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PENELITIAN |*RESEARCH*

Activity of *Ocimum sanctum* Leaf Extract against *Aedes aegypti* Larvae: Midgut Histopathological Alteration

Aktivitas Ekstrak Daun Ocimum sanctum *L. terhadap Larva* Aedes aegypti*: Perubahan Histopatologi Midgut*

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Abstrak. *Ekstrak tumbuhan dapat digunakan sebagai biolarvasida untuk membunuh larva Aedes aegypti, di antaranya adalah ekstrak daun* Ocimum sanctum*. Tujuan penelitian ini adalah menganalisis efek ekstrak metanol daun* O. sanctum *pada larva* Ae. aegypti *dan perubahan pada midgut-nya. Penelitian ini menggunakan desain eksperimental. Ekstrak daun* O. Sanctum *dibuat dengan metode evaporasi dan dibuat pada konsentrasi 0,1%, 0,25%, 0,5%, 0,75%, dan 1%. Percobaan diulang sebanyak 4 kali pada setiap konsentrasi. Pengamatan jumlah larva mati dilakukan setelah 24 jam perlakuan. Berdasarkan hasil pengamatan histopatologi, terdapat kerusakan pada jaringan epitel midgut larva* Ae. aegypti*. Nilai LC50 ekstrak daun* O. sanctum *sebesar 0,66%, sedangkan nilai LC90 yang diperoleh sebesar 1,38%. Hasil menunjukkan bahwa untuk membunuh larva* Ae. aegypti *hingga 90% dari jumlah larva, dibutuhkan konsentrasi ekstrak lebih dari 1%.*

Kata Kunci: Aedes aegypti, *LC50, LC90, ekstrak daun* Ocimum sanctum*, midgut*

Abstract. Plant extracts can be used as biolarvacide to kill *Aedes aegypti* larvae, one of which is *Ocimum sanctum* leaf extract. The aim of the study was to analyze the effect of *O. sanctum* leaf methanol extract on *Ae. aegypti* larvae and histopathological alteration of midgut. The study used an experimental design. *O. sanctum* leaf extract was made by evaporation methods at 0.1%, 0.25%, 0.5%, 0.75%, and 1% concentration. The experiment was repeated four times for each concentration. Observation of larvae mortality was done after 24 hours of treatment. The results of histopathological observation showed that there was the alteration in epithelial midgut *Ae. aegypti* larvae. The LC50 value of *O. sanctum* was 0.66%, while the LC90 value obtained was 1.38%. The results showed that the mortality of *Ae. aegypti* larvae up to 90% required more than 1% of extract concentration.

Keywords: *Aedes aegypti*, LC50, LC90, *Ocimum sanctum*, leaf extract, midgut

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INTRODUCTION

Dengue Hemorrhagic Fever (DHF) is still a public health problem in the world, with about half of the world population at risk of dengue infection.[1] In Indonesia, the number of DHF patients from year to year is increasing. DHF was caused by Dengue Virus (DENV) that was transmitted to humans by *Aedes aegypti* mosquitoes as the main vector and *Ae. albopictus* as secondary vector.[1]

The strategy to reduce incidents and transmission of DHF is by controlling vector. Plant extracts can be used as biolarvacide to kill *Ae. aegypti*, one of which is *Ocimum sanctum* leaf extract. Some research mentioned that *O. sanctum* leaf extract can kill *Ae. aegypti* larvae up to 90.4% at 2500 ppm concentration with LC50 1290.39 ppm and LC90 3173.53 ppm.[2] It was reported by Annes that the activity of *O. sanctum* extract showed a high larvacidal effect on *Ae. aegypti* with LC50 value at concentration 425.76 ppm.[3] It has been reported by Astriani and Widawati that the *O. sanctum* leaf has the potential as a biolarvacide against *Ae. aegypti*.[4]

The aim of the study was to analyze the larvicidal effect of *O. sanctum* leaf extract on *Ae. aegypti* larvae. In addition, histopathological alteration in the midgut *Ae. aegypti* larvae were observed after being tested with the extract.

MATERIAL AND METHODS

This research was an experimental design study, conducted at Parasitology Laboratory, Faculty of Medicine, Universitas Indonesia, from December 2017 to February 2018. The material tested in this study was methanol extract of *O. sanctum* leaf and *Ae. aegypti* larvae instar IV from the Parasitology Laboratory, Department of Parasitology, Faculty of Medicine, Universitas Indonesia. Equipments and materials used for this research include blenders, trays, glass jars, glass funnels, filter paper, spoons, glass bottles, rotary evaporators, analytical balance, plastic cups, glass objects, pipettes, aquadest, dimethyl sulfoxides, paraffin, microscope, hematoxylin, eosin, bousins, ethanol, toluene, and HCl.

The making of *O. sanctum* leaf extract was done at Faculty of Agricultural Technology, Institut Pertanian Bogor. The first stage of the process was to collect 1 kg of good quality *O. sanctum* leaves from the *O. sanctum* farmers in Tegal Regency (green leaf color, unbroken twigs, undamaged and young leaves). *O. sanctum* leaves were washed with water and then dried. After drying, the leaves were blended into powder then put into a sealed plastic and macerated with 70% methanol solvent for 72 hours. After that, prepared a glass bottle and above the mouth of the glass bottle was given a glass funnel with filter paper. Maserat was taken and filtered. The filter results were concentrated into a thick extract using a rotary evaporator.[5]

The qualitative phytochemical analysis of *O. sanctum* leaf extract was conducted at the Chemistry Laboratory, Faculty of Medicine, Universitas Indonesia. The saponin test was carried out by mixing 10 ml of extract with 10 ml of distilled water then shaking it in a tube for 15 minutes. A foam layer of 2 cm indicated the presence of saponins. The flavonoid test was carried out by mixing magnesium fragmented and HCl concentrated with crude extract. The presence of flavonoids was indicated by the appearance of pink which appears in the mixture of solutions. The alkaloid test was carried out by mixing 2 ml of 1% HCl with extract, then heated. The mixture was added by Mayer and Wagner reagent. The presence of alkaloids was characterized by the turbidity produced by the solution sediment. The triterpenoid test was carried out by mixing 2 ml of chloroform, concentrated H_2SO_4 with extract. The red color produced in the lower chloroform layer indicated the presence of triterpenoid. The essential oil test was carried out by taking 5 ml of extract solution and then evaporating to get residue. The typical odor produced by the residue shows the presence of essential oils. The tannin test was carried out by mixing 2 ml of 2% $FeCl_3$ solution with extract of *O. sanctum* leaves. The presence of tannin is shown in blue-green or black in the solution mixture.[6–7]

The activity of *O. sanctum* leaf extract against *Ae. aegypti* larvae were carried out using controls (aquadest) and five concentrations (0.1%, 0.25%, 0.5%, 0.75, 1%) which had previously been determined based on the results of the preliminary study. Each concentration was replicated four times. The extract solution was made by dissolving a thick extract (mg) in Dimethyl Sulfoxide into a liquid extract. After that, distilled water was added to the liquid extract up to 200 ml volume. Then pour it into a plastic cup. Each solution concentration was filled with 25 larvae of *Ae. aegypti* instar IV. The control group used aquadest plus 1 ml of Dimethyl Sulfoxide. Observed mortality of larvae for up to 24 hours.[8]

The making midgut histopathological preservation *Ae. aegypti* larvae were carried out at Histopathology Laboratory, Faculty of Veterinary Medicine, Institut Pertanian Bogor. The first step was to wash the larvae with the aquadest before it was fixed with the bouins solution. The next stage was the dehydration stage of ethanol and toluene. Then the larvae

were attached to paraffin, sliced and given hematoxylin and eosin. The final stage was examined under a microscope against the observed larvae.[9]

The analysis of LC50 and LC90 were using Probit analysis. The effect of *O. sanctum* leaf extract exposure on larval was analyzed using Chi-square with SPSS 20. Midgut histopathological alteration of *Ae. aegypti* larvae were observed descriptively before and after treatment on the mortality of *Ae. aegypti* larvae at LC50 concentrations.

RESULT

Based on Figure 1 the mortality of *Ae. aegypti* larvae showed that the higher the concentration, the higher the average mortality of *Ae. aegypti* larvae. In the treatment of *O. sanctum* leaf extract, the highest mortality of larval was 70 larvae (70%) occurred at a concentration of 0.75%, while the lowest mortality occurred at a concentration of 0.25% that was 13 larvae (13%). In observations carried out using a concentration of 0% as a negative control, the results of the mortality of *Ae. aegypti* larvae were not obtained. The 24 h activity of *O. sanctum* leaf extract against *Ae. aegypti* larvae were presented in Table 1.

The LC50 values of *O. sanctum* leaf extract appeared to be effective against *Ae. aegypti* larvae (LC50 0.66%; LC90 1.38%). It showed that at 0.66% concentration the tested larvae had 50% mortality out of the sample and 90% mortality of the sample at a concentration of 1.38%. Based on Chi-square analysis, the activity of *O. sanctum* leaf extract was also significant aganinst *Ae. aegypti* larvae ($p < 0,01$).

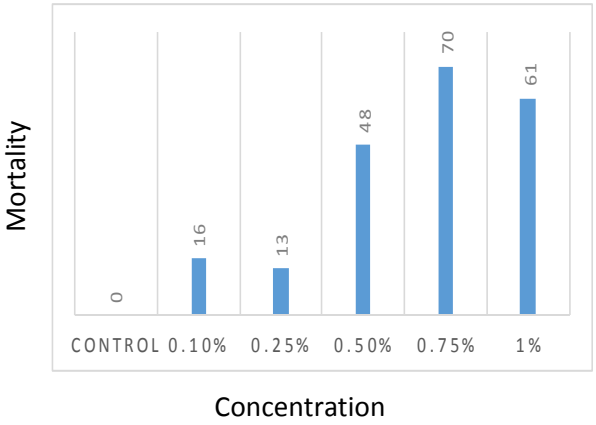

Figure 1. The Mortality of *Ae. aegypti* Larvae on Five Concentrations of *O. sanctum* Leaf Extract

Table 1. Probit Analysis of *O. sanctum* Leaf Extract against *Ae. aegypti* Larvae

Leaf Extract	LC50 (Lower – Upper Bound)	LC90 (Lower – Upper Bound)
O. sanctum	0.66% (0.50– 0.89)	1.38% (1.07–2.24)

Table 2. The Result of Qualitative Phytochemical Analysis of *O. sanctum* Leaf Extract

Phytochemical	Test Result	
	Indication	Positive/ Negative
Saponin	A foam layer	+
Flavonoid	Pink color in the solution mixture	+
Alkaloid	The turbidity produced by the solution sediment	+
Triterpenoid	The red color produced in the lower chloroform layer	+
Essential oil	The typical odor produced by the residue	+
Tannin	The blue-green or black color in the solution mixture	+

Phytochemical analysis of *O. sanctum* leaf extracts showed the precence of saponin, flavonoid, alkaloid, triterpenoid, essential oil, and tannin (Table 2).

Normal midgut showed that there was no destruction in the midgut epithelial cells of larvae (Figure 2). The effects of *O. sanctum* leaf extract towards the anterior and posterior midgut epithelial cells were examined and showed that there was destruction in the midgut epithelial cells of the treated larvae (Figure 3).

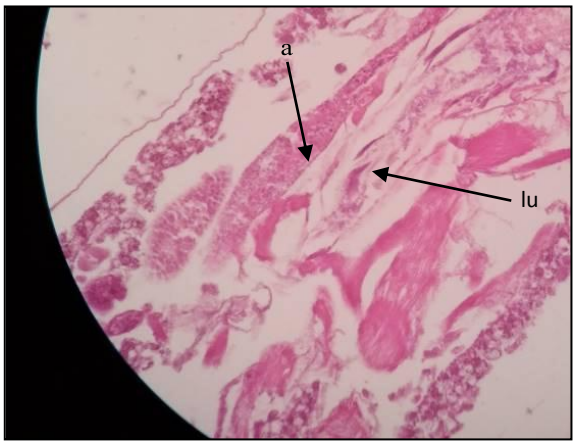

Figure 2. Longitudinal Section of Normal Midgut of *Ae. aegypti* Larvae Instar IV (10x).
a:Midgut Epithelial; lu:Midgut Lumen

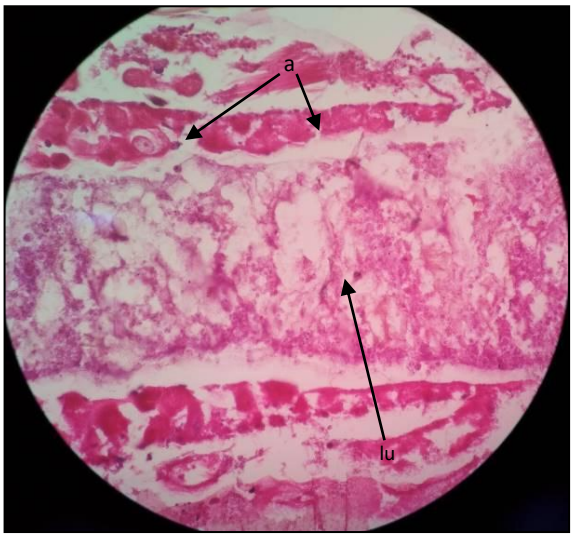

Figure 3. Longitudinal Section of Midgut of *Ae. aegypti* Larvae Instar IV Treated with *O. sanctum* Leaf Extract (LC50) After 24 h (10x). a: Alteration in the Midgut Epithelial; lu:Midgut Lumen

DISCUSSION

The plant extracts have been known as important bioinsecticides as an alternative to insect control.[10] Many previous research reports on the larvicidal activity of *O. sanctum* leaf extract which is comparable to this study. The essential oil of *O. basilicum* showed LC50 at 11.97 ppm for *Ae. albopictus* and LC90 at 21.17 ppm. The results revealed that the chemical compounds of *O. basilicum* are methyl eugenol (18.74%), limonene (1.34%), camphor (1.06%), bornyl acetate (0.51%), linalyl acetate (0.54%), cis-geraniol (0.86%), linalool (52.42%).[11] A report from Pandey *et al.*[12] that the chemical compounds of *O. sanctum* extract are eugenol (41.7%), limonene (3.8%), and E-caryophyllene (24.4%).

Table 1 showed that LC50 value is 0.66% and LC90 value is 1.38%. LC50 and LC90 values of *O. sanctum* leaf extract in this study were lower than the extract of *O. sanctum* leaf in the study of Husna *et al.*[13] The results of the study showed that the LC50 value of leaf extract of *O. sanctum* leaf against *Ae. aegypti* larvae were 0.97% and the LC90 value is 1.42%.[13] Other research has also been done using leaf extracts of *O. sanctum* was study by Kartika and Isti'anah[2] that showed *O. sanctum* leaf extract at a concentration of 2500 ppm can kill *Ae. aegypti* larvae up to 90.4% with LC50 values of 1290.39 ppm and LC90 3173.53 ppm. In addition, Anees[3] showed that the activity of *O. sanctum* leaf extract showed LC50 values at a concentration of 425.76 ppm and had a high larvacidal effect on *Ae. aegypti* larvae.

Low extract activity in this research, when compared to other research, is caused by bioactivity of plant extracts taken from different ecological and geographical conditions. This condition can affect the production of different carbon-based secondary metabolites even though they are still in the same species and genus.[14] Exposure to *O. sanctum* leaf extract showed an increase in mortality of *Ae. aegypti* larvae along with increasing concentration of extract. *O. sanctum* leaf extract has the ability to kill *Ae. aegypti* larvae at a higher concentration. According to Koraag *et al.*[15] that the lower the LC50 value of a plant extract, the more toxic the plant.

O. sanctum leaf extract does not only cause mortality in larvae but can cause alteration to the midgut of *Ae. aegypti* larvae. Alteration in the midgut of *Ae. aegypti* larvae can be found anteriorly and posteriorly of the midgut epithelial cells. These alterations are indicated by the destruction of the midgut larval epithelium. Midgut alteration is similar to previous researches which reported that *Culex quinquefasciatus* larvae treated with *O. basilicum* extracts had lost it swimming and foraging activities; failed to maintain the body in balance; and gut rupturing after 48 hours of continuous extracts exposure.[16] Yu *et al.*[14] added that the larvae treated with seaweed extracts had the cytopathological alteration of the midgut epithelium.

Midgut alterations in this research are different from other researches. This is due to the chemical compounds of plant extracts being tested giving different damage effects. The midgut alteration is thought to be due to saponin compounds contained in the leaf extract of *O. sanctum*. Saponin compounds can act as an insecticide with modes of action such as reduced food intake, indigestion, weight reduction, developmental retardation, a decrease in the rate of reproduction, and mortality.[17] The mode of action of Saponin looks at the properties of its molecules to interact with the structural cholesterol (membrane) or with metabolic cholesterol (food). Saponins have a wide spectrum of action, due to their toxicity to various insect.[18] According to Singh and Chaudhuri,[19] the composition of *O. sanctum* extract is complex which comprises 60 active chemical compounds composed of phenolics, flavonoids, phenylpropanoids, terpenoids, fatty acid derivatives, essential oil, fixed oil, and steroids. The *O. sanctum* extract also has activity as a mosquitocidal.

Desai *et al.*[20] stated that a large number of the biological effects of saponins has been associated to their action on the permeability of cell membranes. They have a specific ability to form pores in membranes. The hemolytic action of

saponins are believed to be the result of the affinity of the aglycone moiety for the phospholipids present in the cell membrane with which they form insoluble complexes. The amount of glycosides required for permeabilization is much lower for cholesterol-rich lipid layers than cholesterol-free membranes.[20] Procopio *et al*.[21] stated that *Schinus terebinthifolius* leaf extract (1.0%) promoted intense disorganization of larval midgut epithelium, including deformation and hypertrophy of cells, disruption of microvilli and vacuolization of cytoplasms, affecting digestive, enteroendocrine, regenerative, and proliferating cells.

In the larval stage of *Ae. aegypti*, in the midgut posterior region slightly wider than the anterior with composed by a tube. The midgut part is capable of secretion, synthesis, absorption, and transportation.[22] The midgut cells are actively involved in the production and secretion of digestive enzymes and the absorption of nutrients. Most nutrients in the gut lumen are absorbed through columnar cells.[23–24]

Development of bioinsecticides to control mosquitoes and other pests can be examined from Flora of Indonesia. It becomes potential because Flora from Indonesia has a rich plant diversity. Further research is needed to search for more selective larvacidal compounds, analysis of larvacides action mode and assessment of effects on non-target organisms.

CONCLUSION

In conclusion, our study revealed that *O. scantum* leaf extract had activity as larvicide. Treated *Ae. aegypti* larvae exhibited an alteration in the midgut epithelial cells.

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AUTHORS CONTRIBUTION

Nurhadi Eko Firmansyah is the main contributor in this article. All authors listed in this article have contributed according to their expertise showed in the following roles:

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Project Administration	:	Rizal Subahar
Resources	:	Rizal Subahar Nurhadi Eko Firmansyah
Supervision	:	All authors
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Writing-Original Draft Preparation	:	Nurhadi Eko Firmansyah Rizal Subahar
Writing-Review & Editing	:	Nurhadi Eko Firmansyah Rizal Subahar Heri Wibowo

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