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Larvicidal Activity of Ethanol Extracts of Bidara (*Ziziphus mauritiana*) Leaves and Fruits Against *Aedes aegypti*

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ABSTRACT

Dengue fever vector control uses abate chemicals can harm the ecosystem. One of the plants that can be used as a natural larvicide is bidara. Bidara leaves and fruits easily available and contains flavonoids, saponins, alkaloids, and tannins. The compound have an impact on the mortality of Aedes aegypti mosquito larvae. Contains study design, subject, methods, data collection and data analysis. Research on ethanol extract of bidara leaves and bidara fruit with Poly Ethylene Glycol (PEG) was experimental laboratory design with post-test only controlled group design. The technique of extraction is maseration. The larvae of Aedes aegypti instar III-IV are used, 800 in total, divided into 8 groups. At 6, 12, 18, and 24 hours, the number of dead larvae was counted .Normality and homegenity tests obtained the results of data not normally distributed and not homogeneous, so it is necessary to do the Kruskall-Wallis non-parametric test followed by Mann-Whitney post hoc .The result is bidara leaf extract with a concentration of 1.5% + 3% PEG resulted in 100% larval death in the 24th hour, meanwhile bidara fruit extract with concentration of 1.5% + 3% PEG resulted 70% larval death in 24th hour. Ethanol extract of bidara leaves has better effectiveness than bidara fruit extract in its ability as a larvicide, where bidara leaf extract with a concentration of 1.5% + 3% PEG resulted in 100% larval death in the 24th hour.

Keywords: Bidara leaves; bidara fruit; Ziziphus mauritiana; Aedes aegypti; larvicide

Introduction

The dengue virus is the etiological agent for Dengue Virus Infection including Dengue Haemoragic Fever (DHF). The primary vector of DHF is the Aedes aegypti mosquito. Infection with DHF remains a major health concern in Indonesia (Jatmiko,

2023; Jatmiko, Aisyah, et al., 2024; Jatmiko, Yulistina, et al., 2024). Up to the 22nd week of 2024, there were about 120,000 dengue cases, which was more than the 114,700 cases that occurred in 2023. There have been 777 dengue-related deaths in 2024 thus far, compared to 894 instances in 2023(antaranews, 2024). To lower morbidity and mortality from DHF infection, vector control must be consistently pursued. There are three different methods for controlling vectors: chemical, biological, and mechanical. Chemical control involves using pesticides to kill or repel vectors. It is often the fastest method for reducing vector population especially during outbreak. Biological control uses natural enemies of the vector to reduce its population. Mechanical control is a method that involves physical removal, modification of the environment or the use of physical barrier to limit vector breeding or prevent contact to human. It is an environmentally friendly and sustainable long-term solution. Larvicide is being used by one of them(Auliaputri, 2022; Nafila; Nurmansyah & Amalia, 2022; Nurhayani et al., 2021). The development of a natural larvicide from Indonesian plants that does not harm the environment is required because the chemical larvicide abate (Temephos 1%) has been used for years, has led to resistance in various locations, and kills pets(Auliaputri, 2022; Bestari et al., 2020; Grisales et al., 2013; Maulana et al., 2022; Mulyatno, Kris Cahyo; Yamanaka, Atsushi; Ngadino; Konishi, 2012; Nirma et al., 2017).

The bidara plant (*Ziziphus mauritiana*) is a native plant in Indonesia. The presence of tannins, saponins, alkaloids, and flavonoids in the bidara plant makes it potentially a larvicide. The type of tannin in bidara is hydrolysable tannins and condensed tannins/proantosianidin. The type of saponin in bidara is saponin triterpenoid and saponin steroid. Alkaloid type in bidara is peptide alkaloid and cyclopeptide alkaloid (ziziphin, frangufolin and mauretin). Type of flavonoid in bidara is sianidin and quercetin (Marcellia et al., 2024). These active ingredients have the potential to harm mosquito larvae's respiratory and digestive systems(Adiningsih & Ganning, 2021; Chairunnisa et al., 2019; Ulfa, 2024). An emulsifier has not been employed in research on bidara leaf and bidara fruit extracts to create a solution with improved dispersion. To determine the ideal extract concentration and emulsifier, more study is required. This study sought to ascertain the relative efficacy of bidara fruit and leaf ethanol extracts combined with an emulsifier in terms of Aedes aegypti larval mortality.

Methodology

This study approach uses a post-test only control group design, making it a true experiment. The investigation was carried out in October and November of 2024 at the Faculty of Medicine's Pharmacology and Parasitology Laboratory at UMS. Bidara fruit and leaves from Pemalang Regency in the Central Java Province were utilized. We used the maceration method for fruit and leaves extractions. Aedes aegypti larvae in stages III–IV were employed. In addition, 3% PEG and 70% ethanol were utilized. Ethics approval for this study was granted by KEPK FK UMS No.5408/A.1/KEPK-FKUMS/XII/2024.

Tawangmangu Traditional Health Services' UPF Testing Laboratory, Dr. Sardjito Hospital Number TL.02.04/D.XI.6/347.046/2025, provided the plant determination.

Working Procedure

Extraction of bidara leaves and fruits (*Ziziphus mauritiana*)

There are multiple steps involved in creating the extract. After being cleaned and dried, the fresh bidara fruits and leaves are blended until they are simple. After that, the wet simplicia is macerated for seven days with 70% ethanol while being agitated. Filter paper is then used to filter it. After that, the filtrate is boiled in a water bath and evaporated.

Rearing of Aedes aegypti larvae

Balitbangkes Pangandaran provided the Aedes aegypti eggs, which were hatched in the UMS Medical Faculty's Parasitology Laboratory. For the preliminary test, 200 Aedes aegypti larvae in stages III–IV were utilized, and for the larvicide test, 600. According to WHO norms, each glass contained 25 larvae. Repetition was carried out 3 times according to the Federer formula.

Stability Test

The purpose of this stability test is to ascertain the ideal diluent or emulsifier concentration for the extract's efficient dissolution. The degree of dilution, the presence of immediate sediment, the creation of sediment after 24 hours, and solubility are stability indicators that are tested on emulsifiers or diluents against extracts. According to these indicators, PEG was the emulsifier employed in this investigation. The experiment included PEG concentrations of 0%, 1%, 2%, and 3%. The findings of the experiment utilizing PEG with 3% bidara leaf and fruit extract revealed a uniform solution devoid of sediment.

Preliminary Test

In order to proceed to the larvicide test, a preliminary test was carried out to ascertain whether the variation in extract concentration was sufficiently effective. The initial test group included six treatment groups and two control groups: the positive control (1% temephos) and the negative control (aquadest + PEG 3%). The following treatment groups were divided into the following: Q1 (ethanol extract of bidara fruit 0.5% + PEG 3%), Q2 (ethanol extract of bidara fruit 1% + PEG 3%), Q3 (ethanol extract of bidara fruit 1.5% + PEG 3%), and P1 (ethanol extract of bidara leaves 0.5% + PEG 3%). At 6, 12, 18, and 24 hours, the number of dead larvae was counted.

Larvicide Test

The Federer Formula is used to repeat the larvicide test, which measures larval mortality. The larvicide test group includes six treatment groups and two control groups: the positive control (1% temephos) and the negative control (aquadest + PEG 3%). P1 (ethanol extract of bidara leaves 0.5% + PEG 3%), P2 (ethanol extract of bidara leaves 1% + PEG 3%), P3 (ethanol extract of bidara leaves 1.5% + PEG 3%), Q1 (ethanol extract of bidara fruit 0.5% + PEG 3%), Q2 (ethanol extract of bidara fruit 1% + PEG 3%), and Q3 (ethanol extract of bidara fruit 1.5% + PEG 3%) are the treatment groups. At 6, 12, 18, and 24 hours, the number of dead larvae was counted. three times.

Result and Discussion

Results of the Preliminary Test

The results of the Preliminary Test are shown in Table 1.

Table 1. Result of Preliminary Test

Group	Larvae Mortality on hour			
	6	12	18	24
Control (+) temephos 1%	25	25	25	25
Control (-) PEG 3%	0	0	0	0
P1 (Leaves 0.5% + PEG 3%)	2	4	7	10
P2 (Leaves 1% + PEG 3%)	2	5	9	15
P3 (Leaves 1.5% + PEG 3%)	3	7	14	21
Q1 (Fruit 0.5% + PEG 3%)	1	3	5	11
Q2 (Fruit 1% + PEG 3%)	7	17	21	25
Q3 (Fruit 1.5% + PEG 3%)	9	19	24	25

Based on the Table 1, it can be seen that bidara leaves and fruit are effective in killing *Aedes aegypti* larvae, which is shown from 6 hours to 24 hours.

Result of the Larvicide Test

The results of the larvicide test are shown in Figure 1.

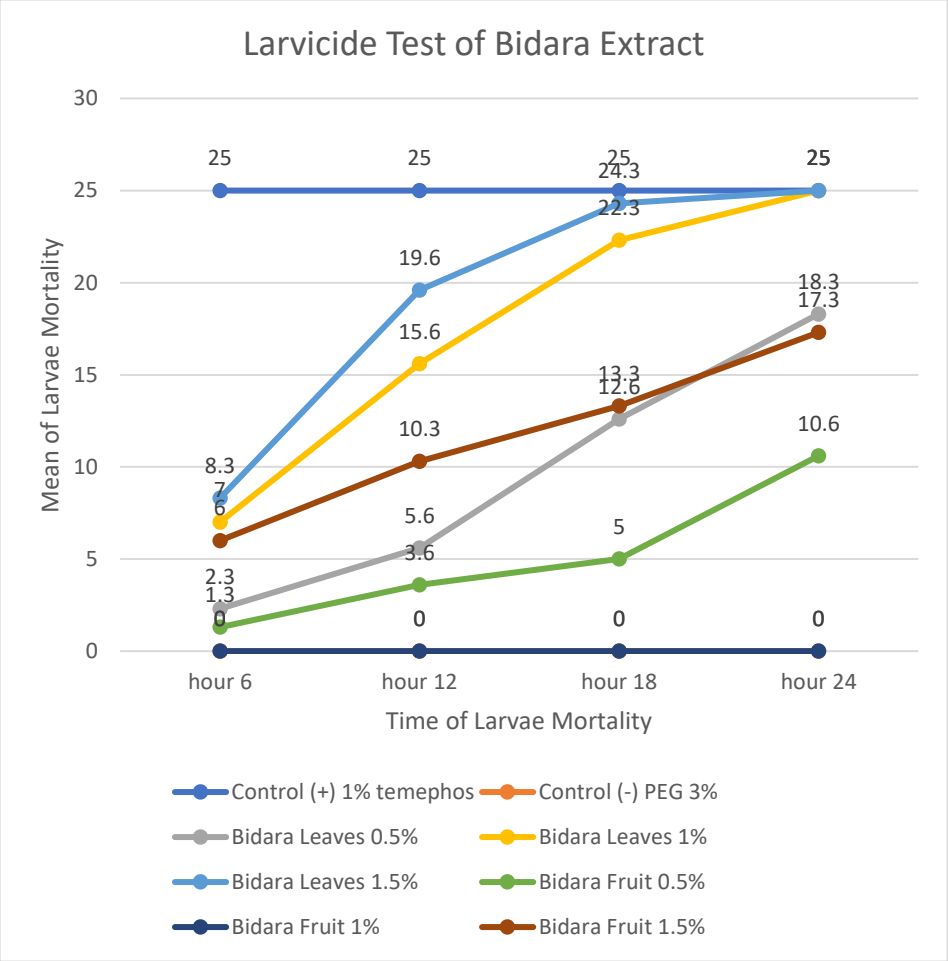

Figure 1. Larvicide Test of Bidara Leaves and Bidara Fruit

A p value of less than 0.05 indicates that the data is not normally distributed, according to the Shapiro-Wilk normality test. The data may not be homogeneous, as shown by the homogeneity test's p value of less than 0.05. A significant difference in the data is interpreted when the Kruskal-Wallis non-parametric test yields a p value <0.05. Thus, the Mann-Whitney post-hoc test can be used to further investigate the data. The Mann-Whitney post-hoc test was employed in this investigation. Finding a P value <0.05, which denotes a significant difference from the comparative data, is the goal of the Mann-Whitney test.

Table 2. Mann-Whitney Test on Larval Mortality at 24 Hours

	C(+)	C(-)	P1	P2	P3	Q1	Q2	Q3
C(+)		0.025	0.037	1.000	1.000	0.034	0.034	0.037
C(-)	0.025		0.037	0.025	0.025	0.034	0.034	0.037
P1	0.037	0.037		0.037	0.037	0.046	0.046	0.513
P2	1.000	0.025	0.037		1.000	0.034	0.034	0.037
P3	1.000	0.025	0.037	1.000		0.034	0.034	0.037
Q1	0.034	0.034	0.046	0.034	0.034		0.043	0.046
Q2	0.034	0.034	0.046	0.034	0.034	0.043		0.046
Q3	0.037	0.037	0.513	0.037	0.037	0.046	0.046	

C(+) : temephos 1%
C(-) : PEG 3%
P1 : Bidara leaves extract 0.5% + PEG 3%
P2 : Bidara leaves extract 1% + PEG 3%
P3 : Bidara leaves extract 1.5% + PEG 3%
Q1 : Bidara fruit extract 0.5% + PEG 3%
Q2 : Bidara fruit extract 1% + PEG 3%
Q3 : Bidara fruit extract 1.5% + PEG 3%

Secondary Metabolite Test Results

Table 3. Secondary Metabolite Test Results on bidara leaves

Compound	Positive sign	Observation results	Conclusion
Alkaloid (Meyer)	Red brown/orange sediment	Red brown/orange sediment	positive
Flavonoid	Yellow sediment	Yellow sediment	positive
Tannin	Dark green/blue	Dark green/blue	positive
Saponin	Stable foam	Stable foam	positive

Table 4. Secondary Metabolite Test Results on bidara fruit

Compound	Positive sign	Observation results	Conclusion
Alkaloid (Meyer)	Red brown/orange sediment	Red brown/orange sediment	positive
Flavonoid	Yellow sediment	Yellow sediment	positive
Tannin	Dark green/blue	Dark green/blue	positive
Saponin	Stable foam	Stable foam	positive

The purpose of this study was to ascertain whether the leaves and fruits of the bidara plant (*Ziziphus mauritiana* L.) have the capacity to kill Aedes aegypti larvae, and if so, whether the potential for killing larvae varies depending on the plant(Adiningsih &

Ganning, 2021). According to earlier reports, the bidara plant (*Ziziphus mauritiana* L.) includes a variety of compounds, including flavonoids, alkaloids, tannins, polyphenols, and saponins. The larvae of Aedes aegypti can be killed by the active chemicals it contains.

Bidara leaf extract is known to contain 0.12% tannins, 0.08% saponins, 0.06% alkaloids, and 0.03% flavonoids(Reni Wulansari, 2022). Because of their highest concentration, these results suggest that tannin and saponin chemicals have the biggest impact. Nevertheless, alkaloid and flavonoid substances also have an impact that causes larval death. According to the study, the highest content compounds that have the biggest impact on larval death are tannin and saponin. Saponins that enter the bodies of larvae will impact food absorption and digestive enzymes, as stated in the study of Karima and Ardiansyah (2021) (stomach poison)(Bunuh et al., 2021). Additionally, saponins can enter through the respiratory system, damaging cell membranes and interfering with metabolic functions. In addition to saponins, alkaloids that infiltrate the larvae's body have the ability to harm and break down the cold cells while also interfering with the neurological system. Bidara leaf tannins have the ability to raise the amount of protein in larvae's digestive systems, which can interfere with the process of protein absorption. Lastly, when flavonoid compounds enter the larvae's body, they paralyze the respiratory nervous system, causing seizures and eventual death from breathing difficulties brought on by respiratory system disruptions.

This study employed a posttest-only controlled group design in a real experimental laboratory setting. Gathering the supplies required to create the extract was the first step in the research procedure. Additionally, the Tawangmangu Traditional Health Service Functional Implementation Unit of Dr. Sardjito General Hospital conducted the plant determination. The materials were dried and powdered until they were smooth after completing the determination process. Ethanol has a high degree of polarity, is readily soluble, and is a universal solvent that can dissolve practically all metabolic chemicals. Ethanol is a universal solvent that can dissolve practically all metabolic chemicals, has a high degree of polarity, and dissolves readily. Because it can offer the best results with a maceration time of about three days, 70% ethanol is utilized as a solvent. This solvent is resistant to microbial development, has a comparatively low cost of use, and can extract molecules with different degrees of polarity(Afifah et al., 2023; Reni Wulansari, 2022).

PEG was employed as an emulsifier in this investigation. When compared to traditional solvents, PEG is a cost-effective and ecologically beneficial substitute. Among the many benefits of this molecule are its stability, superior dissolution of organic compounds, and non-corrosive nature(Mohamadpour, 2023). A number of indications, including the degree of dilution, the presence of direct sediment, sediment after 24 hours, and solubility, were used to evaluate the emulsifier's stability to the extract. According to the test results, PEG combined with bidara leaf and fruit extracts at a concentration of 3% resulted in a homogenous solution rather than sediment. Therefore, in this investigation, bidara leaf and fruit extracts were diluted using PEG at a concentration of 3%.

Comparison between bidara leaves and bidara fruit, which has the greatest potential in killing larvae is bidara leaf extract with a concentration of 1.5% + 3% PEG with 100% larval death in the 24th hour. This study has limitation on thick extract at 1,5% concentration so there was difficulties to count death larvae, hopefully next study it can be concerned.

Conclusion

Ethanol extract of bidara leaves has better effectiveness than bidara fruit extract in its ability as a larvicide, where bidara leaf extract with a concentration of 1.5% + 3% PEG resulted in 100% larval death in the 24th hour.

Declaration of Competing Interest

The authors declare that they have no competing interests.

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