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J. Benrit Vimal
Department of Zoology, Scott Christian College (Autonomous), Nagercoil- 629003.

S. Sam Manohar Das
Department of Zoology, Scott Christian College (Autonomous), Nagercoil- 629003.

Correspondence:
J. Benrit Vimal
Department of Zoology, Scott Christian College (Autonomous), Nagercoil- 629 003.

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 LC_{50} value of 10.70ml/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, pupicidal 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 ^{o}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 LC_{50} 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 gray 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 LC_{50} value recorded for *Ae. aegypti* larvae was 14.34, LCL 13.03 and UCL 15.77 ml/dl and the 48 hrs LC_{50} value recorded was 10.702 ml/dl, LCL 9.45 and UCL 12.10 ml/dl. The 72 hrs LC_{50} 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: LC_{50} and confidence intervals for *Ae. aegypti* larvae exposed to *E. antiquorum* latex extract

S.No.	Hours of exposure	LCL	LC_{50} (ml/dl)	UCL
1	12	14.87	17.98	21.71
2	24	13.03	14.34	15.77
3	36	10.95	12.19	13.56
4	48	9.45	10.70	12.10
5	60	7.22	8.64	10.33
6	72	5.40	6.62	8.11

LCL – Lower Confident Limit
UCL – Upper Confident Limit

Table 2: Phytochemical investigation of *E. antiquorum* latex extract

Sl .No	Compounds	Observation
1	Alkaloids	Present
2	Cynogenic glycosides	Present
3	Phenols	Present
4	Flavonoids	Present
5	Terpenoids	Present
6	Tannins	Absent
7	Saponins	Absent

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 48h LC_{50} 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 LC_{50} value of *Euphorbia antiquorum* L. latex tested in the study against fourth instar *Ae. aegypti* larvae were 6.62 ml/dl. *Jatropha curcas* L. was shown to have high larvicidal activity on the early fourth instar larvae of *Ae. aegypti* and *C. quinquefasciatus* with a LC_{50} 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 LC_{50} of 28mg/l with aqueous latex of *C. procera* against *Anopheles labranchiae* Falleroni, while the ethanolic extract showed LC_{50} 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.

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