

The Effect of *Syzygium polyanthum* Wight Ethanolic Leaf Extract on *Aedes* spp Instar III-IV Larvae

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Abstract: *Aedes* spp mosquitoes are the vectors that most cause diseases. The rise of insecticide resistance is related to the increasing of vectorial capacity. The plant can be used as an alternative source for controlling these vectors, one of which is *Syzygium polyanthum* (Wight). The present study was conducted to investigate the larvicidal properties of *S. polyanthum* leaf on *Aedes* spp instar III-IV. Ethanolic extract of *S. polyanthum* (EESP) was prepared by maceration using ethanol 70%. Larvae were divided into 7 groups (n=25, respectively). Group I to V consisted of different concentrations of EESP (100 (CI);150 (CII);200 (CIII);250 (CIV);300ppm (CV)); VI: Water (W) and VII : Temephos 1% (T). The larvicidal activity was evaluated by calculating the dead larva at 180, 360, 1440 and 2880 minutes to obtain Lethal Concentration 50% (LC50) and Lethal Time 50% (LT50) using Statistical Product and Service Solution (SPSS). The result showed that no larva found dead in W-treated group. Otherwise, the mortality of larva was increased with increasing of EESP concentration (CI to CV). LC50 and LT50 showed 213 ppm and 2410 minutes, respectively. We conclude that EESP has larvicidal activity on *Aedes* spp instar III-IV.

1 INTRODUCTION

Dengue fever is the most important mosquito-borne viral disease of humans (Walker, 2011) which *Aedes* spp mosquitoes act as the main vectors (Owino, 2018). Annually, it is estimated 50-100 million cases with fatality rates between 0.5 and 3.5% in Asian countries (Guzman and Kouri, 2002; Halstead, 2007; Suaya et al, 2009; Walker et al, 2011).

The chemical insecticides for controlling the cycle of the mosquito are known to play a role in increasing mosquitoes resistance (Adrianto, 2018). Therefore, an alternative source with larvicidal properties obtained from the plant could lead to the invention of new agents for vector control (Kamaraj, 2008). Plants that contained an alkaloid, saponin, eugenol, flavonoids, and tannin were reported able to kill *Aedes aegypti* larvae (Laurence et al, 2005; Ardianto, 2008).

Syzygium polyanthum (*S. polyanthum*), a family of *Myrtaceae*, is widely used in Indonesia cuisines (Widyawati, et al, 2015). The potency of this plant as larvacide has been reported by Dwiyanti et al, 2017. Their study showed that water extract of *S. polyanthum* had a killing power against *Aedes* sp larvae.

The bioactivity of plants extracts was related to their active compounds. Thus, the solvents that used to provide plant extracts also will affect the yield of its chemical compounds. The presents study was done to investigate the larvicidal activity of ethanolic extract of *S. polyanthum* (EESP) leaf on *Aedes* spp instar III-IV larvae.

2 MATERIALS AND METHOD

The study was conducted on August-December 2018 at Pharmacology and Therapeutic Departement, Medical Faculty, Universitas Sumatera Utara, Medan, Indonesia.

2.1 Extract Preparation

S. polyanthum leaves were obtained from Titi Kuning, Medan, North Sumatera, Indonesia. The fresh leaves were washed in running water and were dried in the temperature room. The dried leaves or simplicia that had been ground were extracted by

maceration using ethanol 70% to obtain ethanol extract of *Syzygium polyanthum* (EESP).

2.2 *Aedes spp* larvae

Aedes spp were obtained from Loka Litbang Kesehatan Pangandaran Ciamis, West Java, Indonesia (DP.02.01/1/831/2018).

2.3 Experimental Procedure

The larvae were divided into 7 groups (n=25) in 200 ml water (WHO, 2005) with 4 replications of each as follows: CI (EESP 100 ppm + Larvae); CII (EESP 150 ppm + Larvae); CIII (EESP 200 ppm + Larvae); CIV (EESP 250 ppm + Larvae); CV (EESP 300 ppm + Larvae); T (Temephos 1% + Larvae) served as positive control; W (Water + Larvae). Only the active *Aedes spp* larvae instar III-IV were included in the study.

2.4 Data Analysis

Data were analyzed by Kruskal-Wallis and expressed as mean \pm SD. LC50 and LT50 were calculated using probit analysis.

3 RESULTS AND DISCUSSION

Table 1 shows the effect of different concentrations of EESP on *Aedes spp* larvae. The results showed that all larva were dead in T-treated group from the first observation time at 180 min which continued until 2880 min. In EESP-treated groups, the dead larvae were found at 1440 min and 2880 min observations ($p < 0.05$). The number of dead larvae was increased in line with the higher of EESP concentration. At 1440 min observation as follows CI(2.8 \pm 1.7);CII(4 \pm 1.8);CIII(4 \pm 1.8); CIV(15.8 \pm 1.5), and CV(17 \pm 2.4), thus at 2880 min: CI(5 \pm 1.4); CII(8 \pm 1.4); CIII(13 \pm 1.4); CIV(19 \pm 1.4), and CV(21.3 \pm 0.9). Contrarily, in W-treated group, no larval mortality was found. These results revealed the larvicidal activity of EESP after 24 hours of exposure.

Table 1: Effect of ethanolic extract of *Syzygium polyanthum* (Wight) (EESP) leaf on *Aedes spp* larvae instar III-IV mortality.

Group	Number of larvae mortality by time (mean \pm SD)			
	180'	360'	1440'	2880'
CI	0	0	2.8 \pm 1.7	5 \pm 1.4
CII	0	0	4 \pm 1.8	8 \pm 1.4
CIII	0	0	4 \pm 1.8	13 \pm 1.4
CIV	0	0	15.8 \pm 1.5	19 \pm 1.4
CV	0	0	17 \pm 2.4	21.3 \pm 0.9
T	25 \pm 0	25 \pm 0	25 \pm 0	25 \pm 0
W	0	0	0	0

Figure 1 shows that the percentage of *Aedes spp* larvae mortality increased with increasing of EESP concentration ie CI (20%), CII (32%), CIII(52%), CIV(76%) and CV (85%).

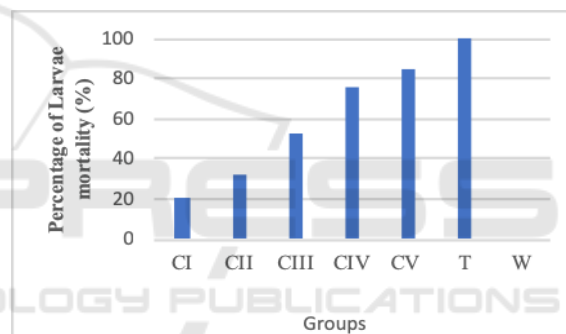


Figure 1: Percentage of *Aedes spp* larvae mortality after 48 hrs treatment.

The present study showed that EESP with a concentration of 213 ppm and a time of 2410 minutes able to cause 50% mortality in the larvae of *Aedes spp* mosquitos (Table 2).

Table 2: LC50 and LT50 of ethanolic extract of *Syzygium polyanthum* (Wight) (EESP) leaf on *Aedes spp* larvae instar III-IV.

Sample	LC50 (ppm)	LT50 (minutes)
EESP	213	2410

The present study used the third and fourth stage of *Aedes* mosquito larvae based on WHO standards. At these stages, the instar not only more resistant to physical and mechanical factors such as displacement, limited space for living in water but also having enough time to turn into an adult mosquito (WHO, 2005; Adrianto, 2008; Tennyson et al. 2013).

Based on resistance and safety issues of chemical products, the efforts were turned to discover a new compound of natural products derived from plantain mosquito's control. New botanical natural products are believed to have following properties ie. effective, environment-friendly, easily biodegradable, inexpensive, and readily available in many areas of the world, no ill effect on non-target organisms and have novel modes of action. More than 2000 plant species known to have bioactivities as an insecticide (Sukumar et al. 1991; Su and Mulla, 1999; Sanei-Dehkordi et al. 2016).

S. polyanthum (Wight) leaf contained an alkaloid, attire oil, flavonoid, steroid, triterpenoid, and saponin (Mangoting et al, 2005; Widyawati, 2015). These phytochemical compounds were potentially toxic to insect. Alkaloids were reported can affect protein kinases which play a role in signal transduction, cell and tissue development, and acetylcholinesterase inhibition. These compounds may damage the midgut and gastric caecum of larvae so that the larvae die (Ojha et al, 2013; Ni'mah et al, 2015; Velue et al, 2015). Temephos could inhibit the cholinesterase enzyme, leading to impaired nerve activities due to the accumulation of acetylcholine (Yulidar, 2014). Thus, alkaloids, attire oil, saponin, and flavonoids were also reported may damage the nervous and respiratory system of larvae.

LC50 of EESP in the present study showed much lower than methanol extract of *S. polyanthum* (213 vs 6576.68 ppm) as reported by Tinneke and Puput, 2015, while Dwijanti, 2017 showed that water extract of *S. polyanthum* showed larvicidal activity at dose 2.5- 55%. The solvent used for the extraction process of those different extracts affects the content of active compounds that can kill larvae.

4 CONCLUSIONS

The present study showed that *Syzygium polyanthum* (Wight) leaf ethanolic extract have larvicidal activity on *Aedes* spp instar III-IV.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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