

## Egg hatchability and larvicidal activity of *Swertia chirata* Buch. - Hams. ex Wall. against *Aedes aegypti* L. and *Culex quinquefasciatus* Say.

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**Abstract:** Crude *Swertia chirata* extracts (hexane, ethyl acetate & methanol) were tested for egg hatchability and larvicidal activity against two vector mosquitoes, *Aedes aegypti* and *Culex quinquefasciatus*. The lowest egg hatchability was recorded at 1000 ppm of ethyl acetate extract which showed 12.6, 20.4 and 25.4 % at 0-6, 6-12 and 12-18 h respectively against *Cx. quinquefasciatus*; it was 11.4, 17.8 and 23.4 % at 0-6, 6-12 and 12-18 h respectively against *Ae. aegypti*. The LC<sub>50</sub> values for the larvicidal activity of ethyl acetate extract of *S. chirata* against first, second, third and fourth instar larvae of *Cx. quinquefasciatus* were 164.91, 220.10, 284.05 and 326.46 ppm and against *Ae. aegypti* 192.67, 237.30, 339.06 and 329.29 ppm respectively.

**Keywords:** *Swertia chirata*, *Culex quinquefasciatus*, *Aedes aegypti*, egg hatchability, larvicidal activity

### Introduction

Mosquito is one of the blood sucking insects in the group of arthropods and it transmits parasites and pathogens which cause devastating impact on human beings. It spreads many dreadful diseases such as filariasis, malaria, dengue, yellow fever and Japanese encephalitis, which contribute significantly to disease burden, death, poverty, and social debility in tropical countries (Jang *et al.*, 2002). *Cx. quinquefasciatus* is an important vector of Bancroftian filariasis. There are 45 million cases of lymphatic filariasis in India alone (Bowers *et al.*, 1995). Estimates suggest that about 120 million people over 73 countries are infected with human lymphatic filariasis (Southgate, 1984; WHO, 1997).

*Aedes aegypti* is a vector of arboviruses responsible for major diseases like dengue, dengue haemorrhagic fever and chikungunya. It infects more than 100 million people every year. Dengue fever has become an important public health problem as the number of reported cases continue to increase, especially with more severe forms of the disease, dengue haemorrhagic fever and dengue shock syndrome, or with unusual manifestations (Hendarto & Hadinegoro, 1992; Pancharoen *et al.*, 2002). About two-fifth of the world's population are at risk of catching dengue according to the World Health Organization (WHO, 2003).

Global use of chemical insecticides viz., organophosphates such as Temephos and Fenthion and insect growth regulators such as Diflubenzuron and Methoprene are generally used for the control of mosquito larvae (Yang *et al.*, 2002). Although effective,

their repeated use has disrupted natural biological control systems led to outbreaks of insect species, widespread development of resistance, and undesirable effects on nontarget organisms, and fostered environmental and human health concerns (Yang *et al.*, 2002; Pitasawat *et al.*, 2007). Natural products are preferred because of their innate biodegradability and harmlessness (Ananthkrishnan, 1988). A large number of plant essential oils may be potential sources of mosquito larvicides, because they constitute a rich source of bioactive components (Lahlou *et al.*, 2001; Cetin *et al.*, 2004). Over the last 50 years, thousands of plants have been screened as potential sources of larvicidal, repellents and insecticidal activities (Sukumar *et al.*, 1991).

*Swertia chirata* belongs to the family Gentianaceae, native of temperate Himalayas, found at an altitude of 1200-3000 m, from Kashmir to Bhutan. The entire plant is used in traditional medicine; however root is documented as the most powerful part for the pharmacological effect (Kirtikar & Basu, 1984). Phytochemical studies have revealed the presence of secondary metabolites such as xanthenes, triterpenoids, glycosides, triterpenoid and alkaloid (Jensen & Schripsema, 2002; Joshi & Dhawan, 2005). The present study deals with the egg hatchability and larvicidal activity of crude solvent extracts of *S. chirata* against the larvae of *Ae. aegypti* and *Cx. quinquefasciatus*.

### Materials and methods

#### *Plant collection and extraction*

Healthy, disease free plants of *S. chirata* (whole plant) were collected from Darjeeling (West Bengal, India). The species was identified and authenticated by Dr. Jeya Jyothi, Taxonomist, Department of Plant Biology and Biotechnology, Loyola College, Chennai and the voucher specimen (LCH 1002) was deposited at the departmental herbarium, Loyola College, Chennai. Freshly collected plant was washed thoroughly, shade dried in open air and grounded into powder. The *S. chirata* plant powder (3 kg) was sequentially soaked in hexane, ethyl acetate and methanol for 72 h respectively with intermittent shaking. After 72 h the solution was filtered and the filtrate was concentrated under reduced pressure using rotary vacuum evaporator. The filtrate was air dried to yield 38 g of hexane extract, 68 g of ethyl acetate extract and 55 g of methanol extract. All the crude extracts above obtained are stored at 4°C in air tight containers until assay.

Table 1. Larvicidal activity of *Swertia chirata* against different larval stages of *Aedes aegypti*

| Instars                | Solvents      | LC <sub>50</sub> | 95% Confidence limit |         | LC <sub>90</sub> | Chi-square |
|------------------------|---------------|------------------|----------------------|---------|------------------|------------|
|                        |               |                  | LFL                  | UFL     |                  |            |
| 4 <sup>th</sup> instar | Hexane        | 1076.94          | 903.40               | 1374.21 | 2181.42          | 26.50      |
|                        | Ethyl acetate | 329.29           | 247.28               | 414.98  | 816.83           | 58.38      |
|                        | Methanol      | 1489.90          | 1194.34              | 2101.58 | 2728.44          | 14.31      |
| 3 <sup>rd</sup> instar | Hexane        | 955.74           | 808.35               | 1195.14 | 2081.92          | 28.60      |
|                        | Ethyl acetate | 339.06           | 302.19               | 490.64  | 766.77           | 67.28      |
|                        | Methanol      | 1307.01          | 1068.12              | 1764.58 | 2504.69          | 18.07      |
| 2 <sup>nd</sup> instar | Hexane        | 866.19           | 699.38               | 1173.78 | 1923.52          | 37.26      |
|                        | Ethyl acetate | 237.30           | 125.09               | 337.30  | 733.96           | 83.54      |
|                        | Methanol      | 1279.15          | 1023.31              | 1807.38 | 2653.54          | 28.23      |
| 1 <sup>st</sup> instar | Hexane        | 875.89           | 707.09               | 1189.42 | 1784.37          | 41.13      |
|                        | Ethyl acetate | 192.67           | 127.46               | 253.68  | 494.91           | 69.41      |
|                        | Methanol      | 996.74           | 775.08               | 1498.81 | 2242.25          | 38.35      |

Values were based on four concentrations and four replications with 25 larvae in each. Significant at  $p < 0.05$  level.

#### Mosquito culture

*Ae. aegypti* and *Cx. quinquefasciatus* larvae, were derived from various places with stagnant water bodies within Chennai, India, and were colonized and maintained continuously for generations in a laboratory free of exposure to pathogens, insecticides or repellents. They were maintained at  $27 \pm 2^\circ\text{C}$ , 75-85% Relative humidity under a photoperiod of 14:10 h (light/dark) in the insectary. Larvae were fed on finely ground dog biscuit and yeast extract in the ratio of 3:1. Water was changed everyday to avoid scum formation, which might create toxicity. Pupae were transferred from the trays to a cup containing tap water and placed in screened cages (30x30x30 cm dimension) where the adults emerged. The adults of *Ae. aegypti* and *Cx. quinquefasciatus* were reared in the glass cages of 30x30x30 cm dimension. The adult colony was provided with 10% sucrose solution and was periodically blood-fed on restrained rats. After three days, ovitrap was kept in the cages and the eggs were collected and transferred to the enamel trays. Two developmental stages, larvae and adult females, were continuously available for the experiments and were

Table 2. Larvicidal activity of *Swertia chirata* against different larval stages of *Culex quinquefasciatus*

| Instars                | Solvents     | LC <sub>50</sub> | 95% Confidence limit |         | LC <sub>90</sub> | Chi-square |
|------------------------|--------------|------------------|----------------------|---------|------------------|------------|
|                        |              |                  | LFL                  | UFL     |                  |            |
| 4 <sup>th</sup> instar | Hexane       | 962.89           | 829.57               | 1167.74 | 1905.51          | 24.51      |
|                        | Ethyl acetat | 326.46           | 224.59               | 429.15  | 887.45           | 67.85      |
|                        | Methanol     | 1588.13          | 1244.69              | 2362.12 | 2937.77          | 15.89      |
| 3 <sup>rd</sup> instar | Hexane       | 930.70           | 772.88               | 1202.66 | 1928.60          | 30.38      |
|                        | Ethyl acetat | 284.05           | 187.98               | 379.08  | 765.55           | 75.40      |
|                        | Methanol     | 1297.58          | 1064.64              | 1737.66 | 2469.81          | 20.65      |
| 2 <sup>nd</sup> instar | Hexane       | 896.75           | 715.00               | 1253.61 | 2003.06          | 38.78      |
|                        | Ethyl acetat | 220.10           | 120.78               | 311.50  | 620.70           | 94.72      |
|                        | Methanol     | 1240.01          | 964.37               | 1888.76 | 2548.19          | 30.85      |
| 1 <sup>st</sup> instar | Hexane       | 789.31           | 633.67               | 1065.92 | 1790.04          | 42.84      |
|                        | Ethyl acetat | 164.91           | 86.34                | 231.31  | 464.21           | 85.50      |
|                        | Methanol     | 1030.91          | 789.85               | 1622.23 | 2295.62          | 42.11      |

Values were based on four concentrations and four replications with 25 larvae in each. Significant at  $p < 0.05$  level.

maintained at the same condition.

#### Egg hatchability test

Egg hatchability was studied following the method of Su and Mulla (1998). Hundred freshly layed (0-6, 6-12 and 12-18 h old) eggs of *Ae. aegypti* and *Cx. quinquefasciatus* were exposed to four (1000, 500, 250 and 125 ppm) concentrations. *Cx. quinquefasciatus* eggs were separated with the help of fine brush. Each concentration was replicated five times. Each crude extract was dissolved in water with an emulsifier (Tween 80) to get the experimental concentrations. Tween 80 was used as negative control and egg

hatchability was observed under the microscope. The hatch rate was assessed in percentage at 120 h post treatment using the following formula.

$$\text{Number of hatched larva} / \text{Total number of eggs in treated water} \times 100$$

#### Larvicidal bioassay

Larvicidal activity was evaluated using WHO method (1975) with slight modifications. Twenty larvae of first, second, third and fourth instar were released separately in a 500 ml glass beaker containing 249 ml of dechlorinated water and 1.0 ml of the desired plant extract concentration. Five replicates for each concentration was run at a time. Tween 80 was used as negative control. Mortality and survival were recorded after 24 hours of the exposure period. The moribund and dead larvae in four replicates were combined and expressed as a percentage of larval mortality for each concentration. Dead larvae were identified when they failed to move after probing with a needle in the siphon or cervical region. Moribund larvae were those incapable of rising to the surface (within reasonable period of time) or showing the characteristic diving reaction when the water was disturbed. The mortality was calculated by EPA Probit analysis programme version 1.5.

#### Results and discussion

In the present investigation the toxicity of *S. chirata* was tested at four different concentrations against the first, second, third and fourth instar larvae of *Cx. quinquefasciatus* and *Ae. aegypti*. The mortality rate was recorded and statistical data regarding LC<sub>50</sub>, LC<sub>90</sub>, 95% confidence limit and chi-square were calculated. The LC<sub>50</sub> values of *S. chirata* against first, second, third and fourth instar larvae of *Cx. quinquefasciatus* were 164.91, 220.10, 284.05 and 326.46 ppm and against *Ae. aegypti*

Table 3. Egg hatchability of *Swertia chirata* against *Culex quinquefasciatus*

| Age of eggs | Solvent       | Concentration |           |           |           |
|-------------|---------------|---------------|-----------|-----------|-----------|
|             |               | 1000 ppm      | 500 ppm   | 250 ppm   | 125 ppm   |
| 00-06 h     | Hexane        | 36.8±2.38     | 46.0±3.60 | 56.6±2.30 | 64.4±2.40 |
| 06-12 h     |               | 46.6±3.20     | 56.4±3.57 | 64.4±3.20 | 73.8±4.20 |
| 12-18 h     |               | 55.6±3.84     | 66.6±2.88 | 79.2±4.54 | 92.4±4.39 |
| 00-06 h     | Ethyl acetate | 12.6±2.40     | 18.6±3.04 | 26.4±3.78 | 33.2±3.83 |
| 06-12 h     |               | 20.4±2.70     | 26.8±2.58 | 33.0±3.80 | 36.2±2.58 |
| 12-18 h     |               | 25.4±3.84     | 36.6±2.70 | 39.6±2.07 | 56.2±3.70 |
| 00-06 h     | Methanol      | 61.2±3.34     | 66.2±3.70 | 71.4±4.92 | 74.4±4.03 |
| 06-12 h     |               | 71.4±3.36     | 75.4±3.36 | 82.6±3.57 | 86.8±3.56 |
| 12-18 h     |               | 84.4±2.96     | 86.4±3.36 | 90.0±4.63 | 93.6±2.96 |
| Control     |               | 98.0±2.12     |           |           |           |

Values are mean of five replications of four concentrations. (Mean ±S.D.)

192.67, 237.30, 339.06 and 329.29 ppm respectively (Table 1 & 2). No mortality was observed in negative control. Maximum mortality was observed in ethyl acetate extract followed by hexane and methanol extract. In the present study first and second instar larvae were highly susceptible than third and fourth instar larvae of *Cx. quinquefasciatus* and *Ae. aegypti*. Maheswaran *et al.* (2008) also have reported similar findings as mentioned above in hexane extract of *Leucas aspera*. Redwane *et al.* (2002) reported that the ethyl acetate extract of *Quercus lusitania* showed 497 and 1531ppm against second and fourth instar larvae of *Cx. pipiens*.

Table 4. Egg hatchability of *Swertia chirata* against *Aedes aegypti*

| Age of eggs | Solvent       | Concentration |           |           |           |
|-------------|---------------|---------------|-----------|-----------|-----------|
|             |               | 1000 ppm      | 500 ppm   | 250 ppm   | 125 ppm   |
| 00-06 h     | Hexane        | 35.4±3.64     | 43.6±4.61 | 54.2±4.08 | 62.2±4.02 |
| 06-12 h     |               | 46.6±3.64     | 53.0±3.16 | 63.2±3.56 | 70.4±5.94 |
| 12-18 h     |               | 53.6±2.96     | 63.2±3.70 | 78.2±4.54 | 91.4±2.50 |
| 00-06 h     | Ethyl acetate | 11.4±1.81     | 16.2±2.77 | 24.6±2.07 | 31.2±3.03 |
| 06-12 h     |               | 17.8±1.48     | 23.6±2.70 | 28.8±2.16 | 32.8±4.96 |
| 12-18 h     |               | 23.4±4.39     | 34.6±3.04 | 37.6±2.96 | 54.0±4.47 |
| 00-06 h     | Methanol      | 62.6±3.84     | 64.2±3.83 | 70.2±2.28 | 74.2±3.96 |
| 06-12 h     |               | 76.8±2.58     | 80.2±1.92 | 83.8±2.48 | 89.2±1.92 |
| 12-18 h     |               | 84.0±4.35     | 87.2±3.11 | 90.6±3.71 | 95.8±1.92 |
| Control     |               | 99.0±1.34     |           |           |           |

Values are mean of five replications of four concentrations. (Mean ±S.D.)

In the laboratory test three different age groups of the eggs of *Cx. quinquefasciatus* and *Ae. aegypti* were tested at four different concentrations of *S. chirata* leaf extract. At 1000 ppm concentration, ethyl acetate extract showed 12.6, 20.4 and 25.4 % egg hatchability at 0-6, 6-12 and 12-18 h against *Cx. quinquefasciatus* and 11.4, 17.8 and 23.4 % egg hatchability at 0-6, 6-12 and 12-18 h against *Ae. aegypti* respectively. At 125 ppm concentration the hatchability was 33.2, 36.2 and 56.2 % at 0-6, 6-12, 12-18 h in *Cx. quinquefasciatus* and 31.2, 32.8 and 54.0 % at 0-6, 6-12, 12-18 h in *Ae. aegypti* followed by hexane and methanol extracts (Table 3 & 4). In the present study the ethyl acetate extract of *S. chirata* retarded embryonic growth and delayed hatch. The treated eggs contained developed embryos the eclosion of the egg was incomplete (Miura *et al.*, 1976). The ovicidal efficacy

compared well with an earlier report; the bioactive compound Azadirachtin (*Azadirachta indica*) showed complete ovicidal activity in eggs of *Cx. tarsalis* and *Cx. quinquefasciatus* exposed to 10 ppm concentration (Su & Mulla 1998). Ouda *et al.* (1998) reported that the seed extract of *Atriplex canescens* showed complete ovicidal at 1,000 ppm concentration in eggs of *Cx. quinquefasciatus*. The present findings corroborated with earlier studies of ethyl acetate extracts of *Glycosmis pentaphylla* leaves which reduced egg hatchability of *Cx. quinquefasciatus* and prolonged developmental duration of mosquito larvae (Muthukrishnan *et al.*, 1999). The botanical insecticides are generally pest-specific and are relatively harmless to non-target organisms including man, easily biodegradable and also harmless to the environment (Rembold, 1994). To the best of our knowledge this is the first report of effective ovicidal and larvicidal activity of different solvent extracts of *S. chirata* against *Cx. quinquefasciatus* and *Ae. aegypti*. Ethyl acetate extract of *S. chirata* showed potential larvicidal activity. In short, our findings suggested that leaves of *S. chirata* and its effective constituents may be explored as a potential natural larvicide. Further investigations for the mode of the constituents' actions, effects on non-target organisms and field evaluation are necessary.

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