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# Ovicidal and pupicidal activity of *Breynia vitis-idaea* (Burm.f.) Fischer. (Euphorbiaceae) against vector mosquitoes (Diptera: Culicidae)

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## Abstract

The present investigation was aimed to determine the ovicidal and pupicidal activity of *Breynia vitis-idaea* leaf extracts against *Aedes aegypti*, *Anopheles stephensi* and *Culex quinquefasciatus* . The ovicidal and pupicidal activity was determined against three mosquito species at concentrations of 50, 100, 150, 200 and 250 ppm and mortality was assessed after 24 hours. Highest percentage of ocividal activity was recorded in ethyl acetate extract extract on *Ae. aegypti*, *An. stephensi* and *Cx. quinquefasciatus* . Maximum pupicidal activity was found in ethyl acetate extract extract on *Ae.aegypti* and *Cx. quinquefasciatus* of 100% ($LC_{50}$ 45.05 and $LC_{50}$ 41.16). These results suggested that the leaf extracts of *B.vitis-idaea* showed potential to be used as an ideal ecofriendly approach for the control of the *Ae. aegypti*, *An. stephensi* and *Cx. quinquefasciatus.*

**Keywords:** Ovicidal, pupicidal, *Breynia vitis-idaea*, leaf extracts, *Aedes aegypti*, *Anopheles stephensi*, *Culex quinquefasciatus*

## Introduction

Mosquitoes transmit serious human diseases, causing millions of deaths every year. Several mosquito species belonging to genera Aedes, Anopheles and Culex are vectors responsible for the pathogens of various diseases like malaria, filariasis, Japanese Encephalitis, Dengue fever and yellow fever. Most parasitic disease is the tropical, indentifying globalization and environmental changes are increasing the danger of contracting arthropod-borne illnesses (Ansari 2000; Tawatsin *et al*., 20001). Most of the mosquito control programs target the larval stage in their breeding areas with larvicides (Knio *et al*., 2008) because the adulticides may only reduce the adult population temporarily ( El Hag *et al*., 1999).

Therefore, a more efficient way to reduce mosquito developing is to target the larvae. Organophosphates and different insecticides are widely used for mosquito control. A drawback with the use of chemical insecticides is that they are non-selective and could be more harmful to other non-target organisms and be a pollutant to the earth. *B.vitis-idaea* belonging to the family Euphorbiaceae. It is a evergreen 1.5-5m tall glabrous tree or large erect shrub with horizontal branches found in the Gangetic plain, Western Peninsula, China, Malay Peninsula and Sri Lanka, India. Leaves are 1-3cm long, elliptic to elliptic-ovate, exchange dark brown or black whendry. Bark is yellowish grey; flowers are small, greenish yellow or

pink. The fruits are thickset, pink to red which turns black when grown and measures 2-3 mm in diameter. The seeds are black and have a very hard seed coat(Pullaiah 2002; Chandrashekar *et al*.,2011; Yoganarasimhan 2000) . In present study, important medicinal plant, *B.vitis-idaea* was selected and investigated for ovicidal, pupicidal activity against *Ae.aegypti, An.stephensi* and *Cx.quinquefasciatus*.

## Materials and Methods

### Plant material

The leaves of *B.vitis idaea* were collected from Puliyansolai hills, Thuraiyur Taluk, Tiruchirapalli District, Tamil Nadu, India. Collecetd plant specimen was identified by Dr. S. John Britto, Director, The Rapinat Herbarium and Centre for Molecular Sytematics, St' Joseph's College, Tiruchirapalli, Tamil Nadu, India and The Voucher specimen (IPH-12) was deposited in Entomology lab, Arignar Anna Government Arts College, Musiri, Tamil Nadu, India.

### Extraction method

The dried leaves (100g) were powdered mechanically using commercial electrical stainless steel blender and extracted sequentially with hexane, chloroform, and ethyl acetate (500ml, Ranchem), in a soxhlet apparatus separately until exhaustion. The extract was concentrated under reduced pressure of 22-26mm hg at 45º C by 'Rotavapour' and the residue obtained was stored at 4ºC in an amber vial. Then the vials were named and covered with silver foil and transported to the laboratory. Until use those vials were kept in cool and dark place at 4ºC.

### Vector rearing

The mosquito larvae of *Ae. aegypti*, *An. stephensi and Cx. quinquefasciatus* were collected from National centre for disease control, Government of India ministry of health and family welfare, Southern India branch, field station, Mettupalayam, Coimbatore district, Tamilnadu, India. The larvae were kept in the plastic buckets half filled with tap water and fed with dog biscuit once a day initially and twice during the later stages of development. Water in rearing container was refreshed every day by removing a little quantity of water from the rearing buckets and replacing with fresh water. This was aimed at preventing scum from forming on the water surface.

### Ovicidal activity

For ovicidal activity, slightly modified method of Su and Mulla (1998) was performed. *Ae.aegypti ,An. stephensi* and *Cx. quinquefasciatus* eggs were collected from Government of India ministry of health and family welfare, Southern India branch, field station, Mettupalayam, Coimbatore district, Tamilnadu, India. The leaf extracts were used in the Hexane, Chloroform and Ethyl acetate extract to achieve various concentrations ranging from 50 to 250ppm. Eggs of these mosquito species (100) were exposed to each concentration of leaf extracts. After 24 h treatment, the eggs from each concentration were individually transferred to distilled water cups for hatching assessment after counting the eggs under microscope. Each experiment was replicated five times along with appropriate control. The hatch rates were assessed 48 h post treatment by the following formula:

$$\% \text{ hatchability} = \frac{\text{No. of hatched larvae}}{\text{Total no. of eggs}} \times 100$$

### Pupicidal activity

The pupicidal activity of plant crude extract was assessed by using the standard method as prescribed by WHO (2005). Similar test concentrations as stated in the previous experiments was prepared and tested against the pupae of *An. stephensi*, *Ae. aegypti* and *Cx. quinquefasciatus*. DMSO (emulsifier) in water treated as control. The pupae of these mosquito species (25 pupae) was introduced in 500-ml plastic cups containing 250 ml of aqueous medium (249 ml of dechlorinated water + 1ml of emulsifier) and the required amount of plant extract was added. The pupal mortality was observed and recorded after 24 h of post treatment. For each experiment, five replicates were maintained at a time. The percentage of mortality was calculated by using Abbott's formula (1925).

### Determination of lethal concentrations

Lethal concentration (LC50) represents the concentration of the test material that caused 50% mortality of all test organisms within the specified period of exposure was determined by exposing various developmental stages of the mosquitoes to different concentrations of the extract. Based on the mortality of the test organisms recorded in these bioassays, LC50 and LC90 was calculated along with their fiducial limits at 95% confidence level by probit

analysis using SPSS software package 16.0 (Statistical Package of Social Sciences) software. Results with $p<0.05$ was considered to be statistically significant.

## Results

The efficacy of leaf crude extracts such as hexane, chloroform and ethyl acetate extracts of *B. vitis-idaea* evaluvated for ovicidal (eggs) and pupicidal (pupae) activities against *Ae. aegypti* , *An. stephensi* and *Cx. quinquefasciatus* and results are presented in tables. Generally, as the concentration increases the rate of eggs and pupal mortality are also increases. It has been noticed that the higher concentrations of *B.vitis-idaea* extracts of ethyl acetate possesses strong ovicidal activity at 250ppm concentration against *Ae. aegypti* , *An. stephensi* and *Cx. quinquefasciatus* (100%) no egg hatchability was recorded. In pupicidal activity, among the three solvent extracts tested against selected mosquitoes at 250ppm higherconcentrations, the ethyl acetate extract was found to be most effective for pupicidal activity provided 100 % (Lc50 45.05), 95.8 % (LC50 47.94) and 100% (LC50 41.16) against *Ae. Aegypti An. stephensi and Cx. quinquefasciatus* respectively. Results of this study show that the *B. vitis-idaea* evaluvated selected extracts may be a potent source of natural ovicidal and pupicidal activities against selected important vector mosquitoes.

**Table 1: Ovicidal activity of crude extract of *B. vitis-idaea* against the Mosquito vectors.**

| Concentrations (ppm) | Crude extract tested | | |
|---|---|---|---|
| | Hexane | Chloroform | Ethyl acetate |
| ***Ae. aegypti*** | | | |
| Control | 100 ±0 | 100 ±0 | 100 ±0 |
| 50 | 48.6 ±3.9 | 42.4 ±2.4 | 24.4±3.3 |
| 100 | 36.6 ±2.7 | 35.8±3.4 | 18.6±1.1 |
| 150 | 25.6±3.2 | 27.2±3.4 | 14.8±4.3 |
| 200 | 13.8±2.9 | 16.2±2.7 | 3.2±2.1 |
| 250 | 4.4±3.9 | 3.8±2.9 | NH |
| ***An. stephensi*** | | | |
| Control | 100 ±0 | 100 ±0 | 100 ±0 |
| 50 | 49.6±2.3 | 30.8±3.1 | 21.2±1.0 |
| 100 | 35.4±3.2 | 21.2±1.4 | 11.8±2.0 |
| 150 | 24.6±2.5 | 15.4±4.2 | 5.6±3.5 |
| 200 | 13.4±2.8 | 9.2±1.3 | NH |
| 250 | 6.8±2.5 | 4.4±3.9 | NH |
| ***Cx. quinquefasciatus*** | | | |
| Control | 100 ±0 | 100 ±0 | 100 ±0 |
| 50 | 44.4 ±3.3 | 36.6 ±3.1 | 21.4 ±1.5 |
| 100 | 25.8 ±3.4 | 31.4 ±1.3 | 12.8 ±2.5 |
| 150 | 16.2 ±2.9 | 25.8 ±3.4 | 4.2 ±3.7 |
| 200 | 11.6 ±3.2 | 11.4 ±2.0 | NH |
| 250 | 4.2 ±3.6 | 6.8 ±3.7 | NH |

Values in a row with a different superscript are significantly different at $p$ <0.05% level. Each value (X – ± SD) represents the mean of six values. NH = No hatchability (100% mortality).

**Table 2: Pupicidal activity of crude extracts of *B. vitis –idaea* against *Ae.aegypti.***

| Plant extract | Concentration (ppm) | Emergence (%) | Pupal Mortality (%) | LC50 (LCL-UCL) | LC90 (LCL-UCL) | X2 (df=4) |
|---|---|---|---|---|---|---|
| Hexane extract | control | 97.8±3.2 | 1.2±1.1 | 59.91 (31.47-79.80) | 220.67 (198.14-254.11) | 1.254 |
| | 50 | 55.6±3.6 | 44.2±1.7 | | | |
| | 100 | 33.2±1.4 | 66.2±2.9 | | | |
| | 150 | 22.6±1.9 | 77.2±2.1 | | | |
| | 200 | 12.6±1.6 | 84.8±3.6 | | | |
| | 250 | 4.6±3.6 | 93.8±3.0 | | | |
| Chloroform | Control | 98.2±3.4 | 1.2±1.0 | 48.64 (16.14-70.49) | 213.05 (190.51-246.87) | 0.946 |
| | 50 | 42.2±1.7 | 52.6±2.3 | | | |
| | 100 | 31.4±1.8 | 64.6±3.2 | | | |
| | 150 | 19.8±2.1 | 75.8±2.9 | | | |
| | 200 | 11.4±4.0 | 88.2±3.9 | | | |
| | 250 | 2.2±2.6 | 95.4±3.5 | | | |
| Ethyl acetate extract | control | 98.8±0.4 | 1.2±1.0 | 45.05 (17.94-63.87) | 176.63 (159.50-200.54) | 4.304 |
| | 50 | 45.2±2.1 | 52.4±2.7 | | | |
| | 100 | 20.8±2.7 | 72.6±3.5 | | | |
| | 150 | 11.2±2.7 | 82.4±3.6 | | | |
| | 200 | 9.4±1.9 | 90.6±1.6 | | | |
| | 250 | 0.0±0 | 100±0 | | | |

*Significant at $P<0.05$. SD = Standard Deviation; LCL = Lower Confidence Limits; UCL = Upper Confidence Limits; $\chi 2$ =Chi square.

**Table 3: Pupicidal activity of crude extracts of *B. vitis –idaea* against *An.stephensi*.**

| Plant extract | Concentration (ppm) | Emergence (%) | Pupal Mortality (%) | LC50 (LCL-UCL) | LC90 (LCL-UCL) | X2 (df=4) |
|---|---|---|---|---|---|---|
| Hexane extract | control | 93.4±3.6 | 5.2±2.8 | 73.21 (37.83-96.51) | 288.06 (250.56-352.43) | 2.201 |
| | 50 | 50.8±3.8 | 47.6±2.6 | | | |
| | 100 | 41.6±2.3 | 55.4±1.5 | | | |
| | 150 | 31.8±1.6 | 64.2±2.5 | | | |
| | 200 | 22.8±2.3 | 75.2±2.8 | | | |
| | 250 | 11.4±1.6 | 88.8±1.9 | | | |
| Chloroform | Control | 94.8±3.7 | 4.8±2.6 | 53.36 (17.37-76.92) | 241.03 (213.25-285.30) | 1.038 |
| | 50 | 41.8±2.2 | 50.8±3.9 | | | |
| | 100 | 32.6±3.2 | 61.8±3.4 | | | |
| | 150 | 23.8±3.8 | 71.2±1.6 | | | |
| | 200 | 10.8±1.9 | 86.2±3.5 | | | |
| | 250 | 8.2±1.9 | 91.2±2.7 | | | |
| Ethyl acetate extract | control | 95.2±2.2 | 4.6±2.6 | 47.94 (16.93-69.02) | 204.56 (183.49-235.53) | 1.944 |
| | 50 | 41.8±2.1 | 54.4±2.8 | | | |
| | 100 | 37.6±1.8 | 61.2±3.6 | | | |
| | 150 | 17.2±3.7 | 80.4±2.6 | | | |
| | 200 | 8.4±3.4 | 89.4±1.6 | | | |
| | 250 | 2.2±1.7 | 95.8±3.6 | | | |

*Significant at $P<0.05$. SD = Standard Deviation; LCL = Lower Confidence Limits; UCL = Upper Confidence Limits; $\chi 2$ =Chi square.

**Table 4: Pupicidal activity of crude extracts of *B. vitis –idaea* against *Cx. quinquefasciatus*.**

| Plant extract | Concentration (ppm) | Emergence (%) | Pupal Mortality (%) | LC50 (LCL-UCL) | LC90 (LCL-UCL) | X2 (df=4) |
|---|---|---|---|---|---|---|
| Hexane extract | control | 93.6±2.3 | 6.4±2.6 | 51.95 (13.87-76.51) | 246.87 (217.62-294.27) | 0.332 |
| | 50 | 50.8±3.8 | 49.2±3.1 | | | |
| | 100 | 41.6±2.3 | 61.6±2.5 | | | |
| | 150 | 31.8±1.6 | 76.2±2.1 | | | |
| | 200 | 22.8±2.3 | 82.6±3.2 | | | |
| | 250 | 11.4±1.6 | 90.2±2.8 | | | |
| Chloroform | Control | 92.4±3.7 | 6.8±2.6 | 50.36 (15.99-73.15) | 226.01 (201.17-264.36) | 0.369 |
| | 50 | 43.2±1.9 | 50.2±3.7 | | | |
| | 100 | 25.8±3.0 | 65.2±2.8 | | | |
| | 150 | 20.4±2.4 | 74.8±3.0 | | | |
| | 200 | 11.8±2.8 | 85.8±3.5 | | | |
| | 250 | 3.4±3.0 | 93.6±3.2 | | | |
| Ethyl acetate extract | control | 95.6±3.2 | 3.8±0.8 | 41.16 (13.07-60.44) | 171.63 (154.77-195.06) | 3.497 |
| | 50 | 43.8±2.9 | 54.4±2.7 | | | |
| | 100 | 14.4±2.9 | 72.6±3.1 | | | |
| | 150 | 9.8±3.8 | 84.6±2.3 | | | |
| | 200 | 5.6±3.7 | 91.4±2.5 | | | |
| | 250 | 0±0 | 100±0 | | | |

*Significant at $P<0.05$. SD = Standard Deviation; LCL = Lower Confidence Limits; UCL = Upper Confidence Limits; $\chi 2$ =Chi square.

## Discussion

Plants are rich sources of bioactive compounds that can be used to increase environmentally harmless vector and pest managing agents. Phytoextracts are promising as possible mosquito control agents, with low cost , effortless to administer, and risk free properties. Our result showed that the crude ethyl acetate solvent leaf extract of *B.vitis idaea* had significant ovicidal and pupicidal properties against *Ae. aegypti* , *An. stephensi* and *Cx. quinquefasciatus* with the $LC_{50}$ values less than 100mg/L respectively. Compared with other results, Petroleum Ether extract of *Andrographis paniculata* against mosquito vectors exhibited more than maximum percentage of ovicidal and pupicidal activity was recorded in petroleum ether extract 250ppm on *Ae.aegypti, Cx.quinquefaciatus* and *An.stephensi* (Jeyasankar and Ramar 2015).The present study reported that the third instar larvae of *Ae.aegypti, Cx.quinquefaciatus* and *An.stephensi* by the ethyl acetate leafe extract of Breyenia vitis –idaea showed the $LC_{50}$ value of 98.2, 107.79 and 115.8ppm respectively(Jeyasankar and Ramar 2014). *Andrographis paniculata* showed larvicidal activity of $LC_{50}$ value of 20.85 and $LC_{95}$ 444.41ppm against *Ae.aegypti, Cx.quinquefaciatus* and *An.stephensi* (Jeyasankar and Ramar 2015). Ovicidal activity by ethyl acetate, aqueous solution, ethanol leaf extract of *Nerium oleander* against *An. stephensi* at 100, 150, 200, 250, and 300 ppm were considered. With each extract at a concentration of 100 ppm, the take of hatchability was very high and nil hatchability was recorded as the concentration of extract was better to 300 ppm in the case of aqueous and ethanol extract (Roni *et al.*,2013). Kovendan *et al.*,(2014) have also reported that the $LC_{50}$ values of ethanol leaf extract of *Morinda citrifoila* against the first to fourth instar larvae and pupae of *An. stephensi* had values of LC50 = 152.05, 190.22, 237.43, 273.12, 305.25 mg / l at 24 hrs . The hexane leaf extract of *Crotons sparciflorus* against III instar larvae and pupae of *Cx. quinquefasciatus* were 145.3, 446.9 ppm respectively(Ramar *et al.,* 2013). Kuppusamy *et al.,* (2008) reported that the ovicidal activity indicated an significant result that the larvae which hatched out of the treated eggs were succumbed to death contained by an hour or two. In the case of ovicidal activity, exposure to the newly laid eggs was more effective than that to the older eggs. Likewise, ovicidal and gravid mortality effect of ethanolic extract of *Andrographis paniculata* against *An. stephensi*. The present study that exposure of *An. stephensi* eggs to the *Coccinia indica* leaf extracts of different solvents not only elicited egg mortality but also deferred

hatchability to larval stages. For this reason of this study clearly reveals that the *B.vitis-idaea* has potency to control the mosquito *Ae.aegypti, Cx.quinquefaciatus* and *An.stephensi* (Rajkumar *et al.*, 2011). As a result of natural products establish to be an valuable approach in eco-friendly mosquito management and control.

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