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*Full Length Research Paper*

# Larvicidal effect of *Hemidesmus indicus, Gymnema sylvestre*, and *Eclipta prostrata* against *Culex qinquifaciatus* mosquito larvae

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**The larvicidal effect of aqueous extracts of *Hemidesmus indicus* roots*, Gymnema sylvestre* and *Eclipta prostrata* leaves were tested against *Culex quinquefasciatus* larvae at the concentrations of 1, 2, 3, 4 and 5% up to three days. All extracts showed larval mortality. Larval mortality was 100% with the use of 5% concentration of root extract of *H. indicus*, leaves extracts of *G. sylvestre* and *E. prostrata* after 2 days. Qualitative analysis of the phytochemicals of aqueous extracts revealed the presence of carbohydrates, saponins, phytosterols, phenols, flavonoids and tannins in all the plants. Quantitative analysis showed that the crude saponin was the major phytochemical constituent present in highest percentage followed by crude tannin in all three plants. It is suggested that all the three plants possess larvicidal properties that could be developed and used as natural insecticides for mosquito control.**

**Key words:** Aqueous extract, larval mortality, qualitative analysis, phytochemicals, natural insecticides.

## INTRODUCTION

Human filariasis is a major public health hazard and remains a challenging socioeconomic problem in many of the tropical countries (Udonsi et al., 1986). Lymphatic filariasis caused by *Wuchereria bancrofti* and transmitted by mosquito *Culex quinquefasciatus* is found to be more endemic in the Indian subcontinent. It is reported that *C. quinquefasciatus* infects more than 100 million individuals worldwide annually (Rajasekariah et al., 1991). *W. bancrofti* is the most predominant filarial nematode, which is usually characterized by progressive debilitating swelling at the extremities, scrotum, or breast (elephantiasis) in an infected individual (Myung et al., 1998).

Mosquitoes constitute a major public health menace, serves as a vector for transmitting diseases to humans. Control of such mosquito-borne diseases is becoming more and more difficult because of increasing resistance to pesticides, lack of effective vaccines and drugs against disease-causing mosquitoes. Hence, an alternative approach for mosquito control is the use of extracts of plant origin (El Hag et al., 1999). Search for natural insecticides, which do not have any ill effects on the non-target population and are easily degradable, remains to be one of the top priority issues for the tropical countries (Redwane et al., 2005).

*Hemidesmus indicus*, commonly called Indian sarsaparilla is a climbing vine found throughout India which belongs to a family Asclepiadaceae. *H. indicus* has long been used as a folk medicine and found to be an ingredient in ayurvedic and unani preparations which are usually prescribed against inflammation, diarrhoea, respiratory disorders, skin diseases, syphilis, fever, bronchitis, asthma, eye diseases, urinary disorders, loss of appetite, burning sensation and rheumatism and especially for epileptic fits in children (Lakshman et al., 2006).

*Gymnema sylvestre* a valuable medicinal plant belongs to a family Asclepiadaceae, widely distributed in all parts of India and Africa, is of much of medicinal value. It has long been used in traditional medicine as a remedy for diabetes mellitus, stomach ache and diarrhea. The plant is popularly known as 'gurmar' for its distinctive property of temporarily destroying the taste of sweetness (Reddy et al., 2004).

*Eclipta prostrata* a small, branched annual herb comm-

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only called as Karichalai belongs to a family Asteraceae, with white flower heads, is native to the tropical and subtropical regions of the world. Leaf juice is used as tonic for jaundice and leaf paste is applied on the affected area for tooth ache (Sandhya et al., 2006).

In the present study, we tested the effectiveness of aqueous extracts of different parts of plants on larvicidal activity against *C. quinquefasciatus* larvae.

## MATERIALS AND METHODS

Roots of *H. indicus* and leaves of *G. sylvestre* and *E. prostrata* were collected from the medicinal herbal garden and their identity were confirmed at VIT University, Tamil Nadu, India. The roots of *H. indicus,* leaves of *G. sylvestre* and *E. prostrata* were washed, dried, powdered and kept in an airtight container for further use. The powdered root and leaves were used separately for the preparation of aqueous extracts of different concentrations; by soaking in 100 ml Millipore sterilized water for 24 h. Then the aqueous extracts were filtered using sterile 0.2 µ membrane filter.

*C. quinquefasciatus* mosquito larvae were collected from water stagnated area, and identified in Zonal Entomological Research Laboratory, Vellore, Tamil Nadu, India. There were then maintained under suitable temperature and humidity. Twenty larvae of the *C. quinquefasciatus* were placed in each of the three 150 ml sterile beaker containing 90 ml of water. After adding the larvae to the beaker, 10 ml of aqueous root extracts of *H. indicus*, leaves extracts of *G. sylvestre* and *E. prostrata* was added in each of the beakers, separately. Then the beaker containing the larvae were kept in the growth room maintained at room temperature. The larvicidal effects of the extracts were monitored by counting the number of dead larvae each day up to three days. Each test was repeated thrice, the percentages of larval mortality and standard error were calculated for each concentration of aqueous extracts of all the three plants.

### Phytochemical screening

Chemical tests were carried out using the aqueous extracts from plants and or the powdered specimens, using standard procedures to identify the constituents as described by Sofowara (1993), Trease and Evans (1989) and Harborne (1973).

### Test for alkaloids

**Mayer's test (Evans, 1997):** To a few ml of the filtrates, a drop of Mayer's reagent was added by the side of the test tube. A creamy or white precipitate indicates the test is positive.

### Test for carbohydrates

**Benedict's test:** To 0.5 ml of the filtrate, 0.5 ml of Benedict's reagent was added. The mixture was heated on boiling water bath for 2 min. A characteristic red colored precipitate indicates the presence of sugar.

### Test for saponins (Kokate, 1999)

The extract was diluted with distilled water and made up to 20 ml. The suspension was shaken in a graduated cylinder for 15 min. 2 cm layer of foam indicates the presence of saponins.

### Test for phytosterols (Finar, 1986)

**Libermann-Buchard's test:** The extract was mixed with 2 ml of acetic anhydride. To this 1 or 2 drop of concentrated sulphuric acid was added slowly along the sides of the test tubes. An array of color change shows the presence of phytosterols.

### Test for phenolic compounds and tannins (Mace, 1963)

**Ferric chloride test:** The extract was diluted to 5 ml with distilled water. To this a few drops of neutral 5% ferric chloride solution was added. A dark green color indicates the presence of phenolic compounds.

**Test for tannins:** About 0.5 mg of dried powdered samples was boiled in 20 ml of water in test tubes then filtered. A few drops of 0.1 % ferric chloride was added and observed for brownish green or blue black coloration.

**Test for flavonoids:** To 5 ml of the dilute ammonia solution a portion of the aqueous extract was added, followed by addition of concentrated sulphuric acid. Appearance of yellow coloration indicates the presence of flavonoids.

**Test for terpenoids (Salkowski test):** 5 ml of the extract was mixed with 2 ml of chloroform and concentrated sulphuric acid to form a layer. A reddish brown coloration of the interface showed the presence of terpenoids.

**Test for phlobatannins:** Formation of red precipitate when aqueous extract of plant sample was boiled with 1% aqueous hydrochloric acid indicates the presence of phlobatannins.

### Determination of total phenols

The fat free sample was boiled with 50 ml of ether for the extraction of the phenolic component for 15 min. 5 ml of the extract was pipetted out into a 50 ml flask, then 10 ml of distilled water was added. 2 ml of ammonium hydroxide solution and 5 ml of concentrated amyl alcohol were also added. The samples were made up to mark and left to react for 30 min for color development and the intensity was measured at 505 nm.

### Alkaloid determination (Harborne, 1973)

To 5 g of the sample in 250 ml beaker, 200 ml of 10% acetic acid in ethanol was added, covered and allowed to stand for 4 h. This was filtered and the extract was concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitated was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed.

### Tannin determination (Van-Burden and Robinson, 1981)

500 mg of the sample was weighed into a 50 ml plastic bottle. 50 ml of distilled water was added and shaken for 1 h in a mechanical shaker. This was filtered into a 50 ml volumetric flask and made up to the mark. Then 5 ml of the filterate was pipetted out into a test tube and mixed with 2 ml of 0.1 M $FeCl_3$ in 0.1 N HCl and 0.008 M potassium ferrocyanide. The absorbance was measured at 120 nm within 10 min.

**Table 1.** Mortality of *Culex quinquefasciatus* mosquito larvae at different concentrations of aqueous extracts of *Hemidesmus indicus* root.

| Root extract concentration (%) | Mortality % | | |
|---|---|---|---|
| | 1st day | 2nd day | 3rd day |
| 0 | 0 ±0 | 0±0 | 0±0 |
| 1 | 23.3±1.6 | 28.3±1.6 | 28.3±1.6 |
| 2 | 25.0±0 | 40.0±0 | 55.0±0 |
| 3 | 35.0±0 | 50.0±0 | 65.0±0 |
| 4 | 53.3±0 | 83.3±3.3 | 96.6±3.3 |
| 5 | 80.0±0 | 100±0 | 100±0 |

Values are the mean of 3 (n = 3 ± SE).

**Table 2.** Mortality of *Culex quinquefasciatus* mosquito larvae at different concentrations of aqueous extract of *Gymnema sylvestre* leaves.

| Leaf extracts concentration (%) | Mortality % | | |
|---|---|---|---|
| | 1st day | 2nd day | 3rd day |
| 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 |
| 1 | 5 ± 0 | 11.6 ± 1.6 | 31.6 ± 1.6 |
| 2 | 20 ± 0 | 25.0 ± 0 | 45.0 ± 0 |
| 3 | 25 ± 0 | 35.0 ± 0 | 45.0 ± 0 |
| 4 | 6.6 ± 1.6 | 71.6 ± 3.3 | 71.6 ± 3.3 |
| 5 | 6.6 ± 1.6 | 100 ± 0 | 100 ± 0 |

Values are the mean of 3 (n = 3 ± SE).

**Saponin determination: (Obadoni and Ochuko, 2001)**

The samples were ground and 20 g of each were put into a conical flask and 100 $cm^3$ of 20% aqueous ethanol were added. The samples were heated over a hot water bath for 4 h with continuous stirring at about 55ºC. The mixture was filtered and the residue re-extracted with another 200 ml 20% ethanol. The combined extracts were reduced to 40 ml over water bath at about 90ºC. The concentrate was transferred into a 250 ml separator funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. 60 ml of n-butanol was added. The combined n-butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation the samples were dried in the oven to a constant weight; the saponin content was calculated as percentage.

**Flavonoid determination (Boham and Kocipai- Abyazan, 1974)**

10 g of the plant sample was extracted repeatedly with 100 ml of 80% aqueous methanol at room temperature. The whole solution was filtered through Whatmann filter paper No 42 (125 mm). The filtrate was later transferred into a crucible and evaporated into dryness over a water bath and weighed to a constant weight.

## RESULTS AND DISCUSSION

The larvicidal activity of aqueous extracts of *H. indicus* root leaves of *G. sylvestre* and *E. prostrata* against *C. quinquefasciatus* mosquito larvae were given in Tables 1 to 3. The larvicidal activity of aqueous extract of *H. indicus* roots showed 28, 55, and 65% of death with the use of 1, 2, and 3% concentrations, respectively, after 3 days. The third day 4% concentration killed more than 95% of the larvae. However, 100% mortality was observed only in 5% concentration alone (Table 1). Aqueous extracts of leaves of *G. sylvestre* causes 31, 45, and 45% after 3 days with respect to 1, 2 and 3% concentration of extract, respectively. The larval mortality was below 50% when 1, 2, and 3% concentrations. *G. sylvestre* showed 100% mortality during the second day with 5% concentrations (Table 2). Aqueous extract from leaves of *E. prostrata* showed greater than 50% mortality when 2% and more concentrations were used. Only the highest concentration (5%) of all three extracts showed 100% mortality (Table 3). Among the three extracts, the root extract of *H. indicus* was found more lethal than other extracts.

The qualitative study carried out on the aqueous extract of all the three plants revealed the presence of medicinally active constituents such as carbohydrates, saponins, phytosterols, phenols, flavonoids and tannins (Table 4). However, cardiac glycosides are absent in all plants. Phlobatannins are present only in *H. indicus* and absent in other plants. Similarly alkaloids are absent in *H. indicus* and *E. prostrata* and present only in *G. sylvestre.*

Crude saponin was the major constituent in *G. sylvestre* (5.5%), *E. prostrate* (4.5%) and *H. indicus* (0.6%) fol-

**Table 3.** Mortality of *Culex quinquefasciatus* mosquito larvae at different concentrations of aqueous extract of *Eclipta prostrata* leaves.

| Leaf extracts concentration (%) | Mortality % | | |
|---|---|---|---|
| | 1st day | 2nd day | 3rd day |
| 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 |
| 1 | 3.3 ± 3.3 | 28.3 ± 3.3 | 36.6 ± 1.6 |
| 2 | 1.6 ± 1.6 | 46.6 ± 1.6 | 51.6 ± 1.6 |
| 3 | 31.6 ± 1.6 | 58.3 ± 1.6 | 68.3±1.6 |
| 4 | 38.3 ± 1.6 | 61.6 ± 3.3 | 70.0 ± 5.0 |
| 5 | 78.3 ± 1.6 | 100 ± 0 | 100 ± 0 |

Values are the mean of 3 (n = 3 ± SE).

**Table 4.** Qualitative analysis of the phytochemicals of aqueous extracts of *Hemidesmus indicus* roots, *Gymnema sylvestre* and *Eclipta prostrata* leaves.

| Phytochemicals | *Hemidesmus indicus* | *Gymnema sylvestre* | *Eclipta prostrata* |
|---|---|---|---|
| Alkaloids | - | + | - |
| Carbohydrates | + | + | + |
| Saponins | + | + | + |
| Phytosterols | + | + | + |
| Phenols | + | + | + |
| Flavonoids | + | + | + |
| Terpenoids | + | - | + |
| Tannins | + | + | + |
| Phlobatannins | + | - | - |

\+ Presence of the compound.
\- Absence of the compound.

**Table 5.** Quantitative analysis of the phytochemicals of aqueous extracts of *Hemidesmus indicus* roots, *Gymnema sylvestre* and *Eclipta prostrata* leaves.

| Phytochemicals | *Hemidesmus indicus* (%) | *Gymnema sylvestre* (%) | *Eclipta prostrate* (%) |
|---|---|---|---|
| Saponins | 0.6 | 5.5 | 4.5 |
| Tannins | 3.0 | 1.0 | 1.0 |

lowed by crude tannins in *H. indicus* (3.0%), *G. sylvestre* (1.0%) and *E. prostrata* (1.0%) (Table 5). Other phytochemicals estimated in all the three study plants are present only in very low concentration. Wiesman and Chapagain (2006) reported that saponin extracted from the fruit of *Balanites aegyptiaca* showed 100% larvicidal activity against *A. aegypti* mosquito larvae. Morrissey and Osbourn (1999) have suggested that the saponin molecules interact with the cuticle membrane of the larvae, ultimately disarranging the membrane could be the most probable reason for the larval death. The deficiency of dissolved oxygen and active presence of the antioxidant saponin molecule might be the reason for larval death. However, much study is required to find out the mechanism by which saponin kills the larvae (Chapagain and Wiesman, 2005). A commercial saponin mixture extracted from *Q. saponaria* showed increasing toxicity (100% larval mortality) in *A. aegypti* and *Culex pipiens* when both saponin concentration and the duration of the experiment were increased (Pelah et al., 2002).

Prenylated xanthones, tetracyclic phenols and saponins are reported to be effective in controlling mosquito *A. aegypti*, the vector of yellow fever (Marston et al., 1993). Aluminium chloride, known for its phenolic complexing activity, obtained from alder leaf also reported to have the larvicidal activity against *A. aegypti* (David et al., 2001). Isoflavonoids from tubers of *Neorautanenia mitis* had larvicidal effect against the malaria and filariasis transmitting mosquitoes, *Anopheles gambiae* and *C. quinquefaciatus*, respectively (Joseph et al. 2004). Similarly monoterpene hydrocarbons showed a marked mosquito larvicidal activity against *C. pipiens* which is obtained from the fresh leaves of *Anthemis melampodina* and *Pluchea dios-*

*coridis* (Massoud et al., 2001). A piperidine alkaloid from *Piper longum* fruit was found to be active against mosquito larvae of *C. pipiens* (Lee, 2000). Similarly an alkaloid derived from the tropical vine *Triphyophyllum peltatum* (Dioncophyllaceae), was found to have larvicidal activity against the malaria vector *Anopheles stephensi* (Francois et al., 1996).

Our results show that plant extracts have high concentrations saponins and tannins might be the reason for larvicidal activity. Further studies are going on in our laboratory to study effect of purified saponins on larvicidal activity. The purified plant meta-bolite of the following plant parts such as roots of *H. indicus* and leaves of *G. sylvestre*, and *E. prostrata* can be used as environment friendly and sustainable insecticides to control mosquito.

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