LARVACIDAL ACTIVITY OF ETHANOL EXTRACT OF SUGAR APPLE (*ANNONA SQUAMOSA*) SEEDS AGAINST *AEDES AEGYPTI*

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ABSTRAK

Tujuan dari penelitian ini adalah untuk menentukan nilai LC_{50} *(lethal concentration)* dari ekstrak etanol biji srikaya (*Annona squamosa*) terhadap larva *Aedes aegypti*. Sampel biji *Annona squamosa* dikumpulkan dari Desa Pringgabaya, Lombok dan Desa Ungasan, Bali. Biji *Annona squamosa* yang sudah kering kemudian digerus dan dimaserasi sebanyak dua kali dengan etanol 95% selama 3 hari. Filtrat yang diperoleh dipekatkan dengan *rotary evaporator* vakum. Ekstrak biji *Annona squamosa* yang diperoleh adalah sebesar 16,20% untuk srikaya Bali dan 14,06% untuk srikaya Lombok. Ekstrak ini selanjutnya diuji aktivitasnya terhadap larva *Aedes aegypti* instar III. Pengamatan hasil uji larvasida dilakukan setelah 24 jam dengan menghitung jumlah kematian larva pada masing-masing kelompok perlakuan. Berdasarkan analisis probit, nilai LC_{50} ekstrak etanol biji *Annona squamosa* dari Lombok adalah 25,37 ppm sedangkan LC_{50} ekstrak biji Srikaya dari Bali adalah 28,64 ppm. Hasil ini menunjukkan bahwa biji *Annona squamosa* memiliki efek toksik terhadap larva *Aedes aegypti* dan dapat digunakan sebagai larvasida.

Kata-kata kunci: aktivitas larvasida, *Annona squamosa*, *Aedes aegypti*, LC_{50}

ABSTRACT

This study aims to determine the LC_{50} (lethal concentration) of sugar apple (Annona squamosa) seeds extract against Aedes aegypti larvae. The seeds were collected in Pringgabaya village, Lombok and Ungasan village, Bali. The air dried seeds was powdered and then soaked in 95% ethanol for 3 days and the residue was further extracted once. The combined filtrate was concentrated by using vacuum rotary evaporator, giving a yield of 16.20% for seeds collected in Bali and 14.06% for those collected in Lombok. The crude extracts were taken for larval bioassay against third instar Aedes aegypti larvae. The larvacidal activity was observed after 24 hours exposure by calculating the mortality of larvae in each concentration. Based on probit analysis, the LC_{50} of ethanolic seeds extract of Annona squamosa collected in Lombok and that in Bali are 25.37 ppm and 28.64 ppm respectively. The result showed that Annona squamosa seeds extract is toxic toward Aedes aegypti larvae, indicating a potential for developing the extract as a novel larvacide.

Keywords: *Larvacidal activity, Annona squamosa, Aedes aegypti, LC_{50}*

INTRODUCTION

Aedes aegypti plays a prodiminat role for the transmission of dengue fever which has been endemic in Indonesia since 1968 [1]. *Abate* (temephos) and other synthetic insecticides are frequently used to control the population of this mosquito. The overuse the synthetic insecticides raises multifarious problems, such as environmental pollution, the resistance and cross-resistance of *Aedes aegypti* as well as the possible toxicity hazards. This problem leads to the interest in the discovery of environmental friendly and safe natural product to control the vector of dengue fever.

Annonaceae family has drawn a lot of attention since the 1980's, due to the presence of acetogenins, of which their structural characteristics feature a variety of biological activities, where the insecticidal activity stands out [2]. Sugar apple *(Annona squamosa)* is one species of the family also contain acetogenin which is toxic towards mosquito.

Annona squamosa was reported to have larvacidal action against larvae and pupae of *Culex quinquefasciatus* [3]. Further, hexane leaves extract of *Annona squamosa* showed LC_{50} 145.39 ppm against of mosquitoe larvae [4]. *Annona squamosa* exhibits larvacidal and growth regulating activities against *Anopheles stephensi* and other mosquitoes [5].

Annona squamosa is sources of fresh fruit and/or fruit jam and could generate tons of waste seeds. These waste products might potentially be developed into simple, locally available plant-based larvacides for *Aedes aegypti*. However, there is no particular research to evaluate larvacidal activity of *Annona squamosa* seeds ethanol extract against *Aedes aegypti*. This study aims to determine the LC_{50} (lethal concentration) of sugar apple (*Annona squamosa*) seeds extract against *Aedes aegypti* larvae.

METHOD

Material

The seeds of *Annona squamosa* were collected in different locations, Ungasan, Bali and Pringgabaya, Lombok. The seeds were air dried for two weeks. They were peeled to obtain the white inner part of the seeds. Then, they were grinded by using blender.

Extraction

The mashed seeds (100 gram) were soaked in 95% ethanol (1000 ml) for 3 days and filtered through filter paper. The residue was further extracted once. The combined filtrate was concentrated using vacuum rotary evaporator at 40^{o}C with pressure at 175 mbar (ethanol) and 75 mbar (water). All evaporation processes in this research were conducted in Forensic Laboratory POLRI Denpasar.

Bioassay *Aedes aegypti*

The Larval bioassay was performed by following the standard method of WHO [6]. The stock solution (1000 ppm) was prepared by dissolving 250 mg crude extractt in 1 mL ethanol and making it to 250 mL by mixing distilled water in the volumetric flask. Then it was diluted into desirable concentration for the test (10 ppm, 20 ppm, 30 ppm, 40 ppm, 60 ppm, 80 ppm, 100 ppm). Control (0 ppm) was treated with distilled water only and run simultaneously. Batches of 25 third and fourth instar larvae were transferred by means of droppers to small disposable test cups containing 100 mL of control and test solutions respectively. After 24 hours exposure, larval mortality was recorded. The average mortality data were subjected to Probit analysis for calculating LC_{50}.

RESULT AND DISCUSSION

Yield percentage of the extract is shown in Table 1. After the extracts were obtained, they taken for larval bioassay. Table 2 shows the mortality cause by each extract.

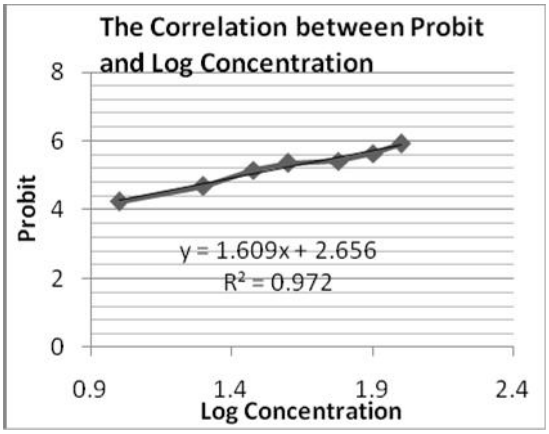
Table 1. Yield Percentage

Origin	Mass of extract (gram)	Percentage
Bali	16.20	16.20
Lombok	14.06	14.06

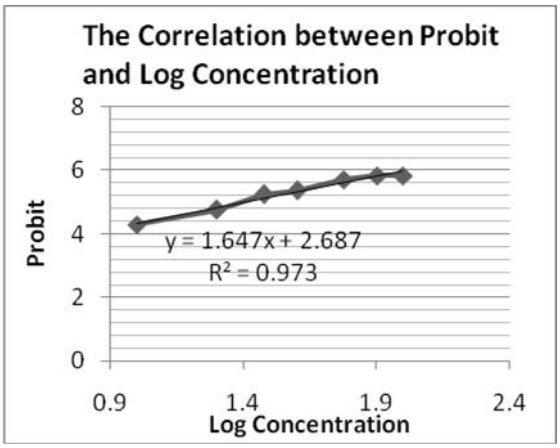
Table 2. *Aedes aegypti* Larvae Mortality

Concentration (ppm)	% Mortality	
	Bali	Lombok
0	0	0
10	22	24
20	38	40
30	56	60
40	64	64
60	66	76
80	74	80
100	82	80

Both ethanol extract of seeds from bali and Lombok show toxic effect toward *Aedes aegypti* larvae after 24 hours exposure in which larvae were found restless. The restlessness behavioral showed by the larvae slowed down their movement and failed to reach the water surface. Figure 1 shows correlation between probit and log concentration of which LC_{50} was determined.



(a)



(b)

Figure 1. Correlation between Probit and Log Concentration of ekstrak biji *Annona squamosa* Bali (a) dan Lombok (b)

Based on probit analysis, the LC_{50} of ethanolic seeds extract of *Annona squamosa* collected in Lombok and that in Bali are 25.37 ppm and 28.64 ppm respectively. The result reveals that Annona squamosa seeds from Bali exhibit higher LC_{50} (lower mortality) than Lombok. Moreover, Bali produce 16.20% seeds extract, higher than Lombok which produce 14.06% seeds extract. This is due to geographic variation among the extracts of *Annona squamosa*. Further, differences in sensitivity of test species used could account for these differences [7]. However, specific environmental data from which any inferences regarding their effects on patterns of toxicity could be drawn is not collected in this study.

Bioactive component which is responsible for larvacidal activity of the *Annona squamosa* seed is acetogenin. There are 24 prominent acetogenin isolated in the seeds [8]. Acetogenin has potent larvacidal activity. It is characterized by a long aliphatic chain bearing a terminal methyl-substituted α,β-unsaturated γ-lactone ring (sometimes rearranged to a ketolactone) with one, two or three tetrahydrofuran (THF) ring located a long the hydrocarbon chain and a number of oxygenated moieties (hydroxyls, acetoxyls, ketones, epoxides) and/or double bonds being present. Larvacidal and cytotoxic potential of prominent acetogenin, squamocin isolated from seed of *Annona squamosa* on the midgut of *Aedes aegypti*. poses LC50 at 6.4 ppm [9].

The structure clearly indicates that the compound is somewhat polar. Therefore, polar solvent, like ethanol is needed to extract the compound. So, the result reveals that ethanol extract of *Annona squamosa* seeds pose toxic effect against *Aedes aegypti*. Further, bioactive components from *Annona squamosa* seeds extract do not produce toxic residue to environment [10].

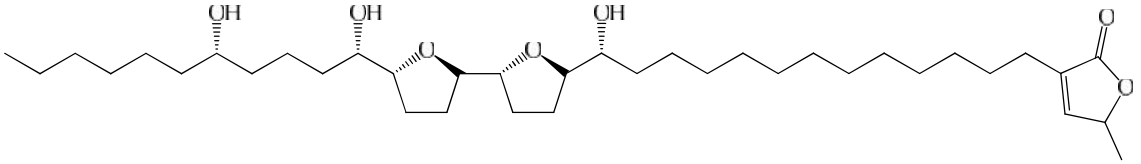


Figure 2. Structure of Squamocin

CONCLUSION

Based on probit analysis, the LC_{50} of ethanolic seeds extract of *Annona squamosa* collected in Lombok and that in Bali are 25.37 ppm and 28.64 ppm respectively. The result showed that *Annona squamosa* seeds extract is toxic toward *Aedes aegypti* larvae, indicating a potential for developing the extract as a novel larvacide.

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