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Larvicidal activity of Phyllanthus emblica Linn. (Euphorbiaceae) leaf extracts against important human vector mosquitoes (Diptera: Culicidae)

Alagarmalai JEYASANKAR[1], PREMALATHA[2] and Kuppusamy ELUMALAI[3*]

[1]

[2]*Department of Zoology & Botany, Arignar Anna Government Arts College, Musiri-621 211 Tamil Nadu, India*
[3]*Unit of Entomotoxicity, Department of Advanced Zoology & Biotechnology, Govt. Arts College Nandanam, Chennai – 600 035, Tamil Nadu, India*

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ABSTRACT

Objective: To determine the larvicidal activity of *Phyllanthus emblica* leaf extracts against *Aedes aegypti* and *Culex quinquefasciatus*. **Methods:** The larvicidal activity was determined against two vector mosquito species at concentrations of 50, 100, 150, 200 and 250 ppm. Larval mortality was assessed after 72 hours. **Results:** The leaf extracts of *P. emblica* was found to be more susceptible against the larvae of *Cx. quinquefasciatus* with a LC50 value of 78.89 ppm. **Conclusions:** These results suggested that the leaf extracts of P .emblica showed potential to be used as an ideal ecofriendly approach for the control of the *Aedes aegypti* and *Culex quinquefasciatus*.

1. Introduction

Mosquitoes are the important single group of insects in terms of public health importance, which transmit a number of diseases, such as malaria, filariasis, dengue, Japanese encephalitis, etc. causing millions of death every year [1]. These diseases not only cause high levels of morbidity and mortality, but also inflict great economic loss and social disruption on developing countries such as India, China, etc. India alone contributes around 40% of global filariasis burden and the estimated annual economic loss is about 720 crore [2-4]. Over and injudicious use of synthetic insecticides in vector control has resulted in environmental hazards through persistence and accumulation of non-biodegradable toxic components in the ecosystem, development of insecticide resistance among mosquito species, biological magnification in the food chain and toxic effects on human health and non-target organisms [5,6]. Plant derived materials are comparatively safer to humans and ecosystem and easily biodegradable [7]. Plant derived natural products have the advantage of being harmless to beneficial non-target organisms and environment when compared to synthetic insecticides [8]. Phytochemicals extracted from various plant species have been tested for their larvicidal activity against mosquitoes [9].

Phyllanthus emblica Linn. (syn. Emblica officinalis), commonly known as Indian gooseberry or amla, family Euphorbiaceae, is an important herbal drug used in unani (Graceo - arab) and ayurvedic systems of medicine. The plant is used both as a medicine and as a tonic to build up lost vitality and vigor. *P. emblica* is highly nutritious and could be an important dietary source of vitamin C, amino acids, and minerals. The plant also contains phenolic compounds, tannins, phyllembelic acid, phyllembelin, rutin, curcum-inoids, and emblicol. All parts of the plant are used for medicinal purposes, especially the fruit, which has been used in Ayurveda as a potent rasayana and in traditional medicine for the treatment of diarrhea, jaundice, and inflammation. Various plant parts show antidiabetic, hypolipidemic, antibacterial, antioxidant, antiulcerogenic,

*Corresponding author: Dr. K. Elumalai, Assistant Professor of Zoology, Unit of Entomotoxicity, Department of Advanced Zoology & Biotechnology, Govt. Arts College Nandanam, Chennai - 600 035, Tamil Nadu, India
mail: kelumalai.amu@gmail.com.
Phone: +91 9443770090. Fax: +91 44 22440049.

hepatoprotective, gastroprotective, and chemopreventive properties [10]. The leaf extracts of this plant exhibited adulticidal and larvicidal properties against the adult cattle tick Haemaphysalis bispinosa Neumann, 1897 (Acarina: Ixodidae), sheep fluke Paramphistomum cervi Zeder, 1790 (Digenea: Paramphistomatidae), fourth instar larvae of malaria vector, Anopheles subpictus Grassi and Japanese encephalitis vector, Culex tritaeniorhynchus Giles (Diptera: Culicidae) [11]. Therefore the present study was carried out to determine the larvicidal activity of *P. emblica* leaf extracts against important vectors *Aedes aegypti* and *Culex quinquefasciatus*.

2. Materials and methods

2.1 Plant collection and extraction

P. emblica leaves collected in and around Tiruchirapalli district, Tamil Nadu, India were brought to the laboratory at PG and Research Depaerment of Zoology, Arignar Anna Government Arts College, Musiri, Tiruchirapalli, Tamil Nadu, India.; shade dried under room temperature and powdered using an electric blender. A total of 1 kg of dried and powdered leaves was subjected to sequential extraction using 3 L of hexane, diethyl ether and ethyl acetate for a period of 72 h to obtain the crude extracts using rotary vacuum evaporator. The hexane, diethyl ether, and ethyl acetate crude extracts thus obtained were lyophilized and a stock solution of 100 000 ppm prepared from each crude extract by adding adequate volume of acetone was refrigerated at 4 ℃ until testing for bioassays.

2.2. Test organisms

All tests were carried out against laboratory reared vector mosquitoes viz., *Aedes aegypti* (Ae. aegypti) and *Culex quinquefasciatus* (Cx. quinquefasciatus) free of exposure to insecticides and pathogens. Cyclic generations of vector mosquitoes were maintained at 25-29 ℃ and 80-90 % relative humidity in the insectarium. Larvae were fed on larval food (powdered dog biscuit and yeast in the ratio of 3:1) and adult mosquitoes on 10 % glucose solution. Adult female mosquitoes were periodically blood-fed on restrained albino mice for egg production.

2.3. Larvicidal activity

Standard WHO protocol with slight modifications was adopted for the study [12]. From the stock solution, concentrations of 50, 100, 150, 200 and 250 ppm were prepared. Twenty five early third instar larvae were introduced in 250 mL beaker containing 200 mL of water with each concentration. A control was prepared by the addition of acetone to water. Mortality was recorded after 72 hours. A total of three trials were carried out with five replicates per trial against vector mosquitoes. However, when the control mortality ranged from 5-20 per cent, the observed percentage mortality was corrected by Abbott's formula [13],

2.4. Statistical analysis

SPSS 11.5 version package was used for determination of LC_{50} and LC_{90} [14]. Data from mortality and effect of concentrations were subjected to analysis of variance. The percentage data obtained was angular transformed. Difference between the treatments was determined by Tukey's test ($P < 0.05$).

3. Results

Larval toxicity of leaf extracts of *P. emblica* against Ae. aegypti, and *Cx. quinquefasciatus* reported in the present study exhibit the mosquitocidal properties in the plant leaf extracts suggesting their use in mosquito population control (Tables 1- 3). The different solvent crude extracts of *P. emblica* showed promising larval mortality against two important mosquito vectors. According to the data, larvae of *Cx. quinquefasciatus* were more susceptible than Ae. Aegypti. The data pertaining to the hexane extract of *P. emblica* against the fourth instar larvae of A. aegypti and C. quinquefasciatus are shown in table 1. The larval mortality of the A. aegypti was more prominent than C. quinquefasciatus as evidenced from the table 1, which showed 86.0% mortality in A. aegypti whereas, 73.6% larval mortality was recorded in C.quinquefasciatus at 250ppm concentration with the LC50 of 111.34 (LCL=93.07 - UCL=133.20) and LC_{50} of 136.78ppm (LCL=113.21 - UCL=165.25) respectively. Similar trend of larval toxicity was also observed in diethyl ether extract of *P. emblica* against the selected two vector mosquito species (Table 2). Besides, the ethyl acetate extract of *P. emblica* exhibited the maximum larvicidal activity (99.6%) with LC50 value of 78.89 ppm against the larvae of Ae. aegypti. The screening of local medicinal plants for mosquito larvicidal activity may eventually lead to their use in natural product-based mosquito abatement practices.

4. Discussion

The results of present study are comparable with similar reports of earlier workers. Bhagan et al [11] who have been reported that ethyl acetate and methanol extracts of *P. emblica* showed highest larval mortality against *C. tritaeniorhynchus* with LC_{50} = 54.82 ppm; LC_{90} 199.89 ppm, respectively and adult mortality was found in leaf methanol extracts against *H. bispinosa* and *P. cervi* with LC_{50} = 256.08; 60.60 ppm; LC_{90} = 1025.60; 287.48 ppm respectively. Sharma et al [15] reported that, petroleum ether extract of Ageratum

Table 1

Larvicidal activity of hexane extracts of *Phyllanthus emblica* against 4th instar larvae of A. aegypti and C. quinquefasciatus

Concentration (ppm)	Mortality* (%)	LC_{50} (ppm)	95%Confidence Limits (ppm)		LC_{90} (ppm)	95%Confidence Limits (ppm)		Degrees of freedom	χ^2 value
			LCL	UCL		LCL	UCL		
Aedes aegypti									
Control	1.2 ± 1.3 a								
50	25.4 ± 2.1b								
100	44.3 ± 2.0 c	111.34	93.07	133.20	617.50	366.90	1039.28	4	4.2548
150	52.4 ± 1.5 d								
200	70.2 ± 1.6 e								
250	86.0 ± 2.4 f								
Culex quinquefasciatus									
Control	1.1 ± 1.2 a								
50	21.2 ± 1.3 b								
100	35.6 ± 1.6 c	136.78	113.21	165.25	939.01	465.67	1893.49	4	0.8128
150	49.4 ± 2.6 d								
200	62.6 ± 1.6 e								
250	73.2 ± 1.2 f								

Value represents mean ± S.D. of five replications. *Mortality of the larvae observed after 72h of exposure period. LC_{50}=Lethal Concentration brings out 50% mortality and LC_{90} = Lethal Concentration brings out 90% mortality. LCL = Lower Confidence Limit; UCL = Upper Confidence Limit; Values in a column with a different superscript alphabet are significantly different at $P < 0.05$ level DMRTTest

Table 2

Larvicidal activity of diethyl ether extracts of *Phyllanthus emblica* against 4th instar larvae of *A. aegypti* and *C. quinquefasciatus*

Concentration (ppm)	Mortality* (%)	LC_{50} (ppm)	95%Confidence Limits (ppm)		LC_{90} (ppm)	95%Confidence Limits (ppm)		Degrees of freedom	χ^2 value
			LCL	UCL		LCL	UCL		
Aedes aegypti									
Control	1.0 ± 0.5 a								
50	17.2 ± 1.6b								
100	50.7 ± 1.2 c	114.77	102.81	125.51	333.50	206.44	243.71	4	4.133
150	66.4 ± 1.8 d								
200	82.3 ± 1.6 e								
250	94.5 ± 2.4 f								
Culex quinquefasciatus									
Control	1.1 ± 0.6 a								
50	39.5 ± 2.6 b								
100	58.1 ± 1.9 c	82.65	65.37	96.36	206.65	189.03	230.90	4	4.058
150	69.2 ± 2.4 d								
200	88.6 ± 1.5e								
250	98.2 ± 2.2 f								

Value represents mean ± S.D. of five replications. *Mortality of the larvae observed after 72h of exposure period. LC_{50}=Lethal Concentration brings out 50% mortality and LC_{90} = Lethal Concentration brings out 90% mortality. LCL = Lower Confidence Limit; UCL = Upper Confidence Limit; Values in a column with a different superscript alphabet are significantly different at $P < 0.05$ level DMRT Test.

conyzoides leaves exhibited larvicidal activity with LC_{50} value of 425.60 and 267.90 ppm after 24 and 48 h of exposure. The toxicity to the third instar larvae of *Cx. quinquefasciatus* by methanolic leaf extract of Memordica charantia, Trichosanthus anguina and Luffa acutangula showed the LC_{50} values of 465.85, 567.81 and 839.81 ppm respectively [16]. The toxicity to the late third instar larvae of *Ae. aegypti* by the hexane leaf extracts of Abutilon indicum and *Cx. quinquefasciatus* by dichloromethane whole plant extracts of Citrullus colocynthis and hexane extracts of aerial parts of Hyptis suaveolens was reported by Arivoli and Samuel [17-19]. Jang et al[20] have reported that the methanol extracts of Cecropia obtusifolia, Cassia tora and Vicia tetrasperma exhibited more than 90% larval mortality at 200 ppm on Ae. aegypti and Culex pipiens. The larvicidal activity of petroleum ether, ethanolic, aqueous extracts of dried leaves and fixed oil from the seeds of Caesalpinia bonduc (Family: Caesalpiniaceae) showed 100% mortality in 1% concentration of petroleum ether and ethanolic extract of leaf, whereas it was 55.0% in 2.5% concentration of aqueous extract and 92.6% in 2.5% concentration of fixed oil against the fourth instar larvae of *Cx. quinquefasciatus*[21]; the petroleum ether extract of Solanum xanthocarpum was observed to be the most toxic with LC50 of 1.41 and 0.93 ppm and LC_{90} of 16.94 and 8.48 ppm at 24 and 48 h after application, respectively against *An. stephensi*[22]. The Ricinus communis seed extract

Table 3

Larvicidal activity of Ethyl acetate extracts of *Phyllanthus emblica* against 4th instar larvae of *A. aegypti* and *C. quinquefasciatus*

Concentration (ppm)	Mortality* (%)	LC_{50} (ppm)	95%Confidence Limits (ppm)		LC_{90} (ppm)	95%Confidence Limits (ppm)		Degrees of freedom	χ^2 value
			LCL	UCL		LCL	UCL		
Aedes aegypti									
Control	0.0 ± 0.0 a								
50	35.7 ± 1.6b								
100	52.8 ± 2.4 c								
150	66.4 ± 2.8 d	80.04	66.27	96.67	332.53	233.06	474.45	4	10.552
200	88.2 ± 1.6 e								
250	99.5 ± 2.4 f								
Culex quinquefasciatus									
Control	0.0 ± 0.0 a								
50	39.2 ± 1.6 b								
100	55.6 ± 2.2 c								
150	59.4 ± 2.5 d	78.89	61.59	101.05	502.10	280.23	899.63	4	18.387
200	74.6 ± 1.4 e								
250	99.6 ± 2.2 f								

Value represents mean ± S.D. of five replications. *Mortality of the larvae observed after 72h of exposure period. LC_{50}=Lethal Concentration brings out 50% mortality and LC_{90} = Lethal Concentration brings out 90% mortality. LCL = Lower Confidence Limit; UCL = Upper Confidence Limit; Values in a column with a different superscript alphabet are significantly different at $P < 0.05$ level DMRT Test.`

exhibited larvicidal effects with 100% killing activities at concentrations 32-64 μg/mL, and with LC_{50} values 7.10, 11.64 and 16.84 μg/mL for *C. quinquefasciatus, An. stephensi* and *Ae. albopictus* larvae, respectively[23]. Venkatachalam and Jebanesan[24] have also reported that the repellent activity of methanol extract of Ferronia elephantum leaves against *Ae. aegypti* activity at 1.0 mg/cm2 and 2:5 mg/cm2 concentrations gave 100% protection up to (2.14±0.16) h and (4.00±0.24) h, respectively, and the total percentage protection was 45.8% at 1.0 mg/ cm^2 and 59.0% at 2.5 mg/cm2 for 10 h. The essential oil of Zingiber officinalis showed repellent activity at 4.0 mg/ cm^2, which provided 100% protection up to 120 min against *C. quinquefasciatus*[31].

The findings of the present investigation revealed that the leaf extracts of *P. emblica* possess larvicidal activity against vector mosquitoes. It may concluded that natural products as extracts from parts of plants of insecticidal and medicinal values have higher efficiency in reducing mosquito menace due to their larvicidal toxicity. Further studies on the screening, isolation and purification of bioactive phytochemical constituents/compounds followed by in-depth laboratory and field bioassays are needed as the present study shows that there is scope to use *P. emblica* leaf extracts to control the immature stages of vector mosquitoes. In conclusion, an attempt has been made to evaluate the role of *P. emblica* against an alternative approach to combat with the important human vector mosquitoes.

Conflict of interests

We declare that we have no conflict of interests.

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