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# Larvicidal activities of six plants extracts against two mosquito species, *Aedes aegypti* and *Anopheles stephensi*

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**Abstract.** Larvicidal activity of crude chloroform, dichloromethane and methanol extracts of the leaves and roots of six Indian plants, *Aegle marmelos* L., *Balanites aegyptica* L., *Calotropis gigantica* L., *Murraya koenigii* L., *Nyctanthes arbor-tristis* L. and *Plumbago zeylanica* L., were tested against the early fourth instar larvae of *Aedes aegypti* L. and *Anopheles stephensi*. The larval mortality was observed after 24 h of exposure. All extracts showed moderate larvicidal effects. However, the highest larval mortality was found in methanol extracts of *P. zeylanica* roots and *B. aegyptica* roots against *Ae.aegypti* ($LC_{50}$ 169.61 mg/lit, 289.59 mg/lit) and *An.stephensi* ($LC_{50}$ 222.34 mg/lit, 102.29 mg/lit), respectively. The methanol extracts of plants were more effective than the other extracts. This is an ideal eco-friendly approach aid for the control of mosquito species, *Ae. aegypti*, and *An.stephensi*.

## INTRODUCTION

Several mosquito species belonging to genera *Anopheles*, *Culex*, and *Aedes* are vectors for the pathogens of various diseases like malaria, filariasis, Japanese encephalitis, dengue, yellow fever, chikungunya, etc. (Redwane *et al*., 2002). Presently organophosphate, organochlorine, and synthetic pyrethroid insecticides are being used for public health control measures. Successive changes in insecticides results in multiple insecticide resistance. Malaria vectors in India are resistant to DDT, Malathion and Deltamethrin (Ragvendra & Subbarao 2002). Phytochemicals derived from plant sources can act as larvicides, insect growth regulators, repellents, and oviposition attractants and can play an important role in the interruption of the transmission of mosquito-borne diseases at the individual as well as at the community level (Choochote *et al*., 2004; Mullai, 2007; Mathew *et al*., 2009). Thus, one of the approaches for control of these mosquito-borne diseases is the interruption of disease transmission by killing or preventing mosquitoes from biting human beings. The search for herbal preparations that do not produce any adverse effects on the non target organisms and are easily biodegradable remains a top research issue for scientists associated with alternative vector control (Redwane *et al*., 2002). As a part of our search for the biodiversity resource available in India for natural products with utilizable bioactivity, we have assayed larvicidal potential of the extracts of *Aegle marmelos* L., *Calotropis gigantica* L., *Murraya koenigii* L., *Nyctanthes arbor-tristis* L., *Balanites aegyptica* L. and *Plumbago zeylanica* L. against *Aedes aegypti* and *Anopheles stephensi* larvae. These are widely distributed in many parts of India. Although

reported for medicinal properties (Table 1), as far as our literature survey could ascertain, this is the first report on the larvicidal activity of the *N. arbor-tristis* L., *B. aegyptica* L. and *P. zeylanica* L. in respective solvent extracts used in present study.

## MATERIAL AND METHODS

The leaves of *A. marmelos* L., *C. gigantica* L., *M. koenigii* L., *N. arbor-tristis* L., and roots of *B. aegyptica* L., *P. zeylanica* L. were collected from the campus of North Maharashtra University, Jalgaon (210 00' 24.5" N, 750 29' 45.5" E, Elevation 218 m). Taxonomic identification was made by Dr. G. S. Chaudhary, Department of Botany, M.J. College, Jalgaon, Maharashtra, India.

**Preparation of plant extracts**

The dried leaves and roots (100 g) were powdered mechanically using commercial electrical stainless steel blender and extracted with chloroform (150 ml, Qualigens), Dichloromethane (200 ml, Qualigens), and methanol (250 ml, Qualigens) in a soxhlet apparatus separately until exhaustion. The extracts were concentrated in a rotary vacuum evaporator (Buchi) and residues obtained were stored at 4°C.

**Test organisms**

For the laboratory trial, larvae of *Ae. aegypti* and *An. stephensi* were collected from stagnant water ponds from slum areas in the Jalgaon city (21° 2' 54" N, 76° 32' 3" E, Elevation 209 m). The mosquito species were identified by an entomologist at the District Malaria control Department, Jalgaon, Maharashtra, India. The larvae were kept in plastic enamel trays containing dechlorinated tap water. They were maintained, and all the experiment were carried out at 27±2°C and 75-85% relative humidity under 14:10 light and dark cycles. Larvae were fed with a diet of finely ground brewer's yeast and dog biscuits (3:1).

**Larvicidal bioassay**

One gram of each crude extract was first dissolved in 100 ml of respective solvent (stock solution). From the stock solution, 1 000 mg/lit each sample was prepared with dechlorinated tap water. Polysorbate 80 (Qualigens) was used as an emulsifier at the concentration of 0.05% in the final test solution. The larvicidal activity was assessed as per procedure of WHO (1996) with some modifications. For bioassay test, larvae were taken in five batches of 25 in 249 ml of water and 1.0 ml of the desired plant extract concentration. The control was set up as described by Elango *et al.* (2009). The numbers of dead larvae were counted after 24 h of exposure, and the percentage mortality was reported from the average of five replicates. The experimental media, in which 100% mortality of larvae occurred, was selected for the dose-response bioassay.

**Dose-response bioassay**

From the stock solution, different concentrations ranging from 10 to 1 000 mg/lit were prepared. Based on the preliminary screening results, crude different solvent sample prepared from root extracts of *B. aegyptiaca*, *P. zeylanica* and leaf extracts of *N. arbor-tristis* were subjected to dose-response bioassay for larvicidal activity against the larvae of *Ae. aegypti* and *An. stephensi*. The numbers of dead larvae were counted after 24 h of exposure, and the percentage mortality was reported from the average of five replicates.

**Data management and statistical analysis**

Average larval mortality data were subjected to probability analysis conducted on mortality counts made after 24 h exposure for calculating $LC_{50}$, $LC_{90}$ along with 95% fiducial confidence intervals using statistical package Minitab (2000). Mortality was calculated using Abbott's formula (Abbott, 1925).

$$\% \text{ Larval Mortality} = \frac{\text{Mortality in Test Concentration - Mortality in Control}}{100 - \text{Mortality in Control}} \times 100$$

## RESULT AND DISCUSSION

Table 1 lists all plants used in present study, with their common name, family, medicinal property and parts used for bioassay. The plant parts were selected based on traditional use and previous report of presence of bioactive compounds (Chopra *et al.*, 1996; Shallan *et al.*, 2005). The activity of crude plant extract is often attributed to the complex mixture of active compounds. The preliminary screening is a good mean of evaluation of the potential larvicidal activity of plants popularly used for this purpose. Larvicidal activity of different crude solvent extracts of six plants are noted and presented in Table 2. Based on the preliminary screening results, three different crude solvent extracts were subjected to bioassay. Out of these, methanol extract of *P. zeylanica* roots was found to possess the most effective larvicidal activity ($LC_{50}$ 169.1 mg/lit) against *Ae. aegypti* followed by dichloromethane extract of *N. arbor-tristis* ($LC_{50}$ 260.72 mg/lit) leaves and methanol extract of *B. aegyptiaca* roots ($LC_{50}$ 289.59 mg/lit). Bioassay of *An. stephensi* with methanol extract of *B. aegyptiaca* roots showed most effective larvicidal activity ($LC_{50}$ 102.29 mg/lit) followed by dichloromethane extracts of *N. arbor- tristis* leaves ($LC_{50}$ 114.56 mg/lit) and methanol extracts of *P. zeylanica* roots ($LC_{50}$ 222.34 mg/lit) (Table 3).

The varying results obtained in lethal concentration were probably due to the differences in the levels of toxicity among the insecticidal ingredients of each plant and the effect of plant extracts can vary significantly depending on plant species, plant part, age of plant part, solvent of extraction and mosquito species (Shallan *et al.*, 2005).

The leaves extract of *N. arbor- tristis* had been earlier reported to possess larvicidal activity against *Culex quinquefasciatus*, *Ae. aegypti* and *An. stephensi* by Mathew *et al.* (2009). However, as evident from the present results crude dichloromethane extract of *N. arbor- tristis* leaves was significantly more effective ($LC_{50}$ 114.5, 260.72 mg/lit) as compared to its extract prepared with chloroform ($LC_{50}$ 780.6, 526.3 mg/lit) against *Ae. aegypti* and *An. stephensi*,

Table 1. List of plants tested for the bioactivity against larvae of *Ae. aegypti* and *An. stephensi* with their reported medicinal properties

| Botanical name | Common name | Family | Medicinal property | Plant part used |
|---|---|---|---|---|
| *Aegle marmelos* L. | wood apple | Rutaceae | Digestive, expectorant, cooling, insecticide. | Leaf |
| *Balanites aegyptica* L. | desert date | Simaroubaceae | Antibacterial, antifungal anthelmintic, insecticide. | Root |
| *Calotropis gigantica* L. | Crown flower | Asclepiadaceae | Useful in acute and sub acute dysentery, bronchitis, insecticide. | Leaf |
| *Murraya koenigii* L. | curry leaf plant | Rutaceae | Used as antiemetic, carminative, antidiarrhoel agent, insecticide. | Leaf |
| *Nyctanthes arbor-tristis* L. | Coral Jasmine | Oleaceae | Used in fever, rheumatism, obstinate sciatica, insecticide. | Leaf |
| *Plumbago zeylanica* L. | White Leadwort | Plumbaginaceae | Alterative, gastric stimulant, appetizer, insecticide. | Root |

Table 2. Larvicidal activity of different plant crude extracts against fourth -instar larvae of *Ae. aegypti and An. stephensi* at 1,000 mg/lit after 24hr

| Name of the Plant | Species | Percent Mortality[a] ± SD | | |
|---|---|---|---|---|
| | | Chloroform | Methanol | Dichloromethane |
| *Aegle marmelos* L. | *Ae. aegypti* | 64.4 ± 2.966 | 81.8 ± 2.387 | 73.2 ± 2.774 |
| | *An. stephensi* | 71.2 ± 1.923 | 84.2 ± 3.114 | 79.6 ± 2.302 |
| *Balanites aegyptica* L. | *Ae. aegypti* | 69.4 ± 1.816 | 100 ± 0.000 | 87.6 ± 1.816 |
| | *An. stephensi* | 70 ± 1.581 | 100 ± 0.000 | 79.2 ± 3.193 |
| *Calotropis gigantica* L. | *Ae. aegypti* | 80 ± 2.738 | 85.6 ± 3.130 | 70.6 ± 1.516 |
| | *An. stephensi* | 84.4 ± 2.073 | 78.2 ± 1.643 | 76.4 ± 2.408 |
| *Murraya koenigii* L. | *Ae. aegypti* | 59.6 ± 1.673 | 78.8 ± 3.193 | 80.4 ± 2.073 |
| | *An. stephensi* | 68.8 ± 1.923 | 85.2 ± 2.387 | 75 ± 2.345 |
| *Nyctanthes arbor-tristis* L. | *Ae. aegypti* | 63.4 ± 2.073 | 81.2 ± 2.280 | 100 ± 0.000 |
| | *An. stephensi* | 72.2 ± 1.923 | 79.4 ± 2.073 | 100 ± 0.000 |
| *Plumbago zeylanica* L. | *Ae. aegypti* | 62.2 ± 2.588 | 100 ± 0.000 | 74.8 ± 1.788 |
| | *An. stephensi* | 69 ± 1.581 | 100 ± 0.000 | 82.8 ± 2.774 |

*Control* nil mortality
[a] Mean of five replicates

Table 3. Larvicidal activity of different solvent crude extracts against fourth instar larvae of *Ae. aegypti* and *An. stephensi* after 24hr

| Name of the Plant | Solvents | Species | $LC_{50}$±SE(mg/lit) (LCL–UCL) | $LC_{90}$±SE(mg/lit) (LCL–UCL) |
|---|---|---|---|---|
| *Balanites aegyptica* L. | Methanol | *Ae. aegypti* | 289.59 ± 10.94 (267.97–311.01) | 677.92 ± 19.64 (641.94–719.40) |
| | | *An. stephensi* | 102.29 ± 6.21 (89.84–114.37) | 275.82 ± 11.73 (254.94–301.51) |
| *Nyctanthes arbor-tristis* L. | Dichloromethane | *Ae. aegypti* | 260.72 ± 9.69 (241.63–279.79) | 587.63 ± 17.12 (556.24–623.77) |
| | | *An. stephensi* | 114.56 ± 8.57 (97.14–130.96) | 367.00 ± 14.52 (340.87–398.40) |
| *Plumbago zeylanica* L. | Methanol | *Ae. aegypti* | 169.61 ± 7.99 (153.76–185.24) | 415.48 ± 14.25 (389.62–445.95) |
| | | *An. stephensi* | 222.34 ± 8.65 (205.31–239.38) | 499.31 ± 15.36 (471.25–531.85) |

*Control* nil mortality, Significant at $P < 0.05$ level, $LC_{50}$ lethal concentration that kills 50% of the exposed larvae, $LC_{90}$ lethal concentration that kills 90% of the exposed larvae, *LCL* lower confidence limit, *UCL* upper confidence limit

respectively. This confirms the effect of extraction solvent on efficacy of plant as reviewed by Shallan *et al.* (2005).

The different parts of the *P. zeylanica* plant have been studied for various medicinal properties (Chopra *et al.*, 1996). The napthoquinone, plumbagin was isolated from the plant reported having chitin synthetase inhibiting activity (Kubo *et al.*, 1983). This suggests that the chemical constituents present in the root extract may arrest the metabolic activities of the larvae. The larvicidal activities of *B. aegyptiaca* have been reported against mosquito larvae (Zarrong *et al.*, 1990; Wiseman & Chapagain, 2006). The high potency of *B. aegyptiaca* as larvicide against mosquito species is reemphasized in the present study.

Our study reflects the larvicidal potency of crude extracts obtained from *B. aegyptiaca*, *N. arbor tristis*, *P. zeylanica* against *Ae. aegypti*, *An. stephensi* larvae which is the basic and most important step in the development of an insecticide of botanical source. There are probabilities that the active principle contained in these plant extracts, especially the methanol extracted fractions will be further more potent as mosquito larvicides as compared with their crude forms. The identification and isolation of these active components is a part of further search for an efficient, ecofriendly, biodegradable insecticide of plant origin and is under consideration in the laboratory.

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