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Larvicidal properties of *Breyenia vitis-idaea* (Burm.f.) Fischer (Euphorbiaceae) against important vector mosquitoes (Diptera: Culicidae)

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Mosquitoes are vectors of many dreadful human diseases. 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 deaths every year[1]. The control of these diseases had targeted partly the reduction in mosquito populations either at developmental or adult stages[2].

Chemical insecticides have been/are being used for the control of vector-borne diseases. The greatest harm from chemical insecticides is that once introduced into the eco system, they may remain there or for a very long duration. Thus, they pose a health hazard to the non-target organisms and human beings and help insects to develop resistance against them. This is the reason that there has always been a need for such an insecticide which is effective, with less side effects and easily degradable in nature, reducing the change to develop resistance against it. These problems have renewed interest in exploiting the pest control potential of plants[3].

Breynia vitis-idaea (Burm.f.) Fischer (Euphorbiaceae) is an evergreen, glabrous tree or large shrub found in the Gangetic Plain, Western Peninsula, China, Malay Peninsula and Sri Lanka. These plants are planted as ornamental hedge in the garden. Bark is yellowish grey, leaves are alternate dark brown or black when dry, flowers are small, greenish yellow or pink, and dull red, purple or white berries. Root, leaves and bark are medicinal. Roots decoction is used as mouthwash. A new sulfur-containing spiroketal glycoside, breynin I, and a new terpenic glycoside, breyniaionoside E, together with 10 known compounds, were isolated from the aerial parts of *B. vitis-idaea* used as a traditional Chinese medicine for the treatment of chronic bronchitis and wounds[4]. Joshi *et al*[5] reported that leaf methanol extract of *B. vitis-idaea* showed promising antioxidant properties. Rasingam *et al*[6] who observed sticks of *B. vitis-idaea* used as herbal tooth sticks by inhabitants of Andaman and Nicobar Islands. Prior to this study, there is no report on the mosquito larvicidal activity of *B. vitis-idaea* in the available literatures. The aim of this study was to investigate the mosquito larvicidal activity of the different solvent extracts of the leaves of *B. vitis-idaea.*

B. vitis-idaea leaves were collected from Puliansolai, Tiruchirapalli district, Tamil Nadu, India and brought to the laboratory at PG and Research Department of Zoology, Arignar Anna Government Arts College, Musiri, Tiruchirapalli, Tamil Nadu during July 2013. The plant was identified at the Rapinat Herbarium and Centre for Molecular Sytematics, St' Joseph's College, Tiruchirapalli, Tamil Nadu and the Voucher specimen (IPH 12) was deposited in Entomology Laboratory, Arignar Anna Government Arts College, Musiri, Tamil Nadu. The collected leaves were 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, chloroform and ethyl acetate for a period of 48 h to obtain the crude extracts using rotary vacuum evaporator. The extract was concentrated under reduced pressure 22–26 mmHg at 45°C by 'Rotavapour' and the residue obtained was stored at 4°C until testing for subsequent bioassays. All tests were carried out against the larvae of laboratory reared vector mosquitoes, *viz. Aedes aegypti*, *Culex quinquefasciatus* and *Anopheles stephensi* (Diptera: Culicidae). Larvae of the mosquito were collected from breeding sites within the study area, and reared under laboratory condition at 25–29°C 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). Pupae were collected daily, and transferred to small bowls containing clean water. The bowls were placed in cage 30 × 30 × 30 cm covered with mosquito net for adult emergence. From the day of emergence, adult mosquitoes were provided with cotton soaked in a 10% sugar solution as a carbohydrate source.

Standard WHO protocol with slight modifications was adopted for the study[7]. From the stock solution, concen-

trations of 50, 100, 150, 200 and 250 ppm were prepared. Twenty-five late III and early IV 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 48 h. 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%, the observed percentage mortality was corrected by Abbott's formula[8]. The SPSS 11.5 version package was used for determination of LC_{50} and $LC_{90.}$ Data from mortality and effect of concentrations were subjected to study the analysis of variance. The percentage data obtained was angular transformed. The difference between the treatments was determined by Tukey's test ($p < 0.05$).

The leaf extracts of *B. vitis-idaea* against *Ae. aegypti*, *Cx. quinquefasciatus* and *An. stephensi* were found to exhibit the larvicidal properties (Tables 1 and 2). The different solvent crude extracts of *B. vitis-idaea* showed promising larval mortality against important mosquito species. According to the data, larvae of *Cx. quinquefasciatus* were more susceptible than *Ae. aegypti* followed by *An. stephensi.* The data pertaining to the ethyl acetate extract of *B. vitis-idaea* against the III instar larvae of *Ae. aegypti*, *Cx. quinquefasciatus* and *An. stephensi* are shown in Table 1. The larval mortality of *Ae. aegypti*, *Cx. quinquefasciatus* and *An. stephensi* was more prominent as evidenced from the Table 1, which showed 100% mortality in all species at 250 ppm concentration with the LC_{50} values of 98.2 ppm (LCL = 68.9 – UCL = 139.9), 107.79 ppm (LCL = 77.4 – UCL = 149.9) and 115.8 ppm (LCL = 86.9 – UCL = 154.5) respectively. Similar trend of toxicity was also observed in chloroform extract of *B. vitis-idaea* against *Cx. quinquefasciatus* with the LC_{50} values of 113.2 ppm (LCL = 81.5 – UCL = 157.1). The methanol extract of *B. vitis-idaea* exhibited the promising larvicidal activity against the larvae of mosquito species are needed to be explored.

Table.1. Larvicidal activity of leaf extracts of *Breyenia vitis-idaea* against III instar larvae of mosquito vectors

Concentration (ppm)	Larval mortality (%)		
	Hexane	Chloroform	Ethyl acetate
	Aedes aegypti		
Control	1.2 ± 0[a]	1.5 ± 1.2[a]	4.4 ± 2.2[a]
50	16.5 ± 2.3[b]	19.2 ± 3.2[b]	26.3 ± 2.3[b]
100	37.3 ± 2.4[c]	33.1 ± 2.5[c]	43.6 ± 2.4[c]
150	55.5 ± 2.5[d]	56.9 ± 3.6[d]	66.7 ± 1.8[d]
200	86.8 ± 1.8[e]	87.7 ± 2.6[e]	89.1 ± 2.7[e]
250	97.9 ± 2.3[f]	99.9 ± 2.1[f]	100 ± 2.8[f]
	Culex quinquefasciatus		
Control	0.0 ± 0[a]	1.3 ± 2.3[a]	6.6 ± 1.2[a]
50	16.4 ± 2.4[b]	18.7 ± 3.2[b]	25.3 ± 3.3[b]
100	30.2 ± 1.5[c]	35.3 ± 1.9[c]	36.2 ± 2.2[c]
150	55.4 ± 1.7[d]	56.6 ± 3.7[d]	69.3 ± 1.5[d]
200	88.5 ± 2.4[e]	88.2 ± 2.5[e]	88.1 ± 2.4[e]
250	98.7 ± 1.2[f]	100 ± 3.2[f]	100 ± 3.4[f]
	Anopheles stephensi		
Control	0.0 ± 0[a]	2.2 ± 1.3[a]	8.2 ± 1.3[a]
50	16.3 ± 1.3[b]	15.4 ± 3.1[b]	19.9 ± 1.4[b]
100	32.3 ± 2.4[c]	38.5 ± 2.1[c]	38.2 ± 2.8[c]
150	49.7 ± 1.5[d]	56.6 ± 2.3[d]	64.8 ± 2.2[d]
200	78.6 ± 2.2[e]	87.5 ± 2.2[e]	88.2 ± 3.4[e]
250	96.9 ± 1.2[f]	92.9 ± 2.8[f]	100 ± 1.2[f]

Each value (mean ± S.D.) represents mean of five values; Values with different alphabets in the column are statistically significant (mANOVA-Duncan's Multiple range test, $p < 0.05$ level).

The results of present study are comparable with similar reports of earlier workers. Jeyasankar *et al*[9] reported that ethyl acetate extract of *Phyllanthus emblica* leaves exhibited larvicidal activity with LC_{50} value of 78.89 ppm

Table 2. Larvicidal activity of leaf extracts of *Breyenia vitis-idaea* against III instar larvae of mosquito

Mosquitoes/Extracts	LC_{50} (ppm)	95% confidence limits (ppm)		LC_{90} (ppm)	95% confidence limits (ppm)		χ^2-value (df=4)
		LCL	UCL		LCL	UCL	
Aedes aegypti							
Hexane	126.18	92.21	172.66	354.23	176.74	674.31	4.278*
Chloroform	111.90	82.77	151.28	258.94	148.54	451.39	4.680
Ethyl acetate	98.21	68.94	139.91	253.54	130.03	494.39	2.796*
Culex quinquefasciatus							
Hexane	115.77	84.93	157.80	268.81	144.79	499.04	4.818
Chloroform	113.18	81.54	157.10	276.24	140.39	543.54	4.206*
Ethyl acetate	107.69	77.38	149.87	262.14	135.98	505.35	3.504*
Anopheles stephensi							
Hexane	125.76	89.34	177.07	329.41	148.49	730.74	4.781
Chloroform	113.28	82.11	156.28	270.89	139.43	526.27	3.379*
Ethyl acetate	115.88	86.91	154.51	251.04	144.22	436.99	2.269*

*Significant at $p < 0.05$ level; LCL–Lower confidence limits; UCL–Upper confidence limits.

(99.6%) on *Cx. quinquefasciatus* and followed by *Ae. aegypti* 80.04 ppm (99.5) after 24 h of exposure. The toxicity to the III 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[10]. The methnol leaf extract of *Solanum trilobatum* exhibited 100% larval mortality in *Cx. quinquefasciatus, Ae. aegypti* and *An. stephensi* at 250 ppm concentration as reported by Premalatha *et al*[11]. Jang *et al*[12] have reported that the methanol extracts of *Cecropia obtusifolia*, *Cassia tora* and *Vicia tetrasperma* exhibited > 90% larval mortality at 200 ppm on *Ae. aegypti* and *Culex pipiens*. The findings of the present investigation revealed that the leaf extracts of *B. vitis-idaea* possess larvicidal activity against important vector mosquitoes. The results reported here open the possibility of further investigation of efficacy on their larvicidal properties of natural product extracts for the control of mosquitoes and thereby prevent the environmental pollution.

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