIP, Muhammadiyah University of Surakarta. Then submit a permit to the Laboratory of the Faculty of Medicine, Muhammadiyah University of Surakarta to conduct research.

The ethanol extract of ketapang leaves (*Terminalia catappa* L.) was prepared in the pharmacology laboratory of the Faculty of Medicine of UMS by maceration. The solvent used is 95% ethanol solvent. The first step is wash the ketapang leaves (*Terminalia catappa* L.) then cut the ketapang leaves, and dried and covered with a black cloth. After the ketapang leaves are dry, then blended until the simplicia became smooth. After the ketapang leaves are mashed, then weighed and given a solvent in the form of 95% ethanol, then stirred. After that, leave it for about 7 days with stirring every day. Then the macerate is concentrated using a Rotatory Evaporator and a Waterbath until a thick extract is formed that can still be poured. Furthermore, the preparation of *Aedes aegypti* mosquito eggs will be hatched and bred until they reach instar III - IV.

The concentration of the ketapang leaf extract used was 1.5% and 2% based on research by Bestari *et al*., (2020), it used ketapang leaf extract (*Terminalia catappa* L.) to kill larvicides. First, the stability test stage was carried out by preparing a 2% TWEEN solution and 0.01% PEG. Then the 2% ketapang leaf ethanol extract in 5 ml and taken it, then added with 2% TWEEN and 0.01% PEG to 5 ml each tube, after that stirring until homogeneous and observed for 24 hours to see whether there was a precipitate or not.

The second step is prepare a stock solution by making a 2% TWEEN and 0.01% PEG solution in 350 ml. Then 2% ketapang leaf ethanol extract (*Terminalia catappa* L.) was added to 350 ml of TWEEN and PEG solutions. Then proceed with the preliminary test and observe for 24 hours to find out whether the concentration used is appropriate in killing the larvae. After that, the stages of the research test with the same steps and concentrations as the preliminary test were carried out and repeated 4 times. Observed and counted the number of larvae deaths in 24 hours every 6 hours.

3. Results and Discussion

Research on the effectiveness of the ethanol extract from ketapang leaves (*Terminalia catappa* L.) on *Aedes aegypti* larvae was carried out in several stages, that are preliminary tests and research tests. The results of the preliminary test obtained an average larvae mortality more than 50% in the second 6 hours, so the research test was continued with the same steps and concentrations as the preliminary test, that is 1.5% and 2%.

Table 1. Larvacidal Test

Treatment group	Larvae mortality Leaf Squeeze Having given Ketapang				Mortality Percentage(%)
	$6^{th}H$	$12^{th}H$	$18^{th}H$	$24^{th}H$	
K (+)	25	25	25	25	100
K (-) Tween	0	0	0	0	0
K (-) PEG	0	0	0	0	0
P1 Tween + 1,5%	5,75	9,75	16,75	23,25	93
P2 Tween + 2%	6,75	12	19	23	92
P1 PEG + 1,5%	4	17,25	23,5	25	100
P2 PEG + 2%	2	13,25	20,25	24,25	97

Based on the research tests in Table 1, there was 100% mortality of *Aedes aegypti* larvae in the positive control group sprinkled with abate and 0% mortality of larvae in the negative control group mixed with distilled water and TWEEN or PEG additions. Mortality was found at 24 hours, that is 100% in group P1 with PEG diluent and a concentration of 1.5% ketapang leaf ethanol extract. The percentage of larvae mortality in group P2 with PEG diluent and ethanol extract concentration of 2% ketapang leaves was 97%, in group P1 with TWEEN diluent and ethanol extract concentration of 1.5% ketapang leaves which was 93%, and in group P2 with TWEEN diluent and concentration 2% ketapang leaf ethanol extract is 92%.

When viewed from the average number of larvae deaths, ketapang leaves can be used as an alternative to replace abate. However, administration of the ethanol extract from ketapang leaves can change the color and the scent from the water, this is not accordance with one of the criteria for larvicides, that it does not cause changes in taste, color and smell in the water after being treated. Based on this, it can be seen that abate as a synthetic larvicide still has better effectiveness compared to natural larvicides, that is ketapang leaves (Nugroho., 2011).

The normality test was carried out using the *Shapiro-Wilk* test. The results of the *Shapiro-Wilk* normality test showed that the data were not normally distributed where the values obtained at 6 hours, 12 hours, 18 hours, and 24 hours had a sig value of <0.05. The data can be said to be normal if the sig value is > 0.05.

Homogeneity test was carried out using the *Levene* test. The results of the *Levene* homogeneity test showed that the data were not homogeneous because the sig <0.05 was obtained. The data obtained were not normally distributed and were not homogeneous, so the non-parametric statistical tests were continued with the *Kruskal-Wallis* test.

The non-parametric test uses the *Kruskal-Wallis* test, because based on the normality test and homogeneity test, the data results are abnormal and not homogeneous. The *Kruskall-Wallis* significance value was obtained <0.05 which indicated that there was a significant difference between the treatment group and the control group (+) and (-).

Table 2. *Mann Whitney* Test

	K (+)	K (-) TWEEN	K (-) PEG	TWEEN + 1,5%	TWEEN + 2%	PEG + 1,5%	PEG + 2%
K (+)		P=0,008*	P=0,008*	P=0,131	P=0,131	P=1.000	P=0,317
K(-) TWEEN	P=0,008*		P=1.000	P=0,013*	P=0,013*	P=0,008*	P=0,011*
K(-) PEG	P=0,008*	P=1.000		P=0.013*	P=0,013*	P=0.008*	P=0.011*
TWEEN+1,5%	P=0,131	P=0,013*	P=0.013*		P=0.877	P=0.131	P=0.405
TWEEN+2%	P=0,131	P=0,013*	P=0.013*	P=0.877		P=0.131	P=0.405
PEG+1,5%	P=1.000	P=0,008*	P=0.008*	P=0.131	P=0.131		P=0.317
PEG+2%	P=0,317	P=0,011*	P=0.011*	P=0.405	P=0.405	P=0.317	

(*)= significantly different

The *Post-Hoc* test using *Mann Whitney* was carried out, because there were significant differences between the data groups on the non-parametric *Kruskal-Wallis* test. The results of the *Mann Whitney* test showed that a comparison between the positive and negative control groups for TWEEN and PEG, the PEG treatment group at 1.5% concentration and the negative control group for TWEEN and PEG obtained p = 0.008. Comparison between the PEG treatment group at 2% concentration and the TWEEN and PEG negative control groups obtained p value = 0.011. Comparison between the TWEEN and PEG negative control groups and the TWEEN treatment group at concentrations of 1.5% and 2% obtained p value = 0.013. The *Mann Whitney* test results obtained sig p <0.05 which indicated a significant difference.

The results of the *Mann Whitney* test showed that a comparison between the negative TWEEN and PEG groups, the positive control group and the PEG treatment group at 1.5% concentration obtained p = 1,000. Comparison of the TWEEN treatment group with a concentration of 1.5% with the PEG treatment group with a concentration of 1.5% and the positive control group obtained p value = 0.131. Comparison of the PEG treatment group with a concentration of 2% and the PEG treatment group with a concentration of 1.5% and the positive control group obtained p value = 0.317. Comparison of the PEG treatment group with a concentration of 2% and the TWEEN treatment group with a concentration of 1.5% and a concentration of 2% obtained p = 0.405. Comparison of the TWEEN treatment group at a concentration of 2% with a concentration of 1.5% obtained p = 0.877. The *Mann Whitney* test results obtained sig p > 0.05 which showed no significant difference.

Comparison of the treatment group with the negative control group showed significant differences, which means that the addition of ketapang extract was effective in *Aedes aegypti* mortality. Comparison of the treatment group with the positive control group found that no significant difference, so judging from the percentage of larvae mortality, it was found that the groups with the highest to lowest effectiveness were PEG+1.5%, PEG 2%, TWEEN 1.5%, and TWEEN 2%.

The results of data analysis in this study are in accordance with the analysis in previous research conducted by Bestari *et al.*, (2020) showing that the results of the normality test with a significance value of p < 0.05, so the data distribution is not normal. The results of the homogeneity

test showed a p value <0.05 which mean that it was not homogeneous. The *Kruskall-Wallis* non-parametric statistical test showed a p value <0.05 meaning that there was a significant difference.

4. Conclusion

The ethanol extract of ketapang leaves (*Terminalia catappa* L.) with TWEEN and PEG diluents was effective to *Aedes aegypti* larvae mortality. The effectiveness of the ethanol extract of ketapang leaves (*Terminalia catappa* L.) with TWEEN diluent is the same as PEG on the mortality of *Aedes aegypti* larvae as shown in the *Mann Whitney Post-Hoc* test with p> 0.05, which means that there is no significant difference. Ketapang leaf ethanol extract (*Terminalia catappa* L.) with TWEEN and PEG diluent has lower effectiveness compared to abate.

Some suggestions for this study are, it is necessary to carry out further research with various fractionations of both polar and non-polar extracts to determine the effectiveness of ketapang leaf extract on *Aedes aegypti* mortality, it is necessary to add more varied extract concentrations to find out trends in mortality data with extract concentrations whether it has increased or not. Then, for the future research may use different extraction methods and use different types of mosquitoes.

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Conflict of Interest

All Authors declare no conflict of interest and agree with the content of the manuscript.

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