

Larvicidal Activity of *Brugmansia candida* against *Aedes aegypti* and *Culex quinquefasciatus* (Diptera: Culicidae)

Setiawan Khoirul Himmi, Didi Tarmadi*, Dita Meisyara, Anugerah Fajar, Titik Kartika, Ikhsan Guswenrivo, Sulaeman Yusuf

Research Center for Biomaterials, Indonesian Institute of Sciences-LIPI, Indonesia

*Email: didi@biomaterial.lipi.go.id

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Abstract. Mosquitoes are well known as vectors of hazardous diseases for human. Plant extracts can be used as an alternative for larval control due to they are a rich source of bioactive chemicals and safe for the environment. The present study investigated the larvicidal activity of crude extracts derived from leaf and flower of *Brugmansia candida* against the second larval instar of *Aedes aegypti* and *Culex quinquefasciatus*. The larval mortality was observed at 24 and 48 h exposure of both leaf and flower extracts, at the concentration of 100, 250, 500, and 1000 ppm. The 24 h exposure of both extracts at the concentration of 500 and 1000 ppm resulted in larval mortality rates were significantly lower than those of 48 h exposure. However, the mortality rate was not significantly different at the lower concentrations of crude extracts. The results also suggested that there was no significant difference in the larvicidal effect between leaf and flower extracts at 24 and 48 h exposure for all concentrations. The LC₅₀ values at 48 h exposure for leaf extract were 789 and 791 ppm for *Ae. aegypti* and *C. quinquefasciatus*, respectively, whereas for flower extract were 772 and 780 ppm for *Ae. aegypti* and *C. quinquefasciatus*, respectively. Overall, *B. candida* showed larvicidal activity against *Ae. aegypti* and *C. quinquefasciatus*. This research contributes to new finding regarding the larvicidal activity of *B. candida*. This finding also supports the next study to develop *B. candida* as an alternative source for larval control agent.

Key words: *Brugmansia candida*; Crude Extract; Larval Mortality; Mosquitoes

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INTRODUCTION

Mosquito is an urban pest insect that bites and feeds on the blood of humans and other animals. They also serve as vectors of several diseases that cause serious health problems in humans (Dehkordi et al., 2016). *Aedes aegypti*, a vector of dengue, and *Culex quinquefasciatus*, a vector of filariasis are widely distributed in tropical and subtropical zones (Samy et al., 2016). Dengue and filariasis are primarily an urban disease of the tropics and hazardous that can be fatal. Mosquito-borne diseases induce negative impacts on an economy, including the loss in commercial and labor outputs, particularly in countries with a tropical climate, including Indonesia. Besides, mosquitoes are capable to change their breeding habitats rapidly and also adapt to various human habitats to support their reproduction. Based on such adverse conditions, a method for controlling the mosquito population is required to protect humans from mosquitoes attack.

Currently, chemical treatment using insecticides has been the primary method that is used extensively in daily life. Nevertheless, the control of mosquitoes with insecticides has several drawbacks such as the environmental damage after its continual use up to

the insecticides diminishing effect for controlling the mosquito due to the increase of mosquito resistance level against insecticides active agent during the last five decades (Govindarajan & Karuppanan, 2011). Also, it was reported that the constant use of chemical insecticides has often led to the disruption of natural biological control systems, outbreaks of insect species, and undesirable effects on humans, mammals, and other non-target organisms (Ghananand et al., 2011). Researchers are developing new products for the management of mosquitoes that are safer for the environment, biodegradable, low in cost, reliable, and functional for preventive and remedial management (Tarmadi et al., 2018).

Plants can be used as an alternative source of insecticidal management agents because they are a rich source of bioactive chemicals (Tarmadi et al., 2018; Ismayati et al., 2019). Earlier researchers reported that several plant extracts possess potency as larvicidal substances, i.e: *Azadirachta indica* (Nour et al., 2012), essential oil from *Mentha spicata* (Govindarajan et al., 2012), *Ocimum basilicum* (Govindarajan et al., 2013), *Pedilanthus tithymaloides* leaf extract (Sundaravadivelan et al., 2013), leaf extract from mangrove plant *Rhizophora mucronata* (Meenakshi

& Jayaprakash, 2014), and seed extract of *Ammi visnaga* (Pavela et al., 2016).

One potential plant to be explored is *Brugmansia candida*. *B. candida* (angel's trumpet) or known in Indonesia as *bunga terompet* is a shrub plant, a member of Solanaceae that is commonly used as ornamental plants. This plant is well known as a poisonous plant due to contain tropane alkaloid (Cardillo et al., 2010; Kerchner & Farkas, 2020). Therefore, this plant opens new possibility for other utilization other than as ornamental plants, for examples as insects control agent.

Based on the past studies, the idea to use *B. candida* as insects control agent has been explored although limited. Some previous study showed that *B. candida* exhibited high termiticidal activities against a subterranean termite, *Coptotermes gestroi* and a dry wood termite, *Cryptotermes cynocephalus* (Tarmadi et al., 2014). This study indicated that there is a potency for *B. candida* to be used to control insect population.

Based on our literature survey, the larvicidal effects of *B. candida* against a dengue vector, *Ae. aegypti* and a filariasis vector, *C. quinquefasciatus* have not yet been reported. Therefore, this study was carried out to investigate the larvicidal effect of *Brugmansia candida* against a dengue vector, *Ae. aegypti*, and a filariasis vector *C. quinquefasciatus*. We expected that the result can give us additional information on how powerful *B. candida* as the source material for larvicidal agent.

METHODS

Plant materials

Leaves and flowers of *B. candida* were collected from Cibodas Botanical Garden, Indonesian Institute of Sciences (LIPI), Cianjur, West Java, Indonesia (Figure 1). The leaves and flowers were shade-dried until its moisture content down to below 10% (Figure 1). The material was then pulverized using a mechanical blender and then sieved using sieve sizes of 40 and 60 mesh, the material sized between 40 and 60 mesh was collected and secured in dry storage for further use.



Figure 1. Collecting and drying process of samples collected from Cibodas Botanical Garden, Indonesian Institute of Sciences (LIPI), Cianjur, West Java.

Extract preparation

Approximately 300 g powdered leaves and flowers were macerated using methanol for three cycles, each cycle lasted for 24 hours and using one liter of Methanol 96% (Merck, Darmstadt, Germany). After each cycle, the liquid extract was collected and the leftover solid material was reintroduced into a new cycle of maceration. The collected liquid extract was then filtered with filter paper (Whatman plc, USA) and the filtrate was evaporated using a rotary evaporator (RV 10 Digital, IKA Works GmbH & Co., Germany) at 40° C to get the dried crude extracts.

Insect rearing

Ae. aegypti and *C. quinquefasciatus* eggs were obtained from the Laboratory of Parasitology and Health Entomology, Faculty of Veterinary Medicine, IPB University (Bogor, Indonesia), and reared at the Laboratory of Biodeterioration and Pest-insect Control, Research Center for Biomaterials LIPI (Bogor, Indonesia). The larvae were reared in plastic trays containing tap water and maintained at a temperature of 28± 2°C and relative humidity of 75–85%. The larvae were fed a diet of dog food pellets.

Larvicidal bioassay

The crude extracts were prepared in concentration units of 100, 250, 500, and 1000 ppm. Tween 80 was used as a surfactant. The larvicidal effect was assessed by using the procedure as described earlier by Tarmadi et al. (2018). Twenty-five second-instar of *Ae. aegypti* and *C. quinquefasciatus* larva were subjected to 100-ml solutions of each concentration (Figure 2). Five replications were prepared and the numbers of dead larvae were counted at 24 and 48 h exposure. Water and Tween 80 solutions (95:5, 100 ml) were used as control solutions.



Figure 2. Larvicidal bioassay: twenty-five second-instar of *Aedes aegypti* and *Culex quinquefasciatus* larva were subjected to the tested extracts solutions.

Statistical analysis

The larval mortality data were subjected to probit analysis for calculating LC₅₀ and LC₉₀ values using the software EPA Probit Analysis Program Version 1.5. Larval mortality rates and larvicidal effect comparison of leaf and flower extracts were analyzed

using SPSS ver. 23 software (IBM, Armonk, NY). Significant differences between means were identified by Tukey's post-hoc test ($P < 0.05$).

RESULTS AND DISCUSSION

Larvicidal effect of leaf extract

The preliminary result of the larvicidal effect of *B. candida* crude extract on *Ae. aegypti* and *C. quinquefasciatus* larvae revealed that the crude extract of *B. candida* leaf distinctly exhibited a larvicidal effect. The result showed that larval mortality increased along with the increasing of the concentration and the exposure periods for both *Ae. aegypti* and *C. quinquefasciatus*. The result was summarized in Figure 3a-d.

The statistical analysis showed that with the time of exposure as the variable of concern (Figure 3a-b), mortality rate of 24 hours exposure was significantly different compared to that of 48 hours at 500 and 1000 ppm-level. At 500 ppm, the statistic result indicated that there was a significant mortality rate difference between 24 hours and 48 hours for *Ae. aegypti* ($F=46$, $P<0.05$) and *C. quinquefasciatus* ($F=25$, $P<0.5$), and at 1000 ppm the result also showed significant differences as well with *Ae. aegypti* ($F=42$, $P<0.05$) and *C. quinquefasciatus* ($F= 28$, $P<0.05$). However, at the lower concentration units which were 100 ppm and 250 ppm, the result showed no statistically significant difference in mortality rate between 24 hours and 48 hours exposure time for both *Ae. aegypti* and *C. quinquefasciatus*.

Another variable of concern was the concentration's impact on the mortality rate of *Ae. aegypti* and *C. quinquefasciatus* (Figure 3c-d). The result suggested that there was no significant difference between larval mortality rate of *Ae. aegypti* and *C. quinquefasciatus* for all concentrations at both 24 hours and 48 hours exposure. This result suggested that the same amount of concentration can be applied to *Ae. aegypti* or *C. quinquefasciatus* and will give a similar mortality rate. It is also important to note that at the end of the observation (48 hours), the leaf crude extract generated 69.6% and 66.4% of larval mortality rate for *Ae. aegypti* and *C. quinquefasciatus*, respectively. Although the larvicidal effect of leaf crude extract was at 60% to 70%, this result showed that the extract of *B. candida* leaf possesses the potential to be used as a larvicidal agent for both *Ae. aegypti* and *C. quinquefasciatus*.

Larvicidal effect of flower extract

The larvicidal activities of the extract of *B. candida* flower against *Ae. aegypti* and *C. quinquefasciatus* at 24 and 48 h exposure time are summarized in Figure 4a-d. The result from the flower extract was

similar to the leaf extract, in which the significant difference was found between 24 hours and 48 hours exposure periods at the concentration of 500 and 1000 ppm (Figure 4a-b). At 500 ppm, the statistic result indicated a significant difference for *Ae. aegypti* ($F=46$, $P<0.05$) and *C. quinquefasciatus* ($F= 25$, $P<0.5$), and at 1000 ppm the result also showed significant difference as well for *Ae. aegypti* ($F=42$, $P<0.05$) and *C. quinquefasciatus* ($F= 28$, $P<0.05$). On the other hand, there was no significant difference in the lower concentrations (100 and 250 ppm).

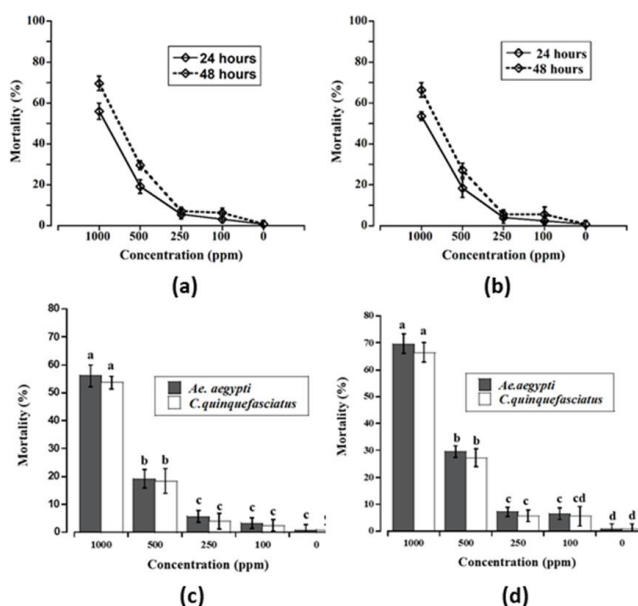


Figure 3. Mortality of *Ae. aegypti* (a) and *C. quinquefasciatus* (b); comparison of larval mortality of *Ae. aegypti* and *C. quinquefasciatus* at 24 h (c) and 48 h (d) after exposure to leaf extract of *B. candida*. Twenty-five larvae of *Ae. aegypti* were used per replication and the data were averaged ($N=5$). Error bars represent standard deviations. Same letters do not differ statistically (Tukey HSD test; $P < 0.05$) following one-way ANOVA.

Regarding the impact of the concentration on the mortality rate of both *Ae. aegypti* and *C. quinquefasciatus*, the study showed that there was no significant difference between larval mortality rate of *Ae. aegypti* and *C. quinquefasciatus* for all the concentrations and the exposure periods (Figure 4c-d). At the end of observation at 48 hours, the larval mortality rate of *Ae. aegypti* and *C. quinquefasciatus* was 71.2% and 68.8%, respectively. The end of the observation results suggested that the flower extract generated the larvicidal effect against both *Ae. aegypti* and *C. quinquefasciatus* at a similar level to crude leaf extract after 48 hours of exposure.

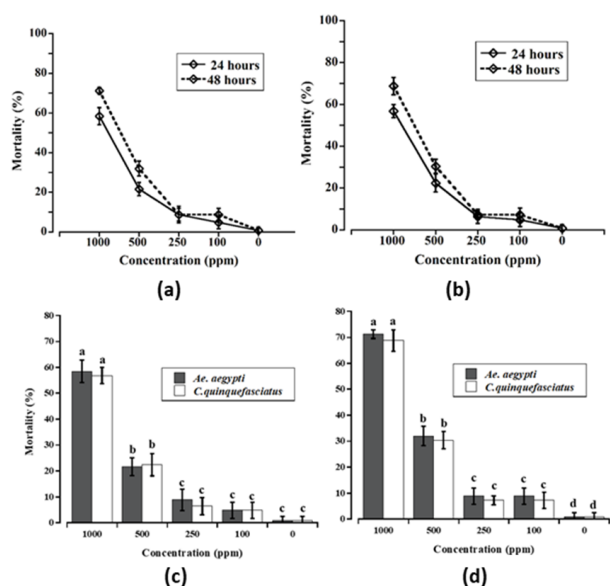


Figure 4. Mortality of *Ae. aegypti* (a) and *C. quinquefasciatus* (b); comparison of larval mortality of *Ae. aegypti* and *C. quinquefasciatus* for 24 h (c) and 48 h (d) after exposure to flower extract of *B. candida*. Error bars represent standard deviations. Same letters do not differ statistically (Tukey HSD test; $P < 0.05$) following one-way ANOVA.

Comparison of larvicidal effect between leaf and flower extracts

The information of larvicidal effect from the leaf and flower extracts showed that generally at the end of the observation, both of the extracts showed a similar mortality rate at around 60 to 70%. But the question remains on whether this similarity applied to all concentrations and time exposure. To answer whether the similarity in mortality rate is applied to all variables, we conducted a comparison test between leaf and flower extracts. The results of the comparison between extracts in all concentrations at 24 hours and 48 hours is shown in Figure 5a-d.

Based on statistical analysis, there was no significant difference among crude extracts for all concentrations at 24-hours and 48-hours exposures against both *Ae. aegypti* and *C. quinquefasciatus* (Figure 5a-d). The data confirmed that leaf and flower extracts show similar larvicidal effect. Further study is necessary to identify what major compounds in the leaf and flower extracts that have larvicidal properties. An earlier study reported that *B. candida* contains scopolamine, anisodamine, and hyoscyamine, in which these substances are tropane alkaloids (Cardillo et al., 2010). The existing larvicidal potential is important because earlier studies have shown that further processing of the extract can lead to an increase of the crude extract potential, in this case, its larvicidal effect. A previous study reported that the crude extract of *Cerbera odollam* possessed a larvicidal effect

against *Ae. aegypti*, and interestingly, the larval mortality for fractionated extract was higher than that for crude extract (Tarmadi et al., 2018). Sasidharan et al. (2011) revealed that active agent in the extract resulted in larval mortality. This type of active agent is highly sought after by the pest control industry because its future to be synthesized in mass scale can be a new source of insecticides to control mosquito population (Duke et al., 2010). One such chemical is pyrethrin derived from *Chrysanthemum cinerariaefolium* which leads to pyrethroid class chemical that is currently used as insecticides (Ujihara, 2019).

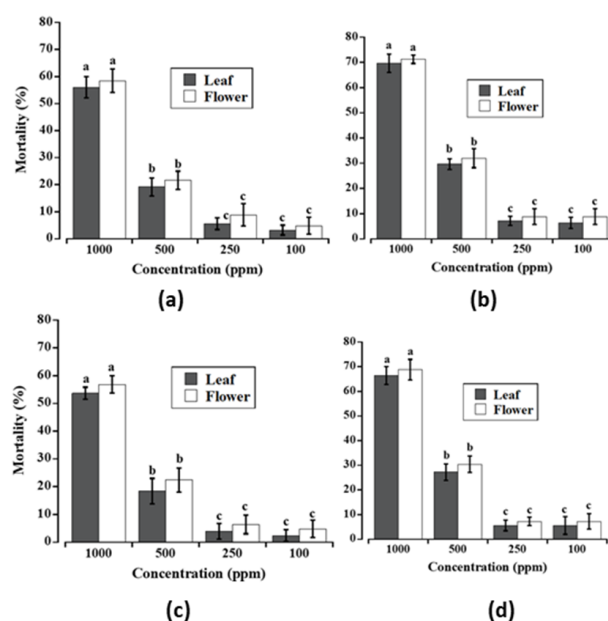


Figure 5. Comparison of crude extracts of *B. candida* for *Ae. aegypti* at 24 h (a) and 48 h (b); *C. quinquefasciatus* at 24 h (c) and 48 h (d). Error bars represent standard deviations. Same letters do not differ statistically (Tukey HSD test; $P < 0.05$) following one-way ANOVA.

An earlier study reported that crude extract can generate higher potency due to the synergistic effect generated by active chemical compounds interaction (Sasidharan et al., 2011). This phenomenon manifested in a higher larvicidal effect of the crude extract compared to the fractionated one, purified extract. In fact, the number of cases that demonstrated such examples were numerous. As examples of synergistic effect in crude extract can be seen in the extracts of *Solanum nigrum* (Rawani et al., 2010) and *Nelumbo nucifera* (Santhoshkumar et al., 2010). These examples demonstrated that the presence of synergistic effect may play a larger role in shaping the larvicidal effect. But some other studies pointed out that although further processing might lessen or remove the synergistic effect, purified extract at various level of purification can target specific chemical compound, which can make the mode of action that induced lar-

vicidal effect to be understood. This notion is important because it can offer us information for the creation of new insecticides.

LC₅₀ and LC₉₀ of crude extracts of *B. candida*

To investigate the larvicidal effect of *B. candida* extracts, we analyzed the LC₅₀ and LC₉₀ values from the larval mortality rate of *Ae. aegypti* and *C. quinquefasciatus* which results are presented in Table 1. At the end of observation at 48 hours, the LC₅₀ values of leaf extract were 789 and 791 ppm against *Ae. aegypti* and *C. quinquefasciatus*, respectively, while the LC₅₀ values of flower extract were 772 and 780

ppm against *Ae. aegypti* and *C. quinquefasciatus*, respectively. An earlier study showed that the LC₅₀ values of the crude extract of *Solanum xanthocarpum* were close to our current study by 788 and 573 ppm for *Ae. aegypti* and *C. quinquefasciatus* respectively (Changbunjong et al., 2010). The previous study on *C. odollam* also yielded an LC₅₀ value around 760 ppm against *Ae. aegypti* (Tarmadi et al., 2018). The current results exhibited a close LC₅₀ value to that of those previous studies, which suggested that *B. candida* extracts possess larvicidal effect on both *Ae. aegypti* and *C. quinquefasciatus*.

Table 1. LC₅₀ and LC₉₀ values from crude extracts of *B. candida* against *Ae. aegypti* and *C. quinquefasciatus* at 24 h and 48 h exposures

Materials	Larvae	Lethal Concentration (LC, ppm)			
		LC ₅₀	LC ₉₀	LC ₅₀	LC ₉₀
		24 h		48 h	
Leaf	<i>Ae. aegypti</i>	980.4	3113.2	789.0	2245.4
	<i>C. quinquefasciatus</i>	998.0	3234.6	791.0	2367.2
Flower	<i>Ae. aegypti</i>	970.7	3101.4	772.0	2210.1
	<i>C. quinquefasciatus</i>	978.0	3212.7	780.0	2330.1

Although the current study showed that *B. candida* possesses larvicidal effect, there were some other plants from earlier studies showed higher larvicidal effects, i.e. *Eclipta alba* (LC₅₀ 127.6 ppm) (Govindarajan & Karuppanan, 2011), *Solanum nigrum* (LC₅₀ 72.6 ppm) (Rawani et al., 2010), and *Acanthospermum hispidum* (LC₅₀ 42.7 ppm) (Vivekanandhan et al., 2018).

Lethal Concentration (LC) value has been an important parameter for deciding how potential the tested material once acted upon its goal. Although the result of *B. candida* LC was not too potential if compared to some plant extracts as shown in previous paragraph, each extract has its superiority especially in its mode of action and the effect it gives to the surrounding area once applied. The understanding on this topic has been scarce and worth pursuing since at its essence it can play as factors to be considered once people want to start developing the new plant-based insecticides, in which *B. candida* can be considered as one of the sources for raw material.

Overall, our finding contributes to new finding due to no information previously regarding the larvicidal activities of *B. candida*. The results clearly showed that *B. candida* extracts exhibited larvicidal activity against *Ae. aegypti* and *C. quinquefasciatus*, therefore, this study supports the next study to develop *B. candida* as an alternative source for mosquito larval control agent.

CONCLUSION

B. candida extracts showed larvicidal activities against *Ae. aegypti* and *C. quinquefasciatus*. The leaf and flower extracts of *B. candida* resulted in larval mortality of *Ae. aegypti* and *C. quinquefasciatus* by 66-71 %. The LC₅₀ values of *B. candida* leaf extract were 789 and 791 ppm for *Ae. aegypti* and *C. quinquefasciatus*, respectively, whereas the LC₅₀ values of *B. candida* flower extract were 772 and 780 ppm for *Ae. aegypti* and *C. quinquefasciatus*, respectively.

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