

# Bukti Kinerja-Naskah Artikel- ejobios

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## Bio-larvicidal effectiveness of *Jasminum sambac* (L.) ait (Oleaceae) and *Stenochlaena palustris* (Blechnaceae) against dengue vector, *Aedes aegypti* (Linn.) (Diptera: Culicidae)

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### Abstract

**Objectives:** This study aim to evaluate the larvicidal effectiveness of *Jasminum sambac* and *Stenochlaena palustris* extracts against *Aedes aegypti*.

**Materials and Methods:** This study use true-experimental design in vitro, a post-test approach only with control group design. The samples were *Ae. aegypti* third/fourth larval instar which selected using simple random sampling and divided into 5 groups (1 control group and 4 treatment groups with concentrations of extract 0.5%, 0.6%, 0.7%, and 0.8%) for each extract. The total sample was a thousand larvae. The number of each group was 25 larvae with 4 times replication. The observation time on 10, 30, 60, 120, 240, 480, and 1440 minute. The LC<sub>50</sub>, LC<sub>90</sub>, LT<sub>50</sub>, and LT<sub>90</sub> values were calculated. The statistical analysis used a probit regression test using MINITAB 14 and combining with PHYTON software.

**Results:** The LC<sub>50</sub> of *J. sambac* and *S. palustris* extracts were under 1% based on each exposure time for each group cases but for LC<sub>90</sub> there was above 1% for concentration 0.5% (*J. sambac*), 0.5% and 0.6% for *S. palustris*. The LT<sub>50</sub> of both extracts were under 1440 minutes except for LT<sub>90</sub> of *S. palustris* which was above it.

**Conclusions:** *J. sambac* and *S. palustris* extracts at 0.5%, 0.6%, 0.7%, and 0.8% showed effective as larvicide against *Ae. aegypti* with effective time starting on 120<sup>th</sup> minute (*J. sambac*) and 60<sup>th</sup> minute (*S. palustris*).

**Keywords:** *Aedes aegypti*, extract, *J. sambac*, larvicide, *S. palustris*

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### INTRODUCTION

Dengue hemorrhagic fever (DHF) is a threatening disease in the midst of the corona virus pandemic in Indonesia (Health Ministry of Indonesia, 2020). Martapura Sub-District is an area with the highest incidence of DHF in Banjar District, South Kalimantan Province (Banjar District Health Office, 2019). The precaution so far is sprinkling temephos larvicide powder in water reservoirs (Desniawati, 2014). However, there has been temephos resistance in several countries such as Colombia (Grisales, 2013), Brazil (Chediak et al, 2016), Cuba (Bisset et al, 2016), and Indonesia (Istiana et al, 2012). If the water containing temephos is consumed for a certain time, it could occurrence of DNA damage in lymphocytes (Benitez-Trinidad et al, 2015). This requires the need for alternative larvicides that are environmentally friendly,

safe, and easily accessible. Local commodity plants in Martapura Sub-District are *Jasminum sambac* (L.) ait (*J. sambac*) and *Stenochlaena palustris* (*S. palustris*). Based on this, it is necessary to prove the effectiveness of *J. sambac* and *S. palustris* extracts as larvicides.

### MATERIALS AND METHODS

#### Study Site

Larval bioassay was carried out at the Entomology Laboratory of the Indonesian Institute for Research and Development of Health in the Kalimantan Regional Work Area. *J. sambac* flowers and *S. palustris* leaves extract

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**Table 1.** The average percentage of larval mortality for each extract, concentrations, and exposure time

Extract	Concentration	Mortality of Larvae (%)						
		10'	30'	60'	120'	240'	480'	1440'
<i>Jasminum sambac</i>	0.5%	0	0	8	16	20	24	52
	0.6%	0	8	12	40	60	76	88
	0.7%	0	12	16	56	76	88	96
	0.8%	0	16	20	84	92	96	100
	Control	0	0	0	0	0	0	0
<i>Stenochlaena palustris</i>	0.5%	0	0	4	24	32	36	48
	0.6%	0	4	16	36	44	52	56
	0.7%	0	12	28	44	52	64	72
	0.8%	0	12	36	56	64	72	80
	Control	0	0	0	0	0	0	0

was produced at the Chemistry Laboratory of Sari Mulia University.

#### Plant Material

The flowers of *J. sambac* were collected during July 2020, from Tunggul Irang Village (3°31'938" S; 114°29'776" E) and the leaves of *S. palustris* were collected in July 2020, from Tunggaran Village (3°37'2.8" S; 114°42'09.2" E) in Sub-District Martapura, Banjar, South Kalimantan Province, Indonesia. The plants were identified with the help of expert in Laboratory of Research and Development Center for Environment and Forestry, Banjarbaru, Indonesia and voucher specimens were deposited.

#### Extract Preparation

The fresh flowers of *J. sambac* and leaves of *S. palustris* (each for 1000 g) were dried for 7-10 days with no direct sunlight then blended into a powder for each plant materials. The powders were put into a part glass jar, and 96% 500 mL ethanol was added until the powders were submerged. After being stirred and allowed to stand for 24 hours, the solutions were filtered and then thickened. The resulting extract for each plant materials stored in a sample bottle.

#### Methods

This research is a true-experimental research conducted in vitro, with a post-test only and a control group design approach. This study was performed according to the international, national, and institutional rules considering animal experiments which is proven by having passed an ethics test from the Ethics Council of Sari Mulia University (Letter of Statement No. 090/KE-LPPM/UNISM/XII/2019).

#### Bioassay

The *Ae. aegypti* larvae instar III/IV obtained from the hatching of adult mosquito eggs developed by the Entomology Laboratory of Health Research and Development of Tanah Bumbu. The larvae were selected using a simple random sampling, which was then divided into 5 groups (1 control group and 4 treatment groups) with *J. sambac* extract or *S. palustris* extract concentrations of 0.5%, 0.6%, 0.7%, and 0.8%. Each group consisted of 25 larvae, with replication of tests at each concentration performed 4 times. The total sample consisted of 1000 larvae.

The solutions, each prepared with its own specified concentration of *J. sambac* flowers or *S. palustris* leaves extract, were transferred into the study tubes with labels K (control), P1 (0.5%), P2 (0.6%), P3 (0.7%), and P4 (0.8%). After the solutions were ready, the larvae were transferred into the study tube. The *Ae. aegypti* larvae mortality then observed for 24 hours. The number of larval mortalities within each sample were counted based on visual inspection. Larvae that have been used after the research process, stored within a solution of 1% temephos until all larvae have died, as confirmed by the observation that larvae do not move in response to stimulation.

#### Statistical Analysis

The larval mortality data for each group (*J. sambac* extract and *S. palustris* extract) were used to calculate the Lethal Concentration (LC<sub>50</sub> and LC<sub>90</sub>) and Lethal Time (LT<sub>50</sub> and LT<sub>90</sub>) values with each 95% fiducial limits of Upper Confidence Limit (UCL) and Lower Confidence Limit (LCL). The data were analyzed by a Probit regression test using MINITAB 14 and combining with PHYTON software to compare those groups value of percentage mortality in each concentrations and times.

## RESULTS

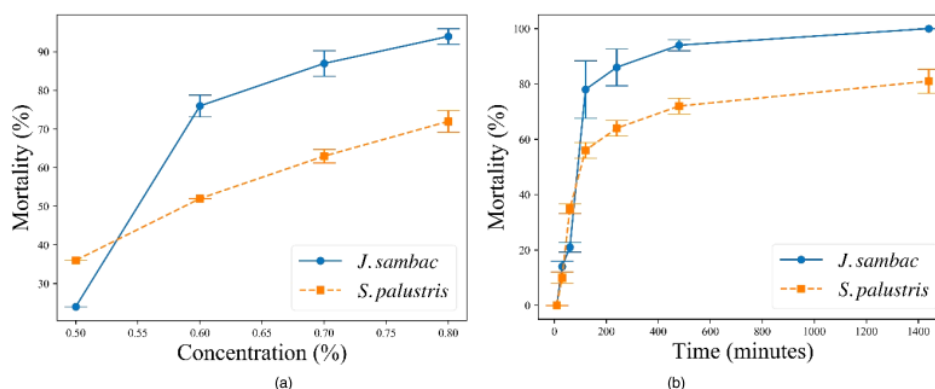
### The Percentage Mortality Larvae of *Jasminum sambac* Flowers Extract and *Stenochlaena palustris* Leaves Extract

The percentage mortality of *Ae. aegypti* larvae exposed to varying concentration of *J. sambac* and *S. palustris* for each time of exposure (10-minute, 30-minute, 60-minute, 120-minute, 240-minute, 480-minute, and 1440-minute) were increased in increasing concentration with increased exposure time (Table 1). The larval mortality of all extracts starts happened on 60-minute for concentration 0.5%, while for concentration 0.6%, 0.7%, and 0.8% the mortality happened on 30-minute.

The larval mortality for both extracts, at a concentration of 0.5%, start to occur at 60-minute. The concentration of 0.6%, 0.7%, and 0.8% of *J. sambac* and *S. palustris* extracts start to occur at 30-minutes. *J. sambac* extract was 100% larvae mortal in concentration 0.8% after 24 h (1440-minute), but for *S. palustris* extract, at the same concentration and time exposure,



**Fig. 1.** The damage of the larval body parts: (a) head; (b) siphon (original picture by the research)



**Fig. 2.** The comparison of percentage mortality  $\pm$  SD of *J. sambac* and *S. palustris*: (a) based on the different concentrations. (b) based on the time exposure

the mortality of larvae lower than that (80%). *J. sambac* and *S. palustris* extracts were observed to cause damage to the body structure of the larvae, including head damage (Fig. 1a) and siphon damage (Fig. 1b).

The comparison of percentage larvae mortality based on each concentration between *J. sambac* and *S. palustris* extract (Fig. 2a) shown that the percentage of larval mortality occurred more or higher in the *J. sambac* treatment group than in *S. palustris* at the same concentration. Besides, the comparison of percentage larvae mortality at the different exposure time (Fig. 2b), the mortality was increased when their exposure time increased. It shown that at early time of the exposure the mortality between those two extracts almost at the same rate, but then when the time increased, the mortality of *J. sambac* extract group was getting higher than *S. palustris* extract group.

#### The Bio-larvicidal Effectiveness of *Jasminum sambac* Flowers Extract and *Stenochlaena palustris* Leaves Extract

Lethal Concentration (LC) analysis is used to assess larvicidal toxicity determined based on the number of larvae that die at each concentration. The LC<sub>50</sub> is defined

as the concentration needed to kill 50% of the test larvae population and LC<sub>90</sub> is to kill 90% population.

The data obtained shows that the LC<sub>50</sub> and LC<sub>90</sub> values in the *J. sambac* and *S. palustris* extracts decreased from the 30th minute to the 1440th minute, except for the 60th minute of *J. sambac* extract which experienced an increase from the 30th minute. The results of the probit analysis show that there is an LC<sub>50</sub> value that exceeds the WHO standard, that is 1% (in *J. sambac* the LC<sub>50</sub> value is at 30 minutes and 60 minutes) while for *S. palustris* it is only at 30 minutes. The LC<sub>90</sub> value in *J. sambac* that exceeded the WHO standard was only at 30 and 60 minutes, while in *S. palustris* most of the LC<sub>90</sub> exceeded the standard, they are at the 30<sup>th</sup>, 60<sup>th</sup>, 120<sup>th</sup>, 240<sup>th</sup>, and 480<sup>th</sup> minutes (Table 2).

Apart from analyzing the Lethal Concentration, the effectiveness of a larvicide is also seen from the Lethal Time (LT). Lethal Time is the length of time that the larvae die. LT<sub>50</sub> is the length of time of exposure that causes 50% mortality of larvae and LT<sub>90</sub> is responsible for 90% of larvae mortality.

The LT<sub>50</sub> value decreased from the lowest concentration (0.5%) to the highest concentration (0.8%), as did the LT<sub>90</sub> value. The LT<sub>50</sub> value of each

**Table 2.** LC<sub>50</sub>, LC<sub>90</sub>, Lower Confidence Limit (LCL), and Upper Confidence Limit (UCL) of *J. sambac* and *S. palustris* Extracts at each time exposure

Extract	Time (minutes)	LC <sub>50</sub> ±SE (%)	LCL-UCL	LC <sub>90</sub> ±SE (%)	LCL-UCL
<i>Jasminum sambac</i>	10	0	0	0	0
	30	1.07 ± 0.11	0.94 - 1.50	1.45 ± 0.21	1.19 - 2.30
	60	1.12 ± 0.14	0.94 - 1.77	1.64 ± 0.29	1.28 - 3.06
	120	0.67 ± 0.01	0.64 - 0.68	0.86 ± 0.02	0.82 - 0.92
	240	0.59 ± 0.01	0.58 - 0.62	0.78 ± 0.02	0.75 - 0.82
	480	0.56 ± 0.01	0.53 - 0.58	0.72 ± 0.01	0.69 - 0.75
	1440	0.49 ± 0.01	0.46 - 0.51	0.63 ± 0.01	0.60 - 0.66
<i>Stenochlaena palustris</i>	10	0	0	0	0
	30	1.09 ± 0.11	0.96 - 1.59	1.42 ± 0.21	1.16 - 2.34
	60	0.88 ± 0.04	0.82 - 0.99	1.20 ± 0.09	1.06 - 1.48
	120	0.75 ± 0.03	0.71 - 0.84	1.19 ± 0.11	1.03 - 1.53
	240	0.68 ± 0.02	0.63 - 0.74	1.15 ± 0.11	0.99 - 1.50
	480	0.60 ± 0.02	0.55 - 0.64	1.01 ± 0.07	0.91 - 1.23
	1440	0.53 ± 0.03	0.46 - 0.58	0.93 ± 0.06	0.84 - 1.10

**Table 3.** LT<sub>50</sub>, LT<sub>90</sub>, Lower Confidence Limit (LCL), and Upper Confidence Limit (UCL) of *J. sambac* and *S. palustris* extracts in the different concentration

Extract	Concentration (%)	LT <sub>50</sub> ± SE (min)	LCL-UCL	LT <sub>90</sub> ±SE (min)	LCL-UCL
<i>J. sambac</i>	0.5	1320.97 ± 96.45	1156.96 - 1547.15	2438.93 ± 196.64	2113.06 - 2912.10
	0.6	441.61 ± 32.01	382.74 - 510.24	1111.92 ± 75.41	983.03 - 1285.88
	0.7	250.5 ± 19.62	213.70 - 291.93	681.620 ± 48.26	599.88 - 794.45
	0.8	126.26 ± 7.48	111.97 - 141.66	273.19 ± 14.91	247.28 - 306.90
<i>S. palustris</i>	0.5	1302.74 ± 113.9	1113.85 - 1580.66	2653.93 ± 257.54	2238.57 - 3299.38
	0.6	985.53 ± 87.79	837.27 - 1195.66	2366.91 ± 231.35	1993.62 - 2946.46
	0.7	647.51 ± 56.36	546.84 - 773.79	1834.03 ± 159.73	1570.96 - 2222.19
	0.8	415.18 ± 41.72	336.41 - 503.28	1432.56 ± 118.03	1236.08 - 1715.21

extract was below the WHO standard (1440 minutes). However, the LT<sub>90</sub> value for *J. sambac* extract exceeds the standard, namely at a concentration of 0.5%, while for *S. palustris* extract all concentrations have an LT<sub>90</sub> value that exceeds the standard (Table 3).

## DISCUSSION

The results showed that the extracts of *J. sambac* and *S. palustris* were proven to be effective as larvicides. This is due to the content of essential oils and secondary metabolite compounds from the ethanol extract of *J. sambac* and *S. palustris*.

Essential oils have a larvicidal effect by disrupting the nervous system of larvae and can damage *Ae. aegypti* eggs (Sulistiyani, 2015). While the content of ethanol extract such as alkaloids can degrade cell membranes and damage cells and can interfere with the larvae's nervous system by inhibiting the action of the acetylcholinesterase enzyme (Ningsih et al, 2016).

Previous research has shown that *J. sambac* extract positively contains alkaloids, flavonoids, saponins and tannins (Hidayah et al, 2020). In addition, *J. sambac* is also known to be one of the potential plants as a natural larvicide among 25 other plants in Indonesia (Chin et al, 2017). Previous studies proved that the *S. palustris* extract positively contained flavonoids, tannins, and alkaloids (Hidayah and Herawati, 2020).

Saponin and flavonoid compounds are poisonous compounds that work as stomach poisoning which can cause digestive system disorders of *Aedes aegypti* larvae and can cause the death of larvae. Saponins can decrease digestive enzyme activity and food absorption (Utami et al, 2016). Research by Jawale (2015) in India

found that all plants containing saponins have shown positive results in killing larvae and are potential as mosquito larvicides. Flavonoids are plant defense compounds that can inhibit insect eating and are also toxic (Utami et al, 2016). In this study, the same mechanism seems to have occurred so that the test larvae exposed to the extracts of *J. sambac* and *S. palustris* died.

Microscopically, the differences between larvae in the control group (live larvae) and larvae in the treatment group (dead larvae) were seen. Larvae of the control group had intact body structure (intake), in contrast to larvae of the treatment group. Larvae that died from exposure to the extract had a rigid body structure, a damaged head and siphon (Fig. 1).

The results showed that at a concentration of 0.5% larvae died only at 60 minutes, while concentrations of 0.6%, 0.7%, and 0.8% of larvae mortality occurred at 30 minutes. This is due to the less toxic content in the concentration of 0.5%, so that the toxin that enters the larvae's body is also less than the concentrations of 0.6%, 0.7%, and 0.8%. Therefore, the mortality of larvae at a concentration of 0.5% is slower.

Research conducted by Lestari (2013) regarding the effectiveness of lime seed extract (*Citrus aurantifolia*) as a larvicide against third instar *Aedes aegypti* larvae with concentrations of 0.25%, 0.5%, 0.75% and 1%, the results obtained were significant differences in the treatment group concentration of 0.25. % with 0.5% and 0.5% with 0.75% but in the treatment group 0.75% and 1% there was no significant difference in causing larvae mortality. This shows that the ethanol extract of *J. sambac* and *S. palustris* has a better level of difference

in causing the death of test larvae when compared to the extract of lime seeds (*Citrus aurantifolia*).

The LC<sub>50</sub> value at 30 minutes to 1440 minutes decreased (Table 2). This decrease indicates that the longer the exposure time, the less concentration is needed to kill larvae (Fig. 2b). This is because the longer the larvae are exposed, the more plant extract content will enter the larvae's body and the faster the larvae will die.

The LC<sub>50</sub> values (Table 2) of *J. sambac* extract at 30 and 60 minutes were above the WHO standard and *S. palustris* extract only at 30 minutes. This shows that the *J. sambac* extract is effective in killing *Aedes aegypti* larvae by as much as 50% at 120 to 1440 minutes exposure, while the *S. palustris* extract is effective earlier, that is at the 60 to 1440 minute.

The results of the LC<sub>90</sub> analysis (Table 2) of *J. sambac* extract showed that the extract was effective in killing 90% larvae in the 120<sup>th</sup> to 1440<sup>th</sup> minutes. While the *S. palustris* extract began to effectively kill the larvae much slower at 1440 minutes.

Judging from the time of exposure, the LT<sub>50</sub> values of the *J. sambac* and *S. palustris* extracts decreased with greater concentration (Table 3). According to WHO, the lethal time limit for a larvicide to be effective is 1440 minutes. This shows that the concentrations of 0.5%, 0.6%, 0.7%, and 0.8% are effective in killing 50% larvae. Based on the LC<sub>90</sub> value (Table 3) the *J. sambac* extract proved ineffective in killing 90% of larvae at a concentration of 0.5%. Meanwhile, *S. palustris* extract was found to be ineffective at killing 90% of larvae at all concentrations. The greater the concentration given, the higher the toxicity or toxicity content exposed to the test larvae so that it takes less time to kill the larvae (Fig. 2a).

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The higher the toxicity of a substance contained in the *J. sambac* and *S. palustris* extracts, the higher the substances absorbed by the *Aedes aegypti* larvae, which were the test larvae in this study. When the absorption exceeds the tolerance limit of larvae, it can cause cell and tissue damage of larvae, which in turn can lead to larvae death (Ningsih et al, 2016). Therefore, the concentrations of 0.5%, 0.6%, 0.7%, and 0.8% are effective in killing *Aedes aegypti* mosquito larvae.

## CONCLUSION

*J. sambac* extract was proven to be effective as a larvicide to kill 50% of *Ae. aegypti* larvae at concentrations of 0.5%, 0.6%, 0.7%, and 0.8% with effective time starting from 120<sup>th</sup> minute and effectively killing 90% larvae at concentrations of 0.6%, 0.7%, and 0.8% with effective time starting from the 120<sup>th</sup> minute. The extract of *S. palustris* is proven to be effective as a larvicide to kill 50% larvae at concentrations of 0.5%, 0.6%, 0.7%, and 0.8% with an effective time in the 60<sup>th</sup> minute but less effective in killing 90% of larvae at these concentrations because it takes time over 1440 minutes.

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