

Oviposition Deterrent Activity and Ovicidal Effect of *Pometia pinnata* Leaves Extract against Dengue Vector, *Aedes aegypti* (Diftera: *Culicidae*)

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Abstract. *Aedes aegypti* is the main vector of dengue fever that most commonly occurred in Indonesia. The application of botanical insecticides is being developed as an alternative vector control. Botanical components have been reported as the potential alternative to inhibit the process of oviposition and fecundity suppression. This study aims to determine the oviposition deterrent activity and ovicidal effects of ethanol extract of *Pometia pinnata* leaves against *Aedes aegypti*. Oviposition deterrent assay was conducted on twenty blood-fed females in a cage (40 cm x 40 cm x 40 cm). One treated oviposition trap and one control oviposition trap were placed in the opposite corner of the cage. Four replicates were performed for each concentration. The eggs were counted and analyzed after 72 hours of trial. Ethanol extract of *Pometia pinnata* leaves have anti-oviposition effect on female mosquitoes *Aedes aegypti* at 400 ppm (effective repellency / ER = 56.38%; oviposition active index/OAI = -0.39) and 500 ppm (ER = 66.4%; OAI = -0.49). In the ovicidal assay, twenty-five eggs are used for determination ovicidal effect and five replicates were performed for each concentration. The hatched larvae were collected and counted daily. The ethanolic extract of *Pometia pinnata* leaves at 0.05%, 0.1%, 0.15%, 0.2%, and 0.25% concentration have ovicidal activity of 11%, 28%, 87%, 94%, and 98% against *Aedes aegypti* eggs, respectively. This study revealed that the ethanol extract of *Pometia pinnata* leaves could be a potential alternative in controlling the dengue vector.

Keywords: oviposition, ovicidal, dengue, *Aedes aegypti*, *Pometia pinnata*.

1 Introduction

Aedes aegypti is the primary vector of dengue fever in tropical countries, such as Indonesia. Dengue hemorrhagic fever remains one of the most important diseases worldwide with 500.000 cases occurred and caused 22.000 people deaths. One of the strategies to reduce the number of dengue vectors is by inhibiting its life cycle [1]. The use of chemical insecticides at mosquitos breeding had been proven to reduce the mosquito populations, but leading to adverse environmental and non-target organism effects and cause resistance [2]. The outspread and evolution of resistance of insecticide is a big problem for the dengue vector controls [3]. The awareness that the use of chemical insecticides has negative impacts encourages research to look out for environment-friendly innovative strategies to target mosquitoes [2,4]. Phytochemicals from plant extract have been reported potential as alternative methods against vector mosquitoes due to their ability to control mosquitoes in various ways such as to cause growth inhibition, ovicidal activity, oviposition deterrence, and fecundity inhibition.

Pometia pinnata is a native plant in Indonesia and commonly called "Matoa" plants. *Pometia pinnata* leaves extracted with ethanol, N-hexane, and ethyl acetate

contain several metabolites such as alkaloid, flavonoid, terpenoid, tannin, saponin, and coumarin [5]. The purpose of this study was to assess the oviposition deterrent and ovicidal activities of *Pometia pinnata* leaves extract against *Aedes aegypti* mosquitoes.

2 Materials and Methods

2.1 Collection of plant material

The matured of *Pometia pinnata* leaves were collected from surrounding area in Universitas Islam Indonesia, Sleman City, Indonesia. The healthy leaves were washed with tap water and dried using cabinet drying. The dried leaves were crushed and using a miller to get a fine powder.

2.2 Preparation of leaves extracts

Dried *Pometia pinnata* powder was extracted using ethanol 96% and allowed to soak for 5 days at room temperature. The supernatant was filtered. A rotary evaporator was used to separate the solvent from its extract and the green residue was obtained. The stock solution was used to prepare the various concentration of the extract.

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2.3 Mosquito rearing

The mosquito and eggs used were obtained from the colony maintained at the Parasitology Laboratory of Medical Faculty, Universitas Islam Indonesia. The mosquito eggs were collected and used to assess ovicidal activity. The blood-fed female mosquitoes were collected and used to assess oviposition deterrent activity.

2.4 Oviposition deterrent assay

For the oviposition deterrent assay, ethanol extract of *Pometia pinnata* was prepared at concentrations of 100 ppm, 200 ppm, 300 ppm, 400 ppm and 500 ppm based on a preliminary test. Five cages (40cm x 40cm x 40cm) were also prepared and twenty blood-fed female of *Ae.aegypti* was transferred into each cage. One treated oviposition trap and one control oviposition trap were placed in the opposite corner of each cage. Four replicates were performed for each concentration. The eggs were counted and analyzed after 3 days of trial [1]. The identification of eggs was carried out using a light microscope with a magnification of 10x. The average numbers of eggs were calculated using a standard formula to assess Effective Repellency (ER) and Oviposition Activity Index (OAI) as follows

$$ER (\%) = \frac{NC - NT \times 100}{NC}$$

$$OAI = \frac{NT - NC}{NT + NC}$$

Where NC is the number of eggs in the control trap and NT is the number of eggs in the treated trap.

The oviposition activity index with a result of +3 and above is considered as an attractant, while a result of -0,3 and below is considered as an anti-oviposition [6,7].

2.5 Ovicidal assay

The various concentrations of *Pometia pinnata* extracts were prepared using DMSO as the solvent. Twenty-five eggs of *Ae. aegypti* were soaked in five different concentrations (0.05%, 0.1%, 0.15%, 0.2%, and 0.25%). The distilled water was used as a negative control for comparison. Percentage of ovicidal effects after being treated with the ethanol extract of *Pometia pinnata* was calculated with the following formula:

$$\frac{A}{B} \times 100\%$$

where A is the number of eggs did not hatch during the treatment and B is the total of eggs before the treatment

3 Results and Discussion

In oviposition deterrent assay, all of the female *Ae. aegypti* were put their eggs in each oviposition trap either containing *Pometia pinnata* leaves extract or containing only distilled water. The average numbers of mosquito eggs in the treated oviposition trap were less than the control oviposition trap (Table 1). This result showed that there was a disruption in the process of laying mosquito eggs in contact with *Pometia pinnata* leaves extracts. The oviposition is an important event in the life cycle of a mosquito. If this stage is inhibited, it will disrupt the life cycle of mosquitoes and will decrease the mosquito population [1,8].

Table 1. The oviposition deterrent activity of ethanol extract of *Pometia pinnata* leaves against *Ae. Aegypti* female mosquitoes.

Concentration (ppm)	Number of Eggs		% ER	OAI
	Treatment	Control		
100 ppm	73±19,6	93,2±21,9	21,71	-0,12
200 ppm	56,7±23,4	78±5,7	27,24	-0,15
300 ppm	47±11,1	72,2±20,7	34,94	-0,21
400 ppm	37,5±9,3	80,2±30,9	56,38	-0,39
500 ppm	25,2±8,5	75,2±24,2	66,4	-049

Data are mean ± standard error (SE) of four replicates. ppm = parts per millions. Different means result with control are tested by Independent samples T-test (p <0.05, level of significance).

The lowest ER values at 100 ppm concentration was 21.71% and the highest at 500 ppm concentration was 66.4%. There is no statistically difference (p>0.05) in the number of eggs in treated oviposition trap with control. In another study showed that *Vitex negundo L.* extracts against *Ae.aegypti*, *Anopheles stephensi* and *Culex*

quinquefasciatus obtained significant results with concentrations of 0.1%, 0.075%, 0.05%, 0.025% and 0.01% [9]. Ethanol extract of *Pometia pinnata* leaves have an anti-oviposition effect on female mosquitoes *Aedes aegypti* at 400 ppm (effective repellence/ER = 56.38%; oviposition active index/OAI = -0.39) and 500 ppm (ER

= 66.4%; OAI = -0.49). Previously, some studies reported effect of plant extracts to reduce the number of mosquitoes laying eggs. Rajkumar and Jebanesan [8] reported that ethanol extract from *Cassia obtusifolia* against *An. Stephensi* mosquitoes at concentration of 400 mg/L showed an %ER of 92.5%. Soonwera and Phasomkusolsil [10] also conducted a study using

Zanthoxylum limonella oil extract against *Ae. aegypti* and *Cx. quinquefasciatus* have negative OAI from -0.89 to -1.00. in another study, the hexane extract of *L. acidissima* recorded 100% oviposition deterrent activity against and *Cx. Quinquefasciatus* mosquitoes [11].

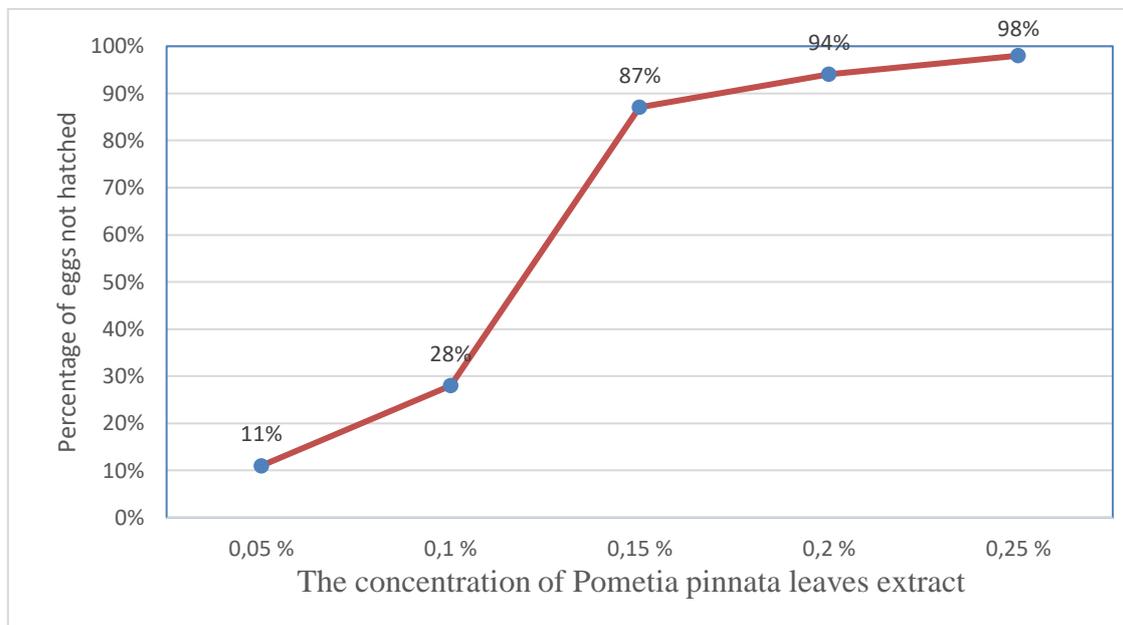


Figure 1. Percent ovicidal activity of *Pomertia pinnata* leaves extracts against *Aedes aegypti* eggs.

In this study, the ethanol extract of *Pomertia pinnata* showed the highest ovicidal effects of 98% at 0.25% concentration against *Ae. aegypti*. When expose to the higher concentration of *Pomertia pinnata* leaves extract, non-hatching eggs showed increase (Figure 1). Another investigator also conducted a study using plants extract to assess ovicidal activities. The crude extract of *C. copticum* showed zero hatchability (100% mortality) at 150 µm/ml for *Culex pipiens* [12]. Wariko and Kumar [2] reported that the petroleum ether and hexane root extract causing only 4.9% and 8.9% hatch respectively at highest concentration against *Ae. aegypti*.

4 Conclusion

In the present study, *Pomertia pinnata* leaves extract show potential effects as ovicidal and anti-oviposition against *Aedes aegypti* mosquitoes. Based on these results, the ethanol extract of *Pomertia pinnata* leaves could be a potential alternative for use in dengue vector control. The phytochemical compound of this extract should be investigated in the future.

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