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Original Article

THE EFFECT OF METHANOL EXTRACT OF MANGO LEAF WITH CMC AND PEG ON AEDES AEGYPTI LARVAE MORTALITY

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ABSTRACT

Objective: This research aims to determine the effectiveness of mango leaf methanol extract (*Mangifera indica L.*) with Carboxymethyl Cellulose (CMC) and Polyethylene Glycol (PEG) diluents on the mortality of Aedes aegypti larvae. CMC and PEG are used to increase the effectivity of mango leaf extract as larvicide.

Methods: Research on methanol extract of mango leaves (*Mangifera indica L.*) with CMC and PEG diluents has an experimental laboratory design with a posttest-only controlled group design. The technique of extraction is maceration. The larvae of Aedes aegypti instar III-IV are used, 700 in total, divided into 7 treatment groups.

Results: The Lethal Concentration (LC50) result of the extract with CMC diluent at the 24th hour was 0.114%, while the LC50 of the extract with PEG diluent was 0.323%. The Lethal Time (LT50) of 0.05% concentration of extract with CMC diluent is 294.755 h and PEG with 140.764 h. At a concentration of 0.1% with CMC diluent is 195.267 h and PEG 194.630 h.

Conclusion: Methanol extract of mango leaf (*Mangifera indica L.*) with CMC and PEG diluents is effective on mortality of Aedes aegypti larvae. CMC-diluted extract demonstrated 2.8-fold higher potency (LC50: 0.114%) than PEG-diluted extract (LC50: 0.323%). CMC need lower concentration than PEG so it can be effective due to lower funding and the environment effect.

Keywords: Aedes aegypti, Mango leaf, CMC, PEG

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INTRODUCTION

Dengue Haemorrhagic Fever is a disease caused by infection of four dengue virus serotypes (DENV 1-4) from the Arbovirus B class that can be transmitted through the bites of *Aedes aegypti* and *Aedes albopictus* mosquitoes to humans directly and is a major health problem in the community. Transmission can only occur when the human body is in a state of viremia, with an incubation period of about 3-5 *d* [1, 2].

Data from 2000-2019 has increased cases reaching 5.2 million people. However, dengue cases decreased in 2020 and 2021 due to the COVID 19 pandemic. Indonesia is a tropical country and endemic to both major mosquito vector species of DENV, *Aedes aegypti* and *Aedes albopictus* [3]. According to the Kemenkes (2022) infected cases in Indonesia from January to September 2022 reached 87,501 cases with 816 deaths.

The rise of *Aedes aegypti* cases spurs efforts to control namely prevention can be done through synthetic insecticides and 3M Plus (Draining, closing water tanks, and recycling used goods) which have proven effective. However, the use of insecticide chemicals can have negative impacts on the community, such as polluting the environment and increasing resistance. On the other hand, for 3M Plus, the use of natural or plant-based larvicides must be intensified because the community still does not know the usefulness and usefulness of natural larvicides, which in fact, have a good effect on the environment rather than using insecticidal chemicals [4-6].

Syah's research (2020) has been tested with 4 concentrations of 96% ethanol extract of mango leaf (*Mangifera indica L.*), namely 125 ppm, 250 ppm, 500 ppm, and 1000 ppm, which are converted into percentages of 0.0125%, 0.025%, 0.05%, and 0.1%, which are found to be effective in eliminating larvae as much as 51%, 78%, 88%, and 99%.

Natural larvicides have the same meaning as natural insecticides derived from plants. Its function is to kill insects quickly in nature. The advantage of using natural larvicides is that they degrade quickly under the influence of sunlight air humidity, thereby reducing the risk of water and soil pollution [8].

One of the plants that can be used as a natural larvicide is mango leaf. On the other hand, mango leaf are easily available and contain flavonoids, saponins, polyphenols, alkaloids, tannins, and steroids that can have a positive impact on preventing the development of *Aedes aegypti* mosquito larvae [9, 10]. Saponin, alkaloid, tannin, flavonoid are the most important secondary metabolites [11]. Flavonoid is a phenolic component that is usually found in stem, leaves, flowers and fruits [12].

The extract obtained will be difficult to dissolve in water because it is solid, therefore, it needs a material to disperse the extract, among others with CMC and PEG diluents. The choice of CMC as an emulsifier was based on research by Nopitasari (2014), which showed that CMC was not the cause of larvae mortality. CMC is one of the cellulose-derived compounds that functions as a solvent, thickener, binder, and suspension agent [13, 14]. PEG is a substance that is easily dissolved in water and can also dissolve in methanol, benzene, dichloromethanes, and has low toxicity [15]. In previous research, there were differences in research results related to the relationship between PEG and insecticidal effects on *Aedes aegypti*, as conducted by Jeffers (2012) explaining that PEG can increase the insecticidal effect on *Aedes aegypti*. Research by Yanuar and Sugiharto (2018) revealed that PEG is not effective in increasing insecticidal ability [16].

Based on the research that has been done, the author is interested in knowing how effective it is to use 90% methanol extraction of mango leaf (*Mangifera indica L.*) given with CMC and PEG diluents as natural larvicides, direct comparison under standardized conditions because there has been no research comparing the two diluents.

MATERIALS AND METHODS

Preparation of materials

The tools used in this research are sieve, basin, stick, tray, blender, measuring cup, plastic cup, analytical balance, hand counter, and label paper. The materials used were Aedes aegypti instar III-IV larvae, distilled water, methanol extract of mango leaf (*Mangifera indica L.*), abate, and CMC and PEG diluents.

Preparation of extract

Mango leaf extract was taken from Kudus, Central Java. Determination of the plants were registered by Balai Besar Penelitian dan Pengembangan Tanaman Obat dan Obat Tradisional (B2P2TOOT) Kementrian Kesehatan Republik Indonesia, No. KM.04.02/2/2024/2022. Dried in the sun with a cloth covered until the leaf feels dry for about 5 *d*. The dried leaf was cut into small pieces then mashed with a blender until it became a fine powder and filtered with a hole diameter of 1 mm placed in a container in the form of a basin covered with white paper. Then, the finished simplisia was carried out the maceration method using 90% methanol solvent for 7 *d* while stirring every day. Then the macerate was concentrated using a Rotatory Evaporator and Water bath until a condensed extract was formed. From crude weight 1220 gr, extract weight os 62.48 g, so extract concentration is (1220/62.48) 5.119%. The condensed extract is given a diluent in the form of CMC and PEG so that it can be well dispersed.

Preparation of larvae

Larvae rearing from lab colony in Parasitology Laboratory of Medical Faculty UMS. Aedes aegypti eggs was obtained from Loka Penelitian dan Pengembangan Kesehatan Pangandaran, Kementrian Kesehatan Republik Indonesia. Determination No. 011/lab. Par/XII/2022.

Larvae testing

Testing of Aedes aegypti instar III-IV larvae placed 25 larvae in each 1 cup treatment divided into 7 treatment groups namely positive control (abate), negative control (CMC), negative control (PEG), (0.05% mango leaf methanol extract+CMC), (0.1% mango leaf methanol extract+CMC), (0.05% mango leaf methanol extract+PEG), (0.1%, mango leaf methanol extract+PEG) with a total larvae is 700. It replicated four times as Federer's Formula.

Ethical approval

Ethical Clearance Letter by Health Research Ethics Committee Faculty of Medicine UMS, No.4602/A.1/KEPK-FKUMS/XI/2022.

Data analysis

The tests carried out in this research used data normality test (Shapirowilk) and homogeneity test continued by statistical tests, which are non-parametric Kruskall Wallis test and Mann whitney post hoc test.

RESULTS AND DISCUSSION

Stability test

The yield obtained was 5.119% from maceration of condensed extracts, then carried out stability tests with CMC and PEG diluents to disperse the condensed extracts and can be homogeneous when added with CMC or PEG.

Indicators used in this research stability test are solubility, immediate precipitate, 24 h precipitate, and dilution. Based on the stability test, the results of the extract can be homogeneous with CMC and PEG diluents shown in table 1.

Table 1: Stability test results

Indicator	CMC (0.01%)	PEG (0.01%)
Solubility	Dissolve	Dissolve
Immediate precipitate	No precipitated	No precipitated
Precipitate after 24 h	A bit of precipitate	A bit of precipitate
Viscosity	Dilute	Dilute

Description: CMC: Carboxymethyl Cellulose, PEG: Polyethylene Glycol

Larvacide effectiveness test

Observations were made by observing dead larvae every 6 h within 1x24 h. The larvacide test obtained the average larvae mortality of 4 replicates presented in fig. 1.

Data analysis using data normality test and homogenity test. Based on the results of the normality and homogeneity tests, it was found that the data were not normally distributed and not homogeneous, so the Kruskal-Wallis and Mann-Whitney tests are presented in table 2 and 3.

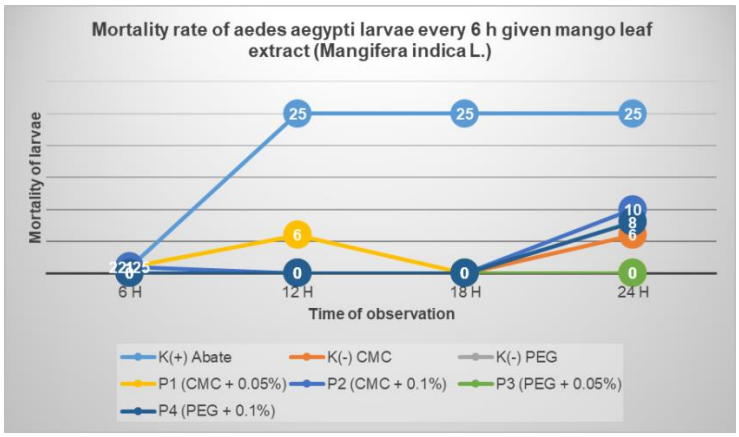

Fig. 1: Mortality rate of aedes aegypti larvae every 6 h given mango leaf extract (*Mangifera indica L.*) with 4 repetitions

Table 2: Non parametric kruskall-wallis test

Treatment	Sig value	Description
6th h	0.003	Significantly different
12th h	0.001	Significantly different
18th h	0.001	Significantly different
24th h	0.001	Significantly different

Table 3: Non-parametric post hoc mann-whitney test

Parameters	K (+) Abate	K (-) CMC	K (-) PEG	P1 (CMC+0.05%)	P2 (CMC+0.1%)	P3 (PEG+0.05%)	P4 (PEG+0.1%)
K (+) Abate		0.008*	0.008*	0.013*	0.014*	0.014*	0.014*
K (-) CMC	0.008*		1.000	0.013*	0.014*	0.014*	0.013*
K (-) PEG	0.008*	1.000		0.013*	0.014*	0.014*	0.013*
P1 (CMC+0.05%)	0.013*	0.013*	0.013*		0.772	0.561	0.243
P2 (CMC+0.1%)	0.014*	0.014*	0.014*	0.772		0.773	1.000
P3 (PEG+0.05%)	0.014*	0.014*	0.014*	0.561	0.773		0.465
P4 (PEG+0.1%)	0.014*	0.013*	0.013*	0.243	1.000	0.465	

Description: CMC: Carboxymethyl cellulose, PEG: Polyethylene glycol

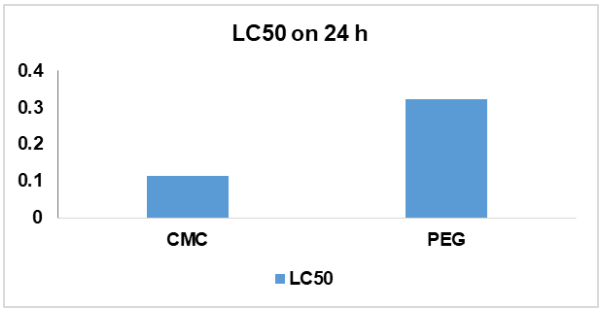

Fig. 2: LC50 on 24 h of mango leaf extract to Aedes aegypti larvae mortality. LC: Lethal concentration

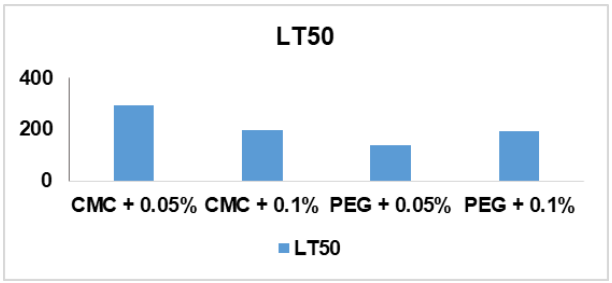

Fig. 3: LT50 of mango leaf extract to Aedes aegypti larvae mortality. LT: Lethal time

Probit analysis

LC50 test aims to determine the concentration required to eliminate 50% of the larvae. LT50 test aims to determine how long it takes to eliminate 50% of the larvae.

DISCUSSION

The research conducted by the author used additional diluents, namely CMC and PEG, but there are still very few studies that discuss diluents that have an effect on larval mortality. Therefore, it is necessary to conduct a stability test obtained the results of the addition of CMC and PEG diluents can affect solubility, immediate sediment, 24 h sediment, and dilution. Based on the stability test in table 1, it was found that the initial concentration of CMC 0.5% and PEG 0.1% was reduced to CMC 0.01% and PEG 0.01%. This is because the solution still looks thick and is feared to affect the mobility of larval movement so that it can cause larvae mortality [17].

Saponin, alkaloid, tannin, flavonoid are the most important secondary metabolites [11] Alkaloids work by disrupting the nervous system of larvae, inhibiting the larvae's appetite and acting as stomach poisons. Flavonoids function as a strong inhibitor of the respiratory system and are toxic to insects [18]. Phytocemistry of mango leaf have been done that mango leaf have alkaloid, flavonoid, saponin, steroid, terpenoid, polifenol, and tanin [19]. HPLC data analysis have mentioned the bioactive coumpound in mango leaf extract as larvicide, identify hydrolysable tannins revelaed the presence of gallatotannin [20].

The results of data analysis in the normality test obtained data are not normally distributed with the p value<0.05 in all treatments. Followed by the homogeneity test with the Levene method, the results of the data are not homogeneous, with the p value<0.05 in all treatments.

The data obtained are not normally distributed and not homogeneous, so it is continued with the Kruskall-Wallis non-parametric test with the results of significant differences presented in table 2. This indicates that H0 is rejected and H1 is accepted by conducting a further post hoc Mann-Whitney test to find out which groups are significantly different and not significantly different, presented in table 3.

Based on table 3 in P1, P2, P3, and P4 obtained p<0.05 with results that can be interpreted as significantly different from k (-) CMC and PEG. This indicates that the methanol extract of mango leaves (*Mangifera indica L.*) with CMC and PEG diluents has effectiveness to eliminate *Aedes aegypti* larvae. Other treatments, namely in P1, P2, P3, and P4, the results obtained p<0.05, which means that there is a significant difference with k (+) abate. Therefore, this indicates that the methanol extract of mango leaves (*Mangifera indica L.*) with CMC and PEG diluents is effective for eliminating *Aedes aegypti* larvae but cannot match the effectiveness of k (+) or abate. In the P1, P2, P3, and P4 treatments, the results obtained p>0.05 can be interpreted as not significantly different. This indicates that the methanol extract of mango leaf (*Mangifera indica L.*) with CMC and PEG diluents has the same effectiveness in eliminating *Aedes aegypti* larvae. The p-value in P1 with P2, P3, and P4 results obtained p>0.05 can be interpreted

as not significantly different. Comparison between P2 with P3 and P4 and P3 with P4 obtained results p>0.05 can be interpreted as not significantly different. This indicates that the methanol extract of mango leaf (*Mangifera indica L.*) with CMC and PEG diluents has the same effectiveness in eliminating *Aedes aegypti* larvae.

Based on this study, the LC50 probit analysis results (fig. 2) of mango leaf methanol extract with CMC diluent at the 24th hour was 0.114%, while the LC50 of mango leaf methanol extract with PEG diluent was 0.323%. The toxicity of an extract to test animals is said to be highly toxic when LC50<1%, toxic 1-10%, moderately toxic 10-50%, slightly toxic 50-99%, and non-toxic at 100% [21]. This research of mango leaf methanol extract with CMC and PEG diluents is categorized as highly toxic to a larva with a more effective level to eliminate larvae, namely in mango leaf methanol extract (*Mangifera indica L.*) with CMC diluent. Lower LC50 of CMC have implication on reducing environment burden, lower funding and lower toxicity potential on non-target.

LT50 probit analysis (fig. 3) obtained that the LT50 of mango leaf methanol extract at a concentration of 0.05% with CMC diluent was 294.755 h and PEG 140.764 h. The concentration of 0.1% with CMC diluent is 195.267 h and PEG 194.630 h. This means that the LT50 at all concentrations cannot be determined because it exceeds the observation time of 24 h, so there is no conformity according to the theory [22], which says the lower the LT50 value, the faster the rate of infection caused by a chemical, the more toxic the extract is. The LT50 values exceeding 24 h suggest the observation period was insufficient. It can be that extending the observation time (e. g., 48–72 h) would yield actionable LT50 data.

The biodegradability and toxicity of Carboxymethyl Cellulose (CMC) and Polyethylene Glycol (PEG) residues in aquatic ecosystems are important considerations for environmental safety. CMC is considered biodegradable. The biodegradability of PEG is dependent on its molecular weight (MW). Lower molecular weight PEGs (up to around 14,600 Da) have been shown to biodegrade in both freshwater and seawater environments, although biodegradation in seawater may take longer.

CONCLUSION

Methanol extract of mango leaf (*Mangifera indica L.*) with CMC and PEG diluents is effective on mortality of Aedes aegypti larvae. CMC-diluted extract demonstrated 2.8-fold higher potency (LC50: 0.114%) than PEG-diluted extract (LC50: 0.323%). CMC need lower concentration than PEG so it can be effective due to lower funding and the environment effect.

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Nil

AUTHORS CONTRIBUTIONS

In this writing, all authors gave the following contributions: Conceptualization and Methodology: Rochmadina S Bestari. Writing and Original Draft Preparation: Fahriza M Trihatmoko. Formal Analysis, Resources: Retno Sintowati. Review: Listiana Masyita Dewi. Editing: Devi U Rosyidah. All authors have read and agreed on the published version of the manuscript.

CONFLICT OF INTERESTS

There is no conflict of interest in the subjects matter or materials discussed in this manuscript.

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