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# Evaluation of Larvicidal Activity of Tridax procumbens (Asteraceae) Leaf Extract Against the Dengue Vector, Aedes aegypti and Bancroftian Filariasis Vector, Culex quinquefasciatus

KEYWORDS: Tridax procumbens, Larvicidal activity, Aedes aegypti and Culex quinquefasciatus.

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**ABSTRACT** *The leaf extract of Tridax procumbens with different solvents Hexane, Acetone, Ethyl acetate and Methanol were tested for larvicidal activity against two important mosquitoes such as dengue vector, Aedes aegypti and Bancroftian filariasis vector, Culex quinquefasciatus. The Tridax procumbens leaves and stem powder was soaked in Hexane, Acetone, Ethyl acetate, Methanol (each solvent 1200 ml). The plant powder soaked with solvents for three days. The filtered sample was poured into a soxhlet apparatus (boiling point range 100°C). After two hours, the filter paper was for concentrated at room temperature until oily paste formed and kept at cool dry place further used. From the stock solution, 500 ppm was prepared with dechlorinated tap water. The numbers of dead larvae were counted after 24 h of exposure and the percentage of mortality was recorded from the average of five replicates. The average larval mortality data were subjected to probit analysis for calculating LC50, LC90 and other statistics at 95% fiducial limits of upper confidence limit and lower confidence limit. Results with p<0.05 were considered to be statistically significant. The results of the leaf extract of Tridax procumbens are promising as good larvicidal activity against the mosquito vector, A. aegypti and C. quinquefasciatus.*

## Introduction

Mosquitoes are known vectors of several disease-causing pathogens, which affect millions of people worldwide. Aedes aegypti is known to carry dengue, yellow fever and Chikungunya; and filarial disease by Culex quinquefasciatus. Mullai et al. (2008) have reported that the leaf extract of Citrullus vulgaris with different solvents (e.g., benzene, petroleum ether, ethyl acetate, and methanol) were tested for larvicidal, ovicidal, repellent, and insect growth regulatory activities against A. stephensi. Govindarajan (2009) reported that the leaf methanol, benzene, and acetone extracts of Cassia fistula were studied for the larvicidal, ovicidal, and repellent activities against A. aegypti. The ethanolic leaf extract of Cassia obtusifolia (Rajkumar and Jebanesan 2009. The results of the present study would be useful in promoting research aiming at the development of new agent for mosquito control based on plant source. This study was undertaken to assess the larvicidal properties of Tridax procumbens leaf extracts of against the medically important mosquito vector, A. aegypti and C. quinquefasciatus.

## Materials and methods

### Mosquito culture

A. aegypti and C. quinquefasciatus colonies were maintained in our insectary (45×45×40 cm) at 27±2°C and 80± 2% RH with a photoperiod of 14:10-h light and dark cycles as per the procedure of (Sharma and Saxena 1994). The egg strips were obtained from Zonal Entomological Research Centre, Vellore (12° 55′ 48″ N, 79° 7′ 48″ E) 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 adults were given a blood meal from a pigeon (Reuben 1987).

### Plant materials

Tridax procumbens (Asteraceae) leaves were collected from Auxilium College campus, Katpadi Vellore District, Tamil Nadu, (South India) in November 2013. The taxonomic identification was made by Ms. S. Isabella Rosaline, Department of Botany, Auxilium College, Katpadi, Vellore District, Tamil Nadu, (South India). The voucher specimen was deposited in our research laboratory for further reference.

### Preparation of plant extracts

The dried leaves (300 g) were powdered mechanically using a commercial electrical stainless steel blender and extracted with in Hexane, Acetone, Ethyl acetate, Methanol (Qualigens) in a soxhlet apparatus (1200 ml) separately until exhaustion (Irungu and Mwangi 1995). The extract was Concentrated under reduced pressure 22-26 mg Hg at 45°C, and the residue obtained was stored at 4°C.

### Larvicidal Bioassay

One gram of solvent extract was first dissolved in 100 ml of respective solvent (stock solution). From the stock solu-

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tion, 500 ppm was prepared with dechlorinated tap water. The larvicidal activity was assessed by the procedure of (WHO, 1996) with some modification and as per the method of (Rahuman et al., 2000). For bioassay test, larvae were taken in five batches of 20 each in 249 ml of water and 1.0 ml of the desired plant extract concentration. The control was set up with respective solvent. The numbers of dead larvae were counted after 24 h of exposure and the percentage of mortality was recorded from the average of five replicates. The experimental media having 100% mortality of larvae alone were selected for dose.

**Dose-response bioassay**

From the stock solution, different concentrations ranging from 31.25 to 500 ppm were prepared. Based on the preliminary screening results, different solvents of plants are among the crude extracts tested, the present results showed the following Hexane, Acetone, Ethylacetate, Methanol extracts of Tridax procumbens.

**Statistical analysis**

The average larval mortality data were subjected to probit analysis for calculating $LC_{50}$, $LC_{90}$ and other statistics at 95% fiducial limits of upper confidence limit and lower confidence limit, and chi-square values were calculated using the software developed by (Reddy et al., 1992). Results with $p<0.05$ were considered to be statistically significant.

**Results**

The solvent Hexane, Ethyl acetate, Acetone and Methanol extracts of the leaves of the plant Tridax procumbens were studied for use as eco-friendly insecticides instead. Results on the larvicidal activities of leaf extracts obtained in this study (Tables 1 and 2) confirm their potential for the control of larval population of mosquito vectors. Hexane, Ethyl acetate, Acetone resulted in moderate mortality; however, the highest larval mortality was Methanol extract observed in two mosquito vectors. Among the solvent extracts tested, the present results showed the following leaf Hexane, Ethyl acetate, Acetone and Methanol against the larvae of Aedes aegypti ($LC_{50}$=393.34, 174.06, 97.93, 52.87, 469.47, 267.22, 210.11 and 139.54 ppm; $LC_{90}$=1180.02, 522.18, 293.79, 158.61, 1408.41, 801.66, 630.33, and 418.62 ppm, $r^2$ = 0.879, 0.999, 0.982, 0.867, 0.877, 0.998, 0.982 and 0.897 ppm, respectively) and against the larvae of Culex quinquefasciatus ($LC_{50}$=450.62, 224.60, 195.03, 94.19, 413.88, 193.83, 137.07 and 75.64 ppm; $LC_{90}$=1351.86, 673.8, 585.09, 282.57, 1241.6, 3581.49, 411.21 and 226.9 ppm, $r^2$ = 0.789, 0.897, 0.888, 0.779, 0.992, 0.862, 0.988 and 0.888 respectively. The $r^2$ regression co-efficient values are significant at $P<0.05$ level. The 95% confidence limits $LC_{50}$ (LCL–UCL) and $LC_{90}$ (LCL–UCL) were also calculated. Larval mortality was observed after 24 h exposure. No mortality was observed in the control group. The results of larvicidal activity clearly indicate that the percentage of mortality being directly proportional to the concentration of the extract. Solvents of the plant extract of Tridax procumbens were used at different concentrations, ranging from 31.25 to 500 ppm, respectively.

**Discussion**

Mosquito-borne diseases, such as filariasis, malaria, dengue, yellow fever, and Japanese encephalitis, contribute significantly to disease burden, death, poverty, and social debility in tropical countries (Jang et al. 2002). Recently, studies reported that the leaf extract of methanol J. curcas against the first to fourth instar larvae showed values of $LC_{50}$=1.200%, 1.290%, 1.358%, and 1.448% and $LC_{90}$=2.094%, 2.323%, 2.444%, and 2.544% larvae of C. quinquefasciatus, respectively (Kovendan et al. 2011). The results of these study revealed that the Methanol, Acetone, Hexane and Ethyl acetate extracts of Tridax procumbens was effective against the third instars larvae of the two species of mosquito (Aedes aegypti and Culex quinquefasciatus) when compared with solvent. In the present study, the maximum mortality was observed in the different solvent extracts, ($LC_{50}$=393.34, 174.06, 97.93, 52.87, 469.47, 267.22, 210.11 and 139.54 ppm; $LC_{90}$=1180.02, 522.18, 293.79, 158.61, 1408.41, 801.66, 630.33, and 418.62 ppm, $r^2$ = 0.879, 0.999, 0.982, 0.867, 0.877, 0.998, 0.982 and 0.897 ppm, against the third instar larva of A. aegypti and C. quinquefasciatus ($LC_{50}$=450.62, 224.60, 195.03, 94.19, 413.88, 193.83, 137.07 and 75.64 ppm; $LC_{90}$=1351.86, 673.8, 585.09, 282.57, 1241.6, 3581.49, 411.21 and 226.9 ppm, $r^2$ = 0.789, 0.897, 0.888, 0.779, 0.992, 0.862, 0.988and 0.888 respectively. In conclusion, an attempt has been made to evaluate the role of plant extracts in mosquito larvicidal activity. The results reported in this study open the possibility of further investigations on the efficacy of the larvicidal properties of natural product extracts.

**Table 1. Larvicidal activity of T. procumbens leaf solvent extracts against third instar larvae of A. aegypti**

| Extract | Concentrations (ppm) | % Mortality ± SD | $LC_{50}$ (LCL-UCL) | $LC_{90}$ (LCL-UCL) | Slope | $r^2$ |
|---|---|---|---|---|---|---|
| Hexane | 500 | 59 | | | | |
| | 250 | 33 | | | | |
| | 125 | 26 | 393.34 (324.57-476.68) | 1180.02 (1162.4-1205.1) | 26 | 0.879 |
| | 62.50 | 19 | | | | |
| | 31.25 | 5 | | | | |
| Ethyl | 500 | 84 | | | | |
| Acetate | 250 | 63 | | | | |
| | 125 | 31 | 174.06 (146.16-207.18) | 522.18 (498.24-562.44) | 31 | 0.999 |
| | 62.50 | 24 | | | | |
| | 31.25 | 17 | | | | |
| Acetone | 500 | 100 | | | | |
| | 250 | 82 | | | | |
| | 125 | 54 | 97.93 (81.69-117.41) | 293.79 (262.19-372.02) | 54 | 0.982 |
| | 62.50 | 36 | | | | |
| | 31.25 | 22 | | | | |
| Methanol | 500 | 100 | | | | |
| | 250 | 98 | | | | |
| | 125 | 86 | 52.87 (40.10-69.71) | 158.61 (121.24-192.02) | 52 | 0.867 |
| | 62.50 | 52 | | | | |
| | 31.25 | 39 | | | | |

Control: Distilled water nil mortality, $LD_{50}$ lethal doses that kills 50% of the exposed adult, $LD_{90}$ lethal doses that kills 90% of the exposed adult, UCL upper confidence limit, LCL lower confidence limit and $r^2$ regression co-efficient.

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**Table 2. Larvicidal activity of T. procumbens leaf solvent extracts against third instar larvae of C.quinquefasciatus**

| Extract | Concentrations (ppm) | % Mortality ± SD | $LC_{50}$ (LCL-UCL) | $LC_{90}$ (LCL-UCL) | Slope | $r^2$ |
|---|---|---|---|---|---|---|
| Hexane | 500 | 54 | | | | |
| | 250 | 39 | | | | |
| | 125 | 19 | 450.62 (290.39-594.85) | 1351.86 (1298.1-1409.2) | 19 | 0.789 |
| | 62.50 | 10 | | | | |
| | 31.25 | 2 | | | | |
| Ethyl | 500 | 68 | | | | |
| Acetate | 250 | 50 | | | | |
| | 125 | 34 | 224.60 (192.27-311.29) | 673.8 (612.5-744.20) | 34 | 0.897 |
| | 62.50 | 19 | | | | |
| | 31.25 | 9 | | | | |
| Acetone | 500 | 74 | | | | |
| | 250 | 56 | | | | |
| | 125 | 39 | 195.03 (158.10-240.46) | 585.09 (498.27-627.85) | 39 | 0.888 |
| | 62.50 | 21 | | | | |
| | 31.25 | 9 | | | | |
| Methanol | 500 | 100 | | | | |
| | 250 | 81 | | | | |
| | 125 | 67 | 94.19 (81.52-108.80) | 282.57 (220.62-312.94) | 67 | 0.779 |
| | 62.50 | 29 | | | | |
| | 31.25 | 50 | | | | |

Control: Distilled water nil mortality, LD50 lethal doses that kills 50% of the exposed adult, LD90 lethal doses that kills 90% of the exposed adult, UCL upper confidence limit, LCL lower confidence limit and r2 regression co-efficient.

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