

# Bioinsecticide Effect of *Pinus merkusii* Tree Bark Extract on *Aedes aegypti* larvae

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## ABSTRACT

**Objective:** *Aedes aegypti* is the vector responsible for the transmission of dengue fever, and it is a cosmopolitan species that proliferates in the water stagnant areas near the houses. Therefore, it is necessary to find new bioinsecticide which is expected to have larvicidal effects. The aims of this study are to evaluate extracts of *Pinus merkusii* tree bark for efficacy against *Aedes aegypti* larvae. **Methods:** This experiment using a completely randomized design with 8 treatment groups. Each group contained five times repetition using 20 third instar larvae of *Aedes aegypti* with concentrations of *Pinus merkusii* tree bark extract are 0, 10, 20, 40, 80, 160, 320, 640 ppm. Larval mortality was observed for 24 h and LC50 was analyzed using Probit analysis. **Results:** Ethanol extract of *Pinus merkusii* tree bark extract showed highest larval mortality against the larvae of *Aedes aegypti* with LC50 = 96.3 ppm; LC90 = 298.4 ppm after 12 h, and LC50 = 58.4

ppm; LC90 = 125.7 ppm after 24 h. **Conclusion:** These findings suggest that extracts from *Pinus merkusii* tree bark have larvicidal effect that can be exploited in development of new bioinsecticides.

**Key words:** *Pinus merkusii*, Larvicidal, *Aedes aegypti*.

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## INTRODUCTION

The most common diseases associated with mosquitoes are dengue fever, chikungunya, yellow fever and the worst, dengue hemorrhagic fever where *Aedes aegypti* (*A. aegypti*) is one of the mosquito species responsible for the transmission of these vector borne diseases. It is widely distributed in the tropical and subtropical zones.<sup>1,2</sup> *A. aegypti* is very closely associated with the human habitat. The geographical range of *A. aegypti* is increasing in part due to rapid urbanization and increased global movement of people and cargo.<sup>3</sup>

The technique in controlling mosquitoes depends on the larval stages (egg, larvae, pupae, and adult) on target. Mosquito control includes targeting the adult mosquito through spraying chemical insecticides or by killing the mosquito larvae before they emerge into adults via using synthetic larvicides or botanical extracts as an alternative larvicide. The indiscriminate use of synthetic insecticides is creating multifarious problems like environmental pollution, insecticide resistance, and toxic hazards to humans.<sup>4</sup> Globally, there have been conscientious efforts to overcome these problems, and great emphasis has been placed recently on enviro-friendly and economically viable methodologies for pest control. Natural products of plant origin are preferred over synthetic insecticides due to their eco-friendly nature. Current research trends use plant extracts as alternative larvicides because they contain various phytochemicals that are specific in killing mosquito larvae without harming other organisms and the environment.<sup>5,6</sup> Instead of using synthetic larvicides, the use of these plant-derived products in controlling mosquito larvae is inexpensive and environment-friendly. In most parts of the world, Synthetic chemical larvicides continue to be applied for controlling mosquitoes but many of these chemicals are toxic to human, animal and plant life and resistance can be problematic in regulating the control. Phytochemicals obtained from the huge diversity of plant

species are important source for safe and biodegradable chemicals, which can be screened for mosquito repellent, larvicidal, and insecticidal activities; and tested for mammalian toxicity. Therefore, researchers are currently exploiting natural substances to be used as insecticides for controlling larval mosquitoes.<sup>7,8</sup>

The phytochemicals of the plants serve as huge storage of compounds that have biological action.<sup>9</sup> Alkaloids, flavonoid, saponins, and tannins are known to possess medicinal and pesticidal properties.<sup>10</sup> These phytochemicals present in the *Pinus merkusii* extract. It has been reported that pinus plant components like phenolics, flavonoids, tannins and other constituents can be used to treat oxidative, inflammatory and microbial.<sup>11</sup> The aim of this study was to evaluate the potential of *Pinus merkusii* tree bark extract against the larvae of *Aedes aegypti* through larvicidal bioassays.

## MATERIALS AND METHODS

### Mosquito culture

*Aedes aegypti* colonies were maintained in our insectary in large enamel basins (45×45×40 cm) and rearing conditions were 28±2°C temperature, 65±5% relative humidity (RH) and photoperiod of 14:10 h light and dark period.<sup>12</sup> The egg strips were obtained from Institute of Tropical Disease Airlangga University Surabaya to start the colony. The strips were immersed in dechlorinated tap water for hatching. Larvae were fed with a diet of finely ground brewer yeast and dog biscuits (3:1). The emerged adults were fed with rabbit blood and with 10% glucose solution. Small porcelain dishes having 50 ml of tap water lined with filter paper was kept inside the cage for oviposition.

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## Preparation of Ethanol Extract of *Pinus merkusii*

Plant material and extract preparation *Pinus merkusii* were collected from Mojolerto, Indonesia. Tree bark were cleaned and chopped into pieces. They were dried under shade at ambient temperature for 5 days and the air-dried pericarps were then ground to powder for extraction. The powdered pericarp (1 kg) was macerated with ethanol 96 % (5 L) for a week at 37°C. The supernatant was then collected and filtered through Whatman No. 1 filter paper in a Buchner funnel under vacuum. The filtrate was concentrated by evaporation with a vacuum rotary evaporator at 45°C. The extract was dried at reduced pressure, stored at 0-4°C and used for the experimentation.

## Phytochemical analysis

The filtrate was tested for the presence of phytochemicals such as alkaloids, flavanoids, saponins, tannins and terpenoids using standard procedures.<sup>13</sup>

## Larvicidal bioassay of *Pinus merkusii* tree bark extract

The tests were conducted at room temperature. The *Pinus merkusii* extracts as larvicide were tested against the third instar larvae of *A. aegypti* mosquitoes.<sup>14</sup> Five replicates of *Pinus merkusii* tree bark extracts dilution with 0, 10, 20, 40, 80, 160, 320 and 640 ppm concentrations were prepared. Each replicate containing 200 ml of the described *Pinus merkusii* tree bark extract was placed in a 500 ml glass beaker. 20 third-instar larvae of *A. aegypti* were transferred into each beaker.<sup>14</sup> After that, the number of dead larvae in each beaker was counted after 1.5; 3; 6; 12 and 24 h. Identification of the *Aedes aegypti* larvae were done by tapping it with a needle in the siphon or cervical area. Each treatment was conducted in five replicates. The larvae were considered dead if, at the end of 24 hrs, they showed no sign of swimming movements even after gentle touching with a glass rod, as described in the World Health Organization's technical report series.<sup>15</sup> The effects of the *Pinus merkusii* extracts were monitored through carefully counting the number of dead larvae after 24 hours of treatment, and the percentage mortality was computed.

$$\text{Percentage mortality} = \frac{\text{Number of dead larve} \quad 100}{\text{Number of larvae introduced}}$$

## Lethal Concentration of *Pinus merkusii* tree bark extract

The LC50 and LC 90 of the *Pinus merkusii* tree bark extract that showed 100% mortality was determined by a similar procedure as mentioned above. 0, 10, 20, 40, 80, 160, 320 and 640 ppm concentration were tested and the observation was recorded after 1.5; 3; 6; 12 and 24 hrs of incubation.

## Statistical Analysis

The statistical tools that were used in this study are the following: the Arithmetic Mean to get the average number of dead mosquito larvae, and Probit Analysis to calculate LC50 and LC90 values to determine Lethal concentrations of the plant extracts on *Aedes aegypti* mosquito larvae after 24 and 48 hours of treatment

## RESULTS

The preliminary phytochemical analysis of *Pinus merkusii* tree bark extracts (Table 1) showed the presence of alkaloids, saponins, flavonoids, triterpenoids and tannins of phytochemicals. Any of these phytochemicals, either singly or in a combination with each other could be responsible for the larvicidal activity of the *Pinus merkusii* tree bark extract.

Different concentrations (0, 10, 20, 40, 80, 160, 320 and 640 ppm) of *Pinus merkusii* tree bark extract solutions were bioassayed against the

**Table 1: Phytochemical analysis of extracts of *Pinus merkusii* tree bark**

<i>Pinus merkusii</i> tree bark extract	
Phytochemicals	Level
Alkaloids	+++
Flavanoids	+++
Saponins	+
Tannins	++
Terpenoids	+++

+: low, ++: immediate, +++: high

third instar larvae of *Aedes aegypti*. The results were recorded after 1, 3, 6, 12 and 24 h of treatment (Table 2). In control treatments, no larvicidal effect was observed; the larvae remained alive, and they moulted into fourth instar larvae. Whereas, when the different *Pinus merkusii* tree bark extract concentrations were tested, different mortality rates were recorded with respect to exposure time.

At 20 ppm *Pinus merkusii* tree bark extract, the larvae remained immobile after 3 h of treatment. When 40 ppm *Pinus merkusii* tree bark extract was tested, 1.8% and 37.4% larval mortality was recorded after 1 h and 24 h of treatment respectively. When 160 ppm *Pinus merkusii* tree bark extract solution was tested, 5.2% and 100% mortality was recorded after 1 h and 24 h of treatment respectively. At 160, 320 ppm complete mortality was recorded after 24 h of exposure, while at 640 ppm, 100% mortality was recorded after 12 h. The maximum result (100%) was recorded with 160 ppm *Pinus merkusii* tree bark extract concentration after 24 h exposure (Table 2).

After 12 h, lethal concentration 50 (50% larvicidal activity) of *Pinus merkusii* tree bark extract was 96.3 ppm while lethal concentration 90 (90% larvicidal activity) was 298.4 ppm. However after 24 h, lethal concentration (50% larvicidal activity) of *Pinus merkusii* tree bark extract was 58.4 ppm while lethal concentration (90% larvicidal activity) was 125.7 ppm (Table 3). The exposure time is very important for larvicidal activity of the *Pinus merkusii* tree bark extract solution.

## DISCUSSION

The medical importance of mosquitoes as vectors for the transmission of serious diseases that cause morbidity, mortality, economical loss, and social disruption such as malaria, lymphatic filariasis, and viral diseases is well documented. *Aedes aegypti*, the primary carrier for viruses that cause dengue and dengue hemorrhagic fever and yellow fever, are widespread over large areas of the tropics and subtropics.<sup>16</sup> The prevention of mosquito breeding through the use of larvicides is the most effective way to fight with this mosquito importation. Synthetic insecticides have been used as larvicide in several countries for the last 30 years.<sup>17</sup> However, the non-selectiveness of insecticides and harmful effects on other organisms is the major hindrance with the use of these chemical insecticides.<sup>18</sup> The need for development of effective insecticides should be taken into consideration due to the toxicity problems, together with the increased incidence of insect resistance. In this study was undertaken to access the toxicant potential of the *Pinus merkusii* against mosquito larvae of *A. aegypti*.

The *Pinus merkusii* tree bark extracts exhibited a concentration dependent activities against *Aedes aegypti* larvae since the percentage mortality were observed to increase with increasing concentrations of the *Pinus merkusii* tree bark extracts. The increase of percentage mortality of the treated *Aedes aegypti* larvae is supported by the presence of phytochemicals in the *Pinus merkusii* extracts which have insecticidal activities. The least percentage mortality was noted in the control group (0 ppm concentration) which is extremely lower compared to those in the experimental

**Table 2: Effect of different concentrations of *Pinus merkusii* tree bark extract solution and exposure time on larvicidal bioassay of third instar larvae of *Aedes aegypti***

<i>Pinus Merkusii</i> Extract (ppm)	Larval Mortality Rate (%) After				
	1.5 h	3 h	6 h	12 h	24 h
0 (Control)	0	0	0	0	0
10	0	0	0	0	0
20	0	1.6 ± 0.3	2.8 ± 0.2	4.3 ± 0.2	11.8 ± 1.6
40	1.8 ± 0.2	4.1 ± 0.5	7.2 ± 0.4	17.4 ± 1.9	37.4 ± 3.9
80	2.6 ± 0.2	6.9 ± 0.2	17.6 ± 2.7	32.3 ± 2.6	69.6 ± 4.8
160	5.2 ± 0.4	13.5 ± 0.7	29.3 ± 3.6	76.8 ± 6.1	100 ± 0
320	7.4 ± 0.3	19.8 ± 1.1	37.3 ± 4.3	91.3 ± 4.7	100 ± 0
640	11.3 ± 0.5	35.8 ± 3.7	48.9 ± 5.2	100 ± 0	100 ± 0

\*The values are mean ± SEM of five replicate.

**Table 3: LC50 and LC90 of extracts of *Pinus merkusii* tree bark**

Time	<i>Pinus merkusii</i> tree bark Extract	
	LC50 (ppm)	LC90 (ppm)
After 12 h	96.3	298.4
After 24 h	58.4	125.7

groups. It reveals that all tested concentrations of the *Pinus merkusii* tree bark extracts (20, 40, 80, 160, 320 and 640) caused mortality of *Aedes aegypti* larvae in comparison to those in the control group.

Result also indicates that 80 ppm concentrations of *Pinus merkusii* tree bark extract have the high percentage of mortality after 24 h. The *Pinus merkusii* tree bark extract high larvicidal activity is supported by the presence of phytochemicals such as alkaloids, saponins, flavonoids, triterpenoids and tannins which are known to have insecticidal and pesticidal properties. The phytochemicals of the plants serve as huge storage of compounds that have biological action. This is supported by the presence of phytochemicals such as alkaloids, saponins, and tannins are known to possess medicinal and pesticidal properties.<sup>10,11,19</sup>

It is further noted that the percentage mortality increased with increasing concentrations of the plant extracts. Moreover, the mortality of mosquito larvae was also increased in relation to the time of exposure. The larvicidal activity of the highest concentration (160 ppm) of the plant extracts on *A. aegypti* mosquito larvae within 24 hours of exposure. Moreover, the results also exhibited that there is a significant difference on the mortality of mosquito larvae between the control group and various concentration of the *Pinus merkusii* tree bark extracts. This result denotes that higher concentration of the *Pinus merkusii* extracts would lead to greater number of mortality in the mosquito larvae.

The lethal concentrations (LC50 and LC90) values of these *Pinus merkusii* tree bark extracts on *Aedes aegypti* mosquito larvae after 24 hours of exposure are 86.7 ppm and 148.9 ppm. It shows that *Pinus merkusii* tree bark extract is the most effective in terms of insecticidal activity. Results show that *Pinus merkusii* is highly lethal to *A. aegypti* larvae.

## CONCLUSION

This study indicates that the ethanolic tree bark extract of *Pinus merkusii* has larvicidal properties and its use as a larvicide against the dengue-vector, *Aedes aegypti* mosquito should be explored. The percentage mortality increased with increasing concentrations of the *Pinus merkusii* tree bark extracts and also increased in relation to the time of exposure. It is worthwhile to study extensively the larvicidal properties of the *Pinus merkusii* by isolating and identifying the active components responsible

for larval mortality, and then test them in field trials in order to assess their potential as an alternative to synthetic chemical larvicides.

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## CONFLICT OF INTEREST

All authors declare no conflict of interest.

## ABBREVIATIONS USED

There are no any typical abbreviations used in manuscripts.

## SUMMARY

- *Pinus merkusii* tree bark extracts showed the presence of alkaloids, saponins, flavonoids, triterpenoids and tannins of phytochemicals
- The lethal concentrations (LC50 and LC90) values of these *Pinus merkusii* tree bark extracts on *Aedes aegypti* mosquito larvae after 24 hours of exposure are 86.7 ppm and 148.9 ppm
- The ethanolic tree bark extract of *Pinus merkusii* has larvicidal properties and its use as a larvicide against the dengue-vector, *Aedes aegypti* mosquito should be explored

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