

**Mosquito Larvicidal Activity of the Essential Oil of *Alstonia Scholaris* (L.)  
R.Br. Against *Aedes Aegypti* L.**

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**Abstract**

The flowers of *Alstonia scholaris* (L.) R.Br. were extracted by maceration using ethanol 95% as a solvent to test the mosquito larvicidal property. The 3<sup>rd</sup>–4<sup>th</sup> mosquito larvae (*Aedes aegypti* L.) were separately treated by drops bioassay against each extract in various concentrations (0 (control), 25, 50, 100, 250 and 500 ppm). The results showed that the extract from maceration exhibited LC<sub>50</sub> against *Ae. aegypti* larvae (24 hours) ca. 176.8 ppm ( $Y = 11.149 + 4.664X$ ). Based on the mortality rate, the concentrations at 500 ppm of the extract from maceration had high insecticidal efficacy on mosquito larvae with 100% mortality after 24 hours exposure. This herbal extract is likely to be applied for controlling the larval mosquitoes.

Keyword: *Alstonia scholaris*, *Aedes aegypti* larvae, maceration

**1. Introduction**

Mosquitos are the most efficient vectors which transmit pathogens causing human malaria, dengue, yellow fever, filariasis, viral encephalitis and other mortal diseases [1]–[3]. Interestingly, *Aedes aegypti* L. (F. Culicidae) is the main species of mosquitoes responsible for dengue fever in several tropical and subtropical countries the number of dengue fever cases has increased significantly in recent years [4], [5]. In Thailand, *Aedes* mosquitoes are the major vectors since there is presently no effective vaccine against dengue. The prevention and control of dengue virus transmission depends on their eradication through two principal measures larviciding and using insecticides [6]–[9]. Most vector surveillance strategies rely merely or only on indicators that have been designed to detect the presence or absence of mosquito larvae or pupae. Elimination of *Ae. aegypti* through source reductions has been proposed but this approach is rather costly, needs full community participation and is

invariably unsuccessful [10]. Additionally, numerous synthetic pyrethroids are commonly used by home owners to control household mosquitoes.

Chemical application could be a crucial cause of insecticide resistance for the house *Ae. aegypti*. The evolution of pyrethroid resistance in this mosquito indicates the limitation of new pyrethroid candidates for *Aedes* mosquito control programs. The development of biologically active materials for mosquitoicides that do not confer cross-resistance to present insecticides is constantly needed. Natural products such as plant-derived insecticides that include a multitude of active ingredients, with distinct modes of action which lessen the chance of resistance in mosquito population [11], are attractive alternative.

Plant extracts, especially essential oils have been used as minor natural resource of insecticides. They constitute a good source of bioactive compounds that are biodegradable into nontoxic products. This minimizes the accumulation of harmful residues and making them more environmentally friendly compared to synthetic compounds [12]. Botanical insecticides used for their larvicidal properties against *Anopheles stephensi*, *Culex quinquefasciatus* and *Aedes aegypti* are ecofriendly [13]. Botanical-based larvicides that are effective and easily available at low cost and do not confer cross-resistance to current insecticides, possess great promise for controlling dengue, especially in cases where vector susceptibility is declining. In this study, we investigated the efficacy of essential oils from *Alstonia scholaris* flowers (F. Apocynaceae) against larval stages of *Ae. aegypti*.

## 2. Material and methods

### 2.1 Mosquito rearing

The 3<sup>rd</sup>-4<sup>th</sup> instar larvae of laboratory-reared *Aedes aegypti* were established in the insectarium of the Program Biology, Faculty of Science and Technology, Pibulsongkram Rajabhat University, Phitsanulok, Thailand and maintained under controlled insectary condition at  $25 \pm 5^\circ \text{C}$ ,  $80 \pm 10\%$  relative humidity, with a 12:12 hour light: dark photophase regime. *Ae. aegypti* was used as a test species because of its easy collection as well as expedience in rearing and maintaining the life cycle. Its sensitivity to larvicides makes *Ae. aegypti* larvae a good indicator of biocidal activity.

### 2.2 Plant material

Fresh flowers of *Alstonia scholaris* were gathered at Pibulsongkram Rajabhat University, Phitsanulok province, Thailand in November 2013. Plant specimen was identified by comparison with reference material at the CMU herbarium, Faculty of Science, Chiang Mai University, Chiang Mai province, Thailand.

### 2.3 Extraction of essential oil

Fresh flowers (500 g) of *Alstonia scholaris* were macerated with ethanol (1.5 L) as solvent for 1 week. After filtration ethanol were evaporated to dryness under reduced pressure. The crude extract obtained was kept in dark glass bottles covered tightly with aluminum foil, labeled then stored in a refrigerator till needed for experiments. The extract was re-dissolved with ethanol and later the water extracts were used directly for the bioassay.

### Larvicidal bioassay

According to WHO recommendations [14], 3<sup>rd</sup>-4<sup>th</sup> instar larvae of *Aedes aegypti* were exposed to extracted oils at various concentrations. Five different extract solutions each with concentration ranging from 25 to 500 ppm (25, 50 100, 250 and 500) were prepared. A set of 25 healthy mosquito larvae was placed in each plastic cups containing 150 ml of water and the test concentration. Four replication for each concentration and control (with water) were tested for larval bioefficacy. The larval mortality at different concentrations and in the control was counted after 24 hours exposure.

### Statistical analysis

The mortality data were subjected to log probit regression analysis [15] to determine the median lethal concentrations (LC<sub>50</sub>) to kill 50 percent of the treated larvae. The percentage of larval mortality was calculated and when control mortality ranged from 5-20% it was corrected using Abbott's [16] formula. To determine the difference in larval mortality between concentrations, ANOVA followed by LSD tests were performed by using SPSS for windows version 16.0 [17]. Results with  $P < 0.05$  were also considered to be statistically significant.

## 3. Results and discussion

The extracts examined in our study had a promising larvicidal efficacy on *Ae. aegypti* after 24 hours exposure with an LC<sub>50</sub> of 188.3 ppm (Table 1). No larval mortality was observed in the untreated groups, whereas dose dependent larval mortalities were substantial in the extract treated groups. Increasing concentrations from 25 to 500 ppm were evident (Table 1). The highest mortality percentage (100%) of larval mosquito was at 500 ppm, followed by 250, 100, 50 and 25 ppm (59, 21, 3 and 1% larval mortalities).

The analysis of variance (ANOVA) F-test shows that there was significant difference in means between the concentration groups ( $P < 0.05$ ). Differences between means using the least significant difference (LSD) tests were also considered significant at the 95% level of confidence. The results indicated that means with the same letter were not significantly different as shown in Table 1.

The larvicidal bioassay exhibited a promising efficacy of plant oil extracted from *A. scholaris* flowers against *Ae. aegypti*. Our results conformed to Kaushik and Saini [13], who used concentrations between 100 to 300 ppm, prepared by maceration method using acetone as the solvent. Nevertheless, the flower extract was significantly more effective ( $LC_{50} = 176.8$  ppm) when compared to its leaf extract with acetone (maceration) ( $LC_{50} = 239.9$  ppm) [13].

The susceptibility of *Ae. aegypti* larvae to a graded series of extracted essential oils under laboratory conditions was dose dependent. Mortality increased when exposed to a higher concentration. According to Kaushik and Saini [13], [18] the toxic effect of this plant oil was probably on the neuromuscular system resulting in abnormal behavior of the treated larvae i.e. restlessness, sluggishness and coiling movement.

**Table 1.** Larvicidal activity extracted essential oil against 3<sup>rd</sup>-4<sup>th</sup> instar larvae of *Ae. Aegypti*.

Means with the same letter were not significant.

<sup>1/</sup> = Regression equation (Y): plant extract concentration (X) at 24 hours

| Conc.<br>(ppm)      | Mortality (%)    | LC <sub>50</sub><br>(ppm) | Regression <sup>1/</sup> | r <sup>2/</sup> | R <sup>23/</sup> |
|---------------------|------------------|---------------------------|--------------------------|-----------------|------------------|
| Control             | 0 <sup>d</sup>   |                           |                          |                 |                  |
| 25                  | 1 <sup>d</sup>   |                           |                          |                 |                  |
| 50                  | 3 <sup>cd</sup>  |                           |                          |                 |                  |
| 100                 | 21 <sup>c</sup>  |                           |                          |                 |                  |
| 250                 | 59 <sup>b</sup>  | 176.8                     | Y = 11.149 + 4.664X      | 0.993           | 0.985            |
| 500                 | 100 <sup>a</sup> |                           |                          |                 |                  |
| F-test              | *                |                           |                          |                 |                  |
| LSD <sub>0.05</sub> | 8.00             |                           |                          |                 |                  |
| C.V. (%)            | 18.5             |                           |                          |                 |                  |

<sup>2/</sup>r = Correlation Coefficient of mosquito larvae mortality and plant extract concentration

<sup>3/</sup>R<sup>2</sup> = Regression Coefficient

\* = Significant difference ( $P < 0.05$ )

These is great medical importance in controlling a main vector of viral diseases i.e. dengue, dengue hemorrhagic and Chikungunya fevers, which are serious health problems in Thailand and other developing countries [5], [18]. There is possibility of developing new types of mosquito larvicides from essential oils for application in mosquito control programs. *A. scholaris* has potential usefulness for other arthropod vector control and should be selected for further study, particularly in controlling dengue and other mosquito-borne diseases.

#### 4. Conclusion

A larvicidal bioassay was carried to evaluate the larvicidal activity of medical plant (*A. scholaris*) extract against *Ae. aegypti*. The essential oils showed good larvicidal property with the LC<sub>50</sub> of 176.8 ppm. Based on the mortality rate, the concentration of 500 ppm had the highest insecticidal efficacy on mosquito larvae with 100% after 24 hours of exposure. Larvicidal activity increased with increased dosage in all trials. The results reported here open the possibility of further investigations of efficacy on its larvicidal property of natural product extract. In order to achieve the best yield with good quality and quantity, the most suitable procedure should be determined.

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