ISSN 0216-3438 (Print). ISSN 2621-1122 (Online)
Jurnal Profesi Medika : Jurnal Kedokteran dan Kesehatan

LARVICIDAL ACTIVITY AND HISTOPATHOLOGICAL MIDGUT ALTERATION OF *AEDES AEGYPTI* LARVAE INDUCED BY METHANOL EXTRACT MAHKOTA DEWA FRUIT (*PHALERIA MACROCARPA* (SCHEFF). BOERL)

Nurul Eliza[1*], Rizal Subahar[2], Agus Aulung[2]

[1]Fakultas Kedokteran, Universitas Gunadarma, Depok
[2]Fakultas Kedokteran, Universitas Indonesia, Jakarta

*Corresponding email: nrlghz@gmail.com

ABSTRACT

Aedes aegypti is the main vector of DHF in Indonesia. The use of synthetic insecticide results in vector resistance. Mahkota Dewa fruits (*Phaleria macrocarpa* (Scheff) Boerl) contain flavonoids, saponins, and tannins. Thus, it has the potential as a natural larvicide against *Ae. aegypti* larvae. The objective of the study was to determine the effectiveness of methanol extract Mahkota Dewa fruit. This research was experimental, used methanol extract Mahkota Dewa fruit at concentration 0.1%, 0.15%, 0.20%, 0.25% and 0.30%, then added 25 larvae each concentration and repeated 3 times. After 24 and 48 hours of exposure, the mortality of larva was counted and then calculated LC50 and anterior midgut histopathology. LC50 values 0,554% (0,391-11,397) and anterior midgut histopathology shows epithelial damage. Mahkota Dewa fruits with methanol extract have activity as larvicide larvae instar III-IV *Ae. aegypti*.

Keywords: Methanol Extract of *Phaleria macrocarpa* (Scheff) Boerl; *Ae. aegypti* larvae; Midgut Anterior.

INTRODUCTION

Vector-borne diseases are a disease transmitted by vectors. [1]Dengue Hemorrhagic Fever is One of the vector-borne diseases that is a health problem in the world, including Indonesia, transmitted by *Ae. aegypti* and *Ae. albopictus*.[2,3] Until now, no drug and vaccine have been found to reduce the mortality rate and morbidity caused by this disease by controlling the population of dengue vectors by using insecticides.[4] However, irrational and planned use contributes to the development of vector resistance to insecticides.

One effort to overcome the problem of insecticide resistance required alternative insecticides derived from plants, which are not toxic to humans, animals, and non-target plants, environmentally friendly, and are not resistant. Mahkota Dewa fruits (*Phaleria macrocarpa* Scheff) have antimicrobial properties, antioxidants[5], larvaside[6]. WHO[7] has bioassay parameters to test the effects of larvicide from plant extracts against mosquito larvae, which are the lethal concentration 50% for 24 hours after exposure to the plant extracts. This study measured the effectiveness of methanol extract of Mahkota Dewa's fruits by measuring LC50 and histopathology anterior midgut instar larvae III-IV *Ae. aegypti.*

METHOD

Extraction

Simplicia 500g was macerated for 24 hours using methanol solution 4 liters and stirred by macerator, strainer, and concentrated with vacuum dry in temperature 50-60^{o}C.

Saponin test

As 0.1g methanol extract dissolved with 15ml of hot water, then shake. A positive test of saponin is characterized by formatting the foam at the surface.

Bioassay

Total of 25 instar larvae III-IV were added in 200cc water with concentrations of 0.10%, 0.15%, 0.20%, 0.25%, 0.30% repeated 3 times for each concentration and for negative

control used water. Larval mortality was calculated at the first 6 hours, 24 hours, and 48 hours. It was then calculating the LC50.

Histopathological Examination with HE staining

Larvae soaked at formaldehyde buffered 10%, then at dehydration using Alcohol 70%, 80%, 90%, 95%, absolute I and II, respectively, for 2 hours. Then, clearing Xylol for 40 minutes repeated three times, followed by blocking paraffin for 30 minutes at 60^0C. The cutting block uses a microtome with a range thickness of 5-7 micrometers, followed by HE staining (hematoxylin-eosin).

RESULTS

Extract of Mahkota Dewa fruit with methanol solvent obtained 52g, and saponin test shows positive (+) formed foam. Furthermore, bioassay was conducted according to WHO rule, using methanol extract at concentration 0,1%, 0,15%, 0,20%, 0,25% and 0,30%. The results of the bioassay are according to table 1.

Kolmogorov Smirnov test obtained average mortality larvae at 24h was 14.40 and 48 hours was 16.53.

Table 1. Bioassay Results methanol Extract of Mahkota Dewa fruit

Conc. (%)	n	Mortality total of larvae						Mean±SD 24h	Mean±SD 48h
		24h			48h				
		1	2	3	1	2	3		
0	25	0	0	0	0	0	0	0	0
0.1	25	4	1	0	4	1	2	1.67±1.73	2.33±1.53
0.15	25	16	13	16	17	17	20	15±5.57	18±1.73
0.20	25	20	16	9	23	21	10	15±1.53	18±7.00
0.25	25	19	21	18	21	23	22	19.33±2.52	22±1.00
0.30	25	19	21	24	21	22	24	21±2.52	22.33±1.53
Mean								14,40±7,472	16,53±8,123
Kolmogorov-Smirnov Z								0,974	1,027
p value								0.299	0.242
LC50 24h (%0								0,554 (0,391-11,397)	
LC90 24h (%)								1,291 (0,632-844,048)	
LT50 24h (hours)								17,082 (12,103-14,229)	

Note: 1,2,3,and 4=repeated, p value= Kolmogorov-Smirnov Z

The distribution data of larval mortality at 24 and 48 hours was normal with P> 0.05 (P = 0.299 and 0.242). Furthermore, by using probit analysis obtained the value of LC50, LC90 and LT50 are respectively 0.554%, 1.291%, and 17h.

Histopathology Midgut larvae *Ae. aegypti* with HE staining, magnification 100x and 200x, anterior midgut larval instar III-IV *Ae. aegypti* control seem homogenous cuboid layer of cells and cell nuclei round flat with bluish with brush border on the apical, and embedded in the basal lamina that looked intact, it is in accordance with figure1A and B. While in the III-IV instar larvae *Ae. aegypti* treatment with extract, epithelial cells are homogeneous and attached to the basement membrane, but some damaged epithelial cells are apart from the basement membrane (Figure 1C and D).

DISCUSSION

Dengue fever, transmitted by *Ae. aegypti* and *Ae. albopictus* does not have a potent vaccine, and therefore one attempt to reduce the transmission of disease by controlling the main vectors.[2] In the 1950s and early 1960s, vector control or eradication programs with large-scale DDT applications in many countries. At the beginning, the program was successful in many countries, but the vector became resistant to the pesticide.[4] It is important to discover new insecticides.

Phaleria macrocarpa (Scheff.) Boerl was a popular medicinal plant in many south Asian countries, Mama lay et al.[5] report on the fruit of this plant that there are numerous phytochemical constituents such as flavonoids, saponins, phenolic compounds, tannins, and terpenoids.

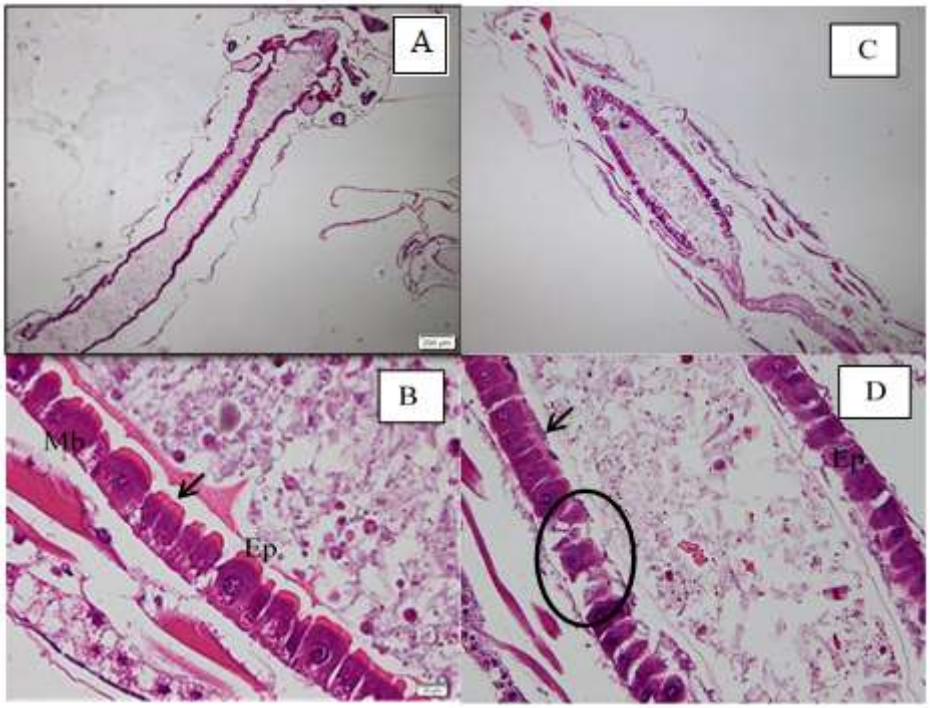

Figure 1. Normal histology midgut *Ae. aegypti* larvae 100x magnification (A) 200x (B). Histopathology of midgut *Ae. aegypti* larvae treatment with methanol extract, magnification 100x (C) 200x (D). Epithelium (Ep), Basal membrane (MB), and Brush border (arrow). Epithelial cell damage (circle).

D'Incao[8] et al. reported that saponins are found in a variety of plants. They are toxic to herbivorous arthropods and mosquitoes because they can alter eating habits, molting processes and lead to the death of insects. In a phytochemical test, contained saponin in this extract, thus making this fruit has the potential to be used as a natural insecticide.

Saponin (sapo = soap) is a glycoside form that is widespread in high-level plants, can cause death, decreased food intake, weight loss, growth disorders, and decreased reproduction in insect pests.[9] Nugroho[6] et al. reported that the Mahkota Dewa fruit extract influenced the development of *Ae. aegypti* larvae, as inhibitor development of instar larvae III *Ae. aegypti* to become an adult stage.

Lethal concentration 50 is the concentration that kills 50% of the total number of larvae. In this study, LC50 and LC90 values for 24 hours were obtained from the probit analysis. Larvae treated were given various concentrations, range 4-5 concentrations, repeated at least three times, mortality of larvae between 10% and 95% in 24 hours or 48 hours were used to determine the LC50 and LC906 values.[7] In this study, values of LC50 and LC90 24 hours are 0.554%, 1.291%. Lethal Time 50 is the time required to kill 50% of larvae at a certain concentration. LT50 was counted at a concentration of 0.30% for 48 h, and LT50 in this study was 17,082h. Chapagain[7] et al. reported saponins isolated from *Balanites aegyptiaca* showed 100% mortality of *Ae. aegypti* larvae at 500 ppm.

Saponins are known to have various biological properties. Namely, membrane-permeabilizing, hemolytic, antioxidant, anti-

inflammatory, immunostimulant, and anticancer affect feed intake, growth, and reproduction in animals and can be used as fungicides molluscicides, and pesticides.[10] Saponins can also cause death, decreased food intake, causing weight loss, growth disorders, decreased reproduction of insect pests, inhibition of sterol absorption, has an antifeedant effect, is a protease inhibitor, and may cause molting defect. However, until now, the mechanism of action of saponin has not been known with certainty. [9,10]

Midgut insects play an important role in the secretion of digestive enzymes and the absorption of nutrients. The anterior part is primarily for digestion and absorption of fats and xenobiotic detoxification.[11] In this study, the anterior midgut larvae treatment with extract of Mahkota Dewa fruit with methanol solvent, shows the cuboidal epithelial appearance still visible homogeneous and attached to the basement membrane with form the nucleus was round and bluish, but there are some damaged epithelial cells and apart from the basal membrane. The presence of epithelial damage to the anterior midgut of the larvae shows that the Mahkota Dewa fruit extract with methanol solvent interferes with the digestion and absorption of food along the digestive tract, and the presence of extracts in the midgut larval was disrupted the structure of the midgut. Epithelium of the insect's midgut was the target of saponins, the epithelial damage-causing starvation and causing insect mortality. [9,12]

CONCLUSION

The methanol extract of the Mahkota Dewa fruit has activity as a natural larvicide and causes damage to the epithelium of instar larvae III-IV *Ae. aegypti*.

REFERENCE

1. Gubler DJ. Vector-borne diseases. Rev Sci Tech.2009;28(2):583–8.
2. World Health Organization. Dengue: guidelines for diagnosis, treatment, prevention, and control. Spec Program Res Train Trop Dis. 2009;x, 147.
3. Depkes RI. Demam Berdarah Biasanya Mulai Meningkat di Januari. 2015;5–6.
4. Rozendaal JA. Vector control and communities. 1997.
5. Lay MM, Karsani SA, Mohajer S, Abd Malek SN. Phytochemical constituents, nutritional values, phenolics, flavonols, flavonoids, antioxidant and cytotoxicity studies on Phaleria macrocarpa (Scheff.) Boerl fruits. BMC Complement Altern Med. 2014;14(1):152.
6. Nugroho TF, Kesetyaningsih TW. Efektivitas Ekstrak Daun Phaleria macrocarpa (Scheff .) Boerl . sebagai Larvasida Aedes aegypti Effectiveness of Phaleria macrocarpa (Scheff .) Boerl . Leaf Extract as Aedes aegypti Larvacide. :118–26.
7. WHO. Guidelines for laboratory and field testing of mosquito larvicides. World Heal Organ. 2005;1–41.
8. D'Incao MP, Gosmann G, Machado V, Fiuza LM, Moreira GRP. Effect of Saponin Extracted from Passiflora alata Dryander (Passifloraceae) on development of the Spodoptera (J.E. Smith) (Lepidoptera, Noctuidae). Int J Plant Res. 2012;2(5):151–9.
9. De Geyter E, Lambert E, Geelen D, Smagghe G. Novel advances with plant saponins as natural insecticides to control pest insects. Pest Technol. 2007;1(2):96–105.
10. De Geyter E, Swevers L, Caccia S, Geelen D, Smagghe G. Saponins show high entomotoxicity by cell membrane permeation in Lepidoptera. Pest Manag Sci. 2012;68(8):1199–205.
11. Linser PJ, Smith KE, Seron TJ, Oviedo MN. Carbonic anhydrases and anion transport in mosquito midgut pH regulation. 2009;1662–71.
12. Wiesman Z, Chapagain BP. Laboratory Evaluation of Natural Saponin as a Bioactive Agent against. Dengue Bull. 2003;27.