Larvicidal Activity of Bintaro (Cerbera odollam) against Culex quinquefasciatus

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Abstract. Mosquitoes are known for their bites which caused discomfort and its role as vector for various dangerous diseases that has been the bane of humanity since antiquity. One of them is Culex quinquefasciatus which facilitates the spread of arbovirus and nematodes that caused debilitating impact for human life. To eliminate mosquito, the study for evaluating plant-based mosquito larvicidal extract of Cerbera odollam extract against C. quinquefasciatus was conducted. Twenty-five second instar of C. quinquefasciatus were subjected to the crude and fractionated extracts of C. odollam, and the larval mortality was observed at 24, and 48 h of exposure. The results showed that the crude extracts of leaves, rinds, and stem bark exhibited low larvicidal activities. Interestingly, crude extract of the seed kernel exhibited high larvicidal activity. We found that the fractionated extract derived from the seed kernel extract using ethyl acetate and n-hexane exhibited high larvicidal activities, suggesting that C. odollam extract could be promoted as a material for larval control.

1. Introduction

It was a well-grounded knowledge that plants may have anti-insect activities including larvicidal activities due to their chemical substances. Earlier studies reported that several plant extracts possess larvicidal qualities, i.e: Aristolochia saccata and Annona squamosal [1]; Nelumbo nucifera [2]; Quercus Lusitania [3]; Saraca indica/asoca, Nyctanthes arbor-tristis, and Clitoria ternatea [4] Solanum villosom [5]; Argemone mexicana, Jatropha curcus, Pergularia extensa and Withania somnifera [6]; Neorautanenia mitis [7]; Solanum nigrum [8]; Solanum xanthocharpum [9]; Abuta grandifolia and Minthostachys setosa [10]; Piper longum [11]; oil of Cryptomeria japonica [12]; Apium graveolens [13]; Murayya koegini, Coriandrum sativum, Ferula asafoetida, and Trigonella foenum gracium [14]; Azadirachta indica [15] and many others. From all of these plants, one of the plants with ubiquitous presence in coastal and riverbank area of Indonesia and low utilization of its biomass is Bintaro (Cerbera odollam).

Bintaro (Cerbera odollam Gaertn) has been known as Suicide tree with various local name such as kattu arrali/othalanga maram in India while in Madagascar, it was known as famentana/kisopo/samanta [16]. For the past two decades, its usage as an agent for homicide/suicide by consuming its kernel has been discussed [16]. Its toxicity comes from combination of cerberin, cereberoside, and odorollin which are active compounds of cardenolide class [17, 18]. Past studies have recorded that the tree association with death was a longstanding one, especially its kernel utilization which was used as instrument of truth in the trial by ordeal against the accused party in Madagascar [16]. In India, it was used as
homicide/suicide agent with some accidental poisoning, especially children, who assumed the fruit to be edible. The Indian data indicated that the poisoning caused by *Cerbera odorllam* was at 50% of the entire plant based poisoning cases in Kerala, India between 1989 to 1999 [16]. *C. odorllam* belongs to apocynaceae family plant which is related to yellow oleander (*Thevetia sp.*) and common oleander (*Narrium sp.*). Both of them is known to be poisonous [16]. The plant can grow to 6 - 15 m in height and has green fleshy lanceolate leaves with delicate, sweet white flowers adorning the plant. The prominent part of the plant is its assuming, harmless looking fruits which looks like small mango [16,17]. The fruit kernel, which was known to be fatal at one kernel serving size, was around 2 x 1.5 cm in size and can actually changing its color from purple to brown after being exposed to air [16,17].

One of the harmful mosquitoes is *Culex quinquefasciatus*. Several diseases such as West Nile fever, West Equine and St. Louis encephalitis and filariasis can be traced back to *C. quinquefasciatus* as the disease vector [19]. *C quinquefasciatus* with the rest of Culex pipians group has been concerning since its wide distribution across the tropic and sub-tropic region of the world with several areas, predominantly in low developing countries, are highly threatened with occasional outbreak [19]. Descriptively, *C. quinquefasciatus* has modest length of 4 mm with brown coloration with the darkest brown in the tarsi while the lightest in center of the head [20]. The mosquito also can be identified with its completely black proboscis and its postspiracular area and the presence of pale scales in abdomen terga II to VI [21].

This study aimed to investigate the larvicidal activity of *Cerbera odorllam* against *C. quinquefasciatus*. Earlier studies reported that *C. odorllam* had insecticidal activities against subterranean termite, *Coptotermes gestroi* and storage pest product, *Sitophilus oryzae* [22-24]; *Trametes versicolor*, *Pycnoporus sanguineus*, and *Schizophyllum commune* [25]; *Streptococcus pyogenes*, *Streptococcus saprophyticus*, and *Salmonella typhi* [26]. Based on earlier studies, no information about the larvicidal effect of *C. odorllam* against *C. quinqufasciatus* has been conducted.

### 2. Materials and methods

#### 2.1. *Cerbera odorllam*

Stem bark, leaves, seed kernels, and rinds of *C. odorllam* were collected form Research Center for Biomaterials-LIPI in Cibinong-Bogor, Indonesia. Specimen identification was conducted by the Herbarium Bogoriense-Research Center for Biology-LIPI.

#### 2.2. Preparation of the crude and fractionated extracts

The preparation of the crude and fractionated extracts were performed as described previously [27]. Briefly: the crude extracts were prepared from 1 kg of dried and powdered of stem bark, leaves, seed kernels, and rinds. The methanol solvent was used for extraction process using a rotary evaporator (RV 10 Digital, IKA Works GmbH & Co., Germany) at 40°C. The fractionated extracts were obtained from crude extract of the seed kernel using n-hexane, ethyl acetate, and distilled water. While the sub-fractions were obtained from ethyl acetate fraction using column chromatography and thin-layer chromatography.

#### 2.3. Bioassay

The concentrations were prepared as follows. The crude extracts were 250, 500 ppm, 1000, and 2000 ppm; the fractionated extracts: 100 ppm, 250 ppm, 500 ppm, 1000, and 2000 ppm; the sub-fractions: 1000 ppm. The bioassay test was conducted as described previously [27]. Twenty-five second instar of *C. quinquefasciatus* larvae were obtained from Research Center for Biomaterials-LIPI were then applied to 100-ml solutions of each concentration in triplicate. The number of living larvae were recorded at 24 and 48 hours of exposure.

#### 2.4. Statistical analysis

The differences in larval mortality were tested by SPSS ver. 23 (IBM, Armonk, NY, USA). Significant differences between means were calculated by Tukey’s post hoc test. Significance levels were set at P < 0.05.
3. Results and discussion

Figure 1 shows the larvicidal activities from crude extracts of *C. odollam* derived from leaves, stem barks, rinds, and seed kernels. The results showed that crude extracts of leaves, stem barks, and rinds generated only 20% mortality of *C. quinquefasciatus* larvae at 48 h of exposure, even at the highest concentration (2000 ppm), suggesting that those crude extracts exhibited low larvicidal activities (Figures 1a-c), even though no mortality in control. Interestingly, the present study found that crude extract of the seed kernel exhibited high larvicidal activity against *C. quinquefasciatus* at 48 h of exposure, in which the mortality of larvae was more than 90% at 48 h of exposure at 2000 ppm, suggesting that crude extract of the seed kernel showed an effect on the mortality of *C. quinquefasciatus* larvae and can be promoted as larval control. An earlier study reported that crude extract of *C. odollam* exhibited high larvicidal effect against *Aedes aegypti* [27]. Other earlier studies reported that plants crude extracts had larvicidal activities against mosquitoes, i.e.: *Aristolochia saccata* and *Annona squamosa* [1]; *Nelumbo nucifera* [2]; *Quercus Lusitania* [3]; *Saraca indicasasoca, Nyctanthes arbor-tristis, and Clitoria ternatea* [4]; *Solanum villosum* [5]; *Argemone mexicana, Jatropha curcus, Pergularia extensa* and *Withania somnifera* [6]; *Neorautanenia mitis* [7]; *Annona crassiflora*, and *Pterodon polygalaeflorus* [28]; *Jatropha curcas* and *Euphorbia tirucalli* [29]; *Annona glabra*, and *Anacardium occidentalis* [30]. Those earlier studies suggested that plants extract, including *C. odollam* extract, had larvicidal effect in larval mortality of *C. quinquefasciatus*. Moreover, our previous study also clearly suggested that *C. odollam* exhibited high larvicidal effect against *Aedes aegypti* [27]. Therefore, *C. odollam* extract could be promoted as larval control against *Ae. aegypti* dan *C. quinquefasciatus*.

To further investigate the larvicidal activity of *C. odollam* against *C. quinquefasciatus*, the present study assessed the fraction extracts derived from the seed kernel as presented in Figures 2 and 3. Figure 2 shows the larvicidal activity from ethyl acetate fraction derived from the seed kernel of *C. odollam*. The results showed that ethyl acetate fraction generated 100% mortality of *C. quinquefasciatus* larvae at even 250 ppm (Figure 2), suggesting that the fraction exhibited high larvalidal activity against *C. quinquefasciatus*. By comparing larvicidal activity of the crude extract from the seed kernel (Figure 1), we observed that the larvicidal activity of ethyl acetate fraction derived from the seed kernel was much higher than that of the crude extract. Those results indicate that the active components that have adverse effects against *C. quinquefasciatus* larvae are more concentrated in the ethyl acetate fraction than those in the crude extract. These findings were similar to results of larvicidal effect of fractionated extracts derived from the crude extract from the seed kernel against *Ae. aegypti* [27].

Figure 3 shows the larvicidal activity of n-hexane fraction derived from the seed kernel of *C. odollam*. The n-hexane fraction resulted in 100% mortality of *C. quinquefasciatus* larvae at 1000 ppm at 48 h of exposure. It indicates that the larval mortality for n-hexane fraction was higher than that of the crude extract from the seed kernel (Figure 1), suggesting that the toxic components are more concentrated in the n-hexane fraction than those in the crude extract. However, by comparing the larval mortality from ethyl acetate fraction (Figure 2) and n-hexane fraction (Figure 3), the results clearly showed that the larval mortality of ethyl acetate fraction was much greater than that of n-hexane fraction, suggesting that larvicidal activity of ethyl acetate fraction was much higher than that of n-hexane fraction. Such finding also indicates that toxic components are more concentrated in the ethyl acetate fraction than those in the n-hexane fraction. The extracts of *C. odollam* are known to have many active components, and therefore, as an attempt to identify the specific active components that have larvicidal effect against *C. quinquefasciatus* larvae, the present study was then further fractionated the ethyl acetate fraction derived from the seed kernel into sub-fractions using column and thin-layer chromatography. Ten sub-fractions were obtained from the process (Figure 4).
Figure 1. Larval mortality of *C. quinquefasciatus* at 24 h and 48 h of exposure (a) = leaves, (b) = stem barks, (c) = rinds, (d) = seed kernels. Error bars represent standard deviations.

Figure 2. Larval mortality of *C. quinquefasciatus* at 24 h and 48 h of exposure for ethyl acetate fraction. Error bars represent standard deviations.
Figure 4 shows the larvicidal effect of the ethyl acetate sub-fractions from the seed kernel against *C. quinquefasciatus* larvae. The results showed that sub-fractions 1, 7, and 10 generated 100% of larval mortality even at 24 h of exposure, while other sub-fractions generated under 100% of larval mortality at 24 h of exposure. The lowest of larval mortality occurred by sub-fraction 5, in which the larval mortality was around 50% and 70% at 24 h and 48 h of exposures, respectively. In addition, the larval mortality of sub-fractions 2 and 3 were higher than that of sub-fraction 5, where the larval mortality for sub-fraction 2 and 3 at 24 h of exposure was 76% and 62%, respectively. Thus, the larval mortality of sub-fractions 8 and 9 were higher than that of sub-fractions 2 and 3. The sub-fractions 8 and 9 resulted in the larval mortality at 83% and 92% at 24 h of exposure, respectively. Therefore, based on the results, sub-fractions 1, 7, dan 10 had high larvividal activity against *C. quinquefasciatus* larvae, suggesting that toxic components are consisted in sub-fractions 1, 7, and 10. Further study is necessary for the identification of the active components in sub-fractions 1, 7, and 10. Previous report suggested that *C. odollam* contains cerberin as main active components and is categorized as a poisoning plant [31]. Other bioactive components in plant that have strong larvicidal activities have been reported by another report, i.e., bioactive compound of ectoquinone isolated from *Cryptomeria japonica* [32]; Clerodane diterpenoids and prenylated flavonoids from *Dodonaea viscosa* [33]; and pectolinaringenin from *Clerodendrum phlomidis* [34].

![Figure 3](image-url) **Figure 3.** Larval mortality of *C. quinquefasciatus* at 24 h and 48 h of exposure of n-hexane fraction from the seed kernel of *C. odollam*. Error bars represent standard deviations.

4. Conclusion
*Cerbera odollam* extracts exhibited high larvicidal activity against mosquito *C. quinquefasciatus*, suggesting that *C. odollam* extract could be developed as a bioinsecticidal material for larval control.
5. References

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