Euphorbia antiquorum latex and its mosquitocidal potency against Aedes aegypti

J. Benrit Vimal and S. Sam Manohar Das

Abstract
Aedes aegypti is the primary vector of chikungunya, yellow fever and dengue. Dengue fever is the major cause of child morbidity in hospitalization in some Asian and African countries. The methanolic latex extract of Euphorbia antiquorum was tested for larvicidal activity against the fourth instar larvae of Ae. aegypti. Latex extract showed high toxicity to the mosquito larvae and gave a LC₅₀ value of 10.70 ml/dl at 48hr exposure.

Keywords: Euphorbia antiquorum, Latex extract, Mosquito larvae, Aedes aegypti.

1. Introduction
Mosquito-borne diseases, such as malaria, filariasis and dengue fever are major public health problems in the South East Asian countries because of their tropical climate, poor drainage system especially during rainy season and the presence of many fish farms, irrigation ditches and rice fields which provide abundant mosquito breeding places. Chemical vector control programs have been carried on for long time, but mosquito-vector diseases exit because of the refusal by house holders to house spraying with synthetic insecticides and evolution of mosquito resistance to conventional insecticides [1, 2, 3].

Due to the disadvantages associated with such synthetic pesticides inducing development of pesticide resistant strains, ecological imbalance and harm to non-target organisms there is a renewed effort to develop substances of plant origin which are considered to be environment friendly due their innate biodegradability and lower toxicity to most organisms [4].

Botanical phytochemicals with mosquitocidal and larvicidal potential are now recognized as potent alternative insecticides to replace synthetic insecticides in mosquito control programs due to their excellent larvicidal, pucidal and adulticidal properties [5, 6, 7]. It has renewed the interest in the research on these compounds considering them as an ecologically safe alternative [8, 9].

The main objective of the present study was to determine the effect of mosquitocidal properties of Euphorbia antiquorum latex extract against the fourth instar larvae of the mosquito Aedes aegypti.

2. Materials and methods
2.1 Plant Material
The present study was carried out in Scott Christian College, Nagercoil during the period of 2013 – 2014. The plant material used for the collection of latex was Euphorbia antiquorum L. belonging to Euphorbiaceae family.

2.2 Collection and preparation of Euphorbia antiquorum latex extract
Latex samples were collected early in the morning from plants by nipping the leaves and stem or by incision of the trunk and branches of the plant and allowing the latex to drain in clean glass tubes separately, brought to the laboratory and kept in the refrigerator till use.

The collected latex was mixed with methanol in the ratio of 1:9 (10%) and centrifuged at 3500 rpm for 5 minutes and the supernatant was collected in a glass vial and stored at 4 °C till further use.

2.3 Procurement of Ae. aegypti larvae
The eggs of Ae. aegypti were procured from Indian council of Medical Research (ICMR) Madurai. Filter paper with attached eggs was dipped into plastic tray containing water over night and the eggs were allowed to hatch.
The larvae were fed with ground biscuit until they moulted to pupal stage. They were transferred into a mosquito cage where the emergent adults were fed with 10% sucrose solution. Five days after emergence, female mosquitoes were allowed to feed on blood of white mice for 2-3 hours. The gravid mosquitoes were allowed to lay eggs.

2.4 Screening for larvicidal activity of methanolic extracts of E. antiquorum against Ae. aegypti

Larvicidal effects of E. antiquorum were assessed using the standard method of [18] with some modifications. Different concentrations of latex were prepared from the latex (01, 03, 05, 07, 09, 11, 13, 15, 17 and 19 ml/dl) diluted in 100 ml of water in plastic cups. In each cup, 10 4th instar larvae were released. Each experiment was conducted with 5 replicates and control. 100 ml tap water was maintained as the control. No food was provided during the treatment. Observations were recorded 12, 24, 36, 48, 60 and 72 hours after treatment. Dead larvae were assured by non-wriggling movement and settlement of the larvae in the bottom of plastic cups. The LC50 value for each concentration was calculated.

2.5 Data analysis

The mortality response of mosquito larvae exposed to E. antiquorum latex extract was subjected to probit analysis [19].

2.6 Phytochemical Screening

Phytochemical screening for major constituents was carried out through standard qualitative methods as described by [20-21]. Presence of alkaloids, cynogenic glycosides, phenols, flavonoids, terpenoids, tannins and saponins were tested for the latex extract of Calotropis procera. The methods are briefly described as follows

2.7 Alkaloids

Portion of the latex was treated with few drops of aqueous solution of hydrochloric acid and 0.5ml of Mayer’s reagent. Formation of white precipitate indicated the presence of alkaloids.

2.8 Cynogenic Glycosides

To 250μl of the latex, equal volume of cold concentrated sulphuric acid was added. Formation of intense colour indicates the presence Cynogenic glycosides.

2.9 Phenolic Compounds

 Phenolic compounds of latex were detected by folin ciocalteau reagent. A portion of the latex was mixed with few drops of diluted folin ciocalteau reagent and aqueous solution of sodium carbonate mixture and was allowed to stand for 10 min. Formation of grey colour indicated the presence of phenol groups.

2.10 Flavonoids

Few drops of 1% aluminium solution was added to a portion of the latex. Yellow colouration indicated the presence of flavonoids.

2.11 Terpenoids

A red to purple color formation indicated the presence of terpenoids when a chloroform portion of latex was treated with an equal volume of concentrated sulphuric acid.

2.12 Tannins

A portion of latex was mixed with few drops of 0.1% ferric chloride and observed for brown green colouration which indicated the presence of tannins.

2.13 Saponins

To 0.5ml of latex, 5ml of distilled water was added. The solution was then vigorously shaken and observed for a stable persistent froth with honeycomb structure which indicated the presence of saponins.

3. Results

The 24 hrs LC50 value recorded for Ae. aegypti larvae was 14.34, LCL 13.03 and UCL 15.77 ml/dl and the 48 hrs LC50 value recorded was 10.702 ml/dl, LCL 9.45 and UCL 12.10 ml/dl. The 72 hrs LC50 value recorded for Ae. aegypti larvae was 6.62, LCL 5.40 and UCL 8.11 ml/dl (Table 1).

Phytochemical analysis of E. antiquorum latex revealed the presence of alkaloids, cynogenic glycosides, phenols, flavonoids and terpenoids and showed the absence of tannins and saponins (Table -2).

Table 1: LC50 and confidence intervals for Ae. aegypti larvae exposed to E. antiquorum latex extract

<table>
<thead>
<tr>
<th>S.No</th>
<th>Hours of exposure</th>
<th>LCL</th>
<th>LC50 (ml/dl)</th>
<th>UCL</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>12</td>
<td>14.87</td>
<td>17.98</td>
<td>21.71</td>
</tr>
<tr>
<td>2</td>
<td>24</td>
<td>13.03</td>
<td>14.34</td>
<td>15.77</td>
</tr>
<tr>
<td>3</td>
<td>36</td>
<td>10.95</td>
<td>12.19</td>
<td>13.56</td>
</tr>
<tr>
<td>4</td>
<td>48</td>
<td>9.45</td>
<td>10.70</td>
<td>12.10</td>
</tr>
<tr>
<td>5</td>
<td>60</td>
<td>7.22</td>
<td>8.64</td>
<td>10.33</td>
</tr>
<tr>
<td>6</td>
<td>72</td>
<td>5.40</td>
<td>6.62</td>
<td>8.11</td>
</tr>
<tr>
<td>LCL – Lower Confident Limit</td>
<td>UCL – Upper Confident Limit</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2: Phytochemical investigation of E. antiquorum latex extract

<table>
<thead>
<tr>
<th>Sl .No</th>
<th>Compounds</th>
<th>Observation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Alkaloids</td>
<td>Present</td>
</tr>
<tr>
<td>2</td>
<td>Cynogenic glycosides</td>
<td>Present</td>
</tr>
<tr>
<td>3</td>
<td>Phenols</td>
<td>Present</td>
</tr>
<tr>
<td>4</td>
<td>Flavonoids</td>
<td>Present</td>
</tr>
<tr>
<td>5</td>
<td>Terpenoids</td>
<td>Present</td>
</tr>
<tr>
<td>6</td>
<td>Tannins</td>
<td>Absent</td>
</tr>
<tr>
<td>7</td>
<td>Saponins</td>
<td>Absent</td>
</tr>
</tbody>
</table>

4. Discussion

The disease causing vector Ae. aegypti L. is a dreadful insect to human beings. Dengue, Chickungunya and yellow fever are the menace spread by this vector. As a trial Ae. aegypti mosquito larvae were selected to study the bioefficacy of E. antiquorum latex extract. The 48th LC50 value recorded for E. antiquorum latex extract against the fourth instar Ae. aegypti was 10.70 ml/dl. By testing the toxicity of E. antiquorum latex extract it was found out the latex produced higher mortality. The 72hrs LC50 value of Euphorbia antiquorum L. latex tested in the study against fourth instar Ae. aegypti larvae was 6.62 ml/dl. Jacrhopa curcas L. was shown to have high larvicidal activity on the early fourth instar larvae of Ae. aegypti and C. quinquefasciatus with a LC50 value of 8.79 and 11.34 ppm respectively [10]. Rajeswari et al. [11] reported that the latex of E. tirucalli possessed a high larvicidal activity against C. quinquefasciatus. The results of the study confirmed that the mortality increased with the increase in concentration of E. antiquorum latex extract and it was also found that latex was a strong killer against Ae. aegypti L. Ramos et al. [12] showed that C. procera latex contains the larvicidal compounds which caused 100% mortality in 3rd stage larvae of Ae. aegypti after 5 min at 29 ppm. [13] obtained an LC50 of 28mg/l with aqueous latex of C. procera against Anopheles labranchiae Falleroni, while the ethanolic extract showed LC50 of 315 mg/l against the Anopheles sp.

The mortality response of A.aegypti to different concentration
of *E. antiquorum* latex is
due to the presence of bioactive compounds which have been found
to have a reasonable efficacy. Tonk *et al.* [14] studied
that bioactive components present in the extract of
*Centratherum anthelminticum* L. possessed high toxicity
against *An. stephensi* 3rd instar larvae. According to Lima *et al.* [15] terpenoids were responsible for the insecticidal action of
mosquito larvae. Larshini *et al.* [16] reported that alkaloids
present in the latex of *C. procera* possess insecticidal
properties. The phytochemicals might have jointly or independently contributed to the toxicity of mosquito larvae.
Nathan *et al.* [17] reported that biopesticides of plant origins are
used against several insect species especially disease
transmitting vectors based on the fact that compounds of plant
origin are safer in usage and also do not leave scum in the
environment.

5. Conclusion
The findings of the present study suggest that *Euphorbia antiquorum* latex extract may be explored as potential natural
mosquitocidal agent.

6. References
1. Wattal BL, Joshi GC, Dos M. Roll of agricultural
insecticides in precipitating vector resistance. J Comm Dis
2. WHO. Vector resistance for pesticides. WHO. Tech Rep
Ser 1992, 818.
3. Nontananda V. Problems facing in the interruption of malaria transmission in Thailand. 1972; Vector Control in
Southeast Asia Singapore: The first SEAMEO Workshop,
15-16.
4. Frederich M, Dogne JM, Angenot L, Demol P. New
9:1435-1456.
5. Mittal PK, Subbarao SK. Prosects of using herbal
products in the control of mosquito vectors. ICMR Bull
2003; 33:1-10.
AA, Rajakumar G. Efficacy of botanical extracts against
Japanese encephalitis vector. Culex tritaeniorhynchus
Parasitol Res 2011; 106:481-492.
extracts and oils of mosquito (Diptera: Culicidae)
repellent plants from Sweden and Guinea-Bissau. J Med
Entomol 2006; 43:113-119.
G, Pandian G. Larvicidal potential of medicinal plant
extracts against *Anopheles subpictus* Grassi and *Culex
tritaeniorhynchus* Giles (Diptera: Culicidae). Parasitol Res
9. Govindarajan M. Bioefficacy of Cassia fistula Linn leaf
extract against chikungunya vector, *Aedes aegypti*
13:99-103.
K. Larvicidal activity of some Euphorbiaceae plant
extracts against *Aedes aegypti* and *Culex quinquefasciatus*
11. Rajeswari Y, Srivastava VK, Ramesh C, Ajay S.
Larvicidal activity of latex and stem bark of *Euphorbia tirucalli*
plant on te mosquito, *Culex quinquefasciatus*. J
common Dis 2002; 34: 264-269.
12. Ramos MV, Bandeira GP, de Freitas CDT, Nogueira
NDP, Alencar NMN, De sousa PAS, Carvalho AFU.
Latex constituents from *Calotropis procera* (R.Br.)
display toxicity upon egg hatching and larvae of *Aedes aegypti* (Linn). Mem Insit Oswaldo cruz 2006;
13. Markouk M, Bekkouche K, Larhsini M, Bousaid M,
Lazrek HB. Evaluation of some moroccan medicinal
plant extracts for larvicidal activity. J Ethnopharmacol
14. Tonk S, Bartarya R, Kumari MK, Bhatnagar VP,
Srivastava SS. Effective method for extraction of
larvicidal component from leaves of *Azadirachta indica*
and *Artemisia annuva* Linn. J Environ Biol 2006; 27:103-
105.
15. Lima MGA, Maia ICC, Sousa BD, Morais SM, Freitas
SM. Effect of stalk and leaf extracts from Euphorbiaceae
species on *Aedes aegypti* (Diptera : Culicidae) larvae. Rev
16. Larshini M, Bousad M, Lazrek HB, Jana M, Amarouch H.
Evaluation of antifungal and molluscicidas properties of
extracts of *Calotropis procera*. Fitoterapia 1997; 68:371-
373.
17. Nathan SS, Chung PG, Murugan K. Effect of botanicals
and bacterial toxin on the gut enzyme of *Cnaphalocrocis
18. World Health Organisation. Instructions for determining
the susceptibility or resistance of mosquito larvae to
19. Finney DJ. Probit Analysis. Cambridge University press,
20. Sofowora A. Phytochemical screening of me dicinal
plants and traditional medicine in Africa 2nd Edition
Phytochemical screening and extraction: A review.