Ovicidal and larvicidal activities of some plant extracts against Aedes aegypti L. and Culex quinquefasciatus Say (Diptera: Culicidae)

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Abstract

Objective: To evaluate the ovicidal and larvicidal activities of hexane, chloroform and methanol extracts of Gymnema sylvestre, Scilla peruvina, Rubia cordifolia (R. cordifolia) and Elytraria acaulis roots against the eggs and larvae of Aedes aegypti L. (Ae. aegypti) and Culex quinquefasciatus Say (Cx. quinquefasciatus) at different concentrations of 62.5, 125, 250 and 500 mg/L.

Methods: The plant materials were shade dried in the laboratory for one week and then coarsely powdered. The root powder of each plant (500 g) was sequentially soaked in hexane, chloroform and methanol for 96 h with intermittent shaking. After 96 h, the solution was filtered and the filtrate was concentrated under reduced pressure by using rotary vacuum evaporator. All the crude extracts thus obtained were stored in air tight glass vials and Petri dishes.

Results: The ovicidal activity results showed that the methanol extract of R. cordifolia root was the most potent compared to other with 82.40% and 70.40% activity against the eggs of Cx. quinquefasciatus and Ae. aegypti, respectively, at 500 mg/L concentration similarly, methanol extract of R. cordifolia root also recorded the highest larvicidal activity with LC50 and LC90 values of 95.69, 347.96 mg/L against Cx. quinquefasciatus and 102.23, 350.20 mg/L against Ae. aegypti larvae, respectively.

Conclusions: Hence, methanol extract of R. cordifolia root can be probed further for effective biological control of mosquitoes.

1. Introduction

Mosquitoes are well established in tropical regions and serve as vectors responsible for transmission of several pathogens to human. Some pathogens are transmitted by the day-biting mosquitoes and some are transmitted by the night-biting mosquitoes. Particularly, in recent years thousands of dengue and chikungunya positive cases have been reported from several countries(1-4). Aedes aegypti (Ae. aegypti), a day-biting mosquito is the primary vector of dengue and chikungunya. Similarly, several thousands of people suffer from filariasis(5,6). Culex quinquefasciatus (Cx. quinquefasciatus) is a night-biting mosquito involved in the transmission of filarial nematode. Targeting the immature stages is the key factor in controlling these two species of mosquitoes(7,8). For this, chemical larvicides such as organophosphates have been used; but several disadvantages have been reported due to harmful effects to human and other associated organisms(9-14). Further, many factors involved in the increase of mosquito population(15), particularly development of resistance to synthetic insecticides lead to greater increase of vector mosquitoes.

Plant extracts are good alternatives to chemical insecticides to control mosquito population(16). Many earlier reports have confirmed the bioactivity of several plant extracts. In the present study, the roots of four plants viz., Gymnema sylvestre (G. sylvestre), Scilla peruvina (S. peruvina), Rubia cordifolia (R. cordifolia) and Elytraria acaulis (E. acaulis) were used for solvent extraction. G. sylvestre is used in the treatment of diabetes, besides being used for arthritis, diuretic, anemia, osteoporosis, hypercholesterolemia(17). Species under the genus Scilla reported to possess antioxidant effect, blood circulatory activation, cough control and abscess reduction(18). R. cordifolia decoction from roots is prescribed to cure jaundice, paralytic affections and urinary troubles(19). Roots of R. cordifolia have also been used as astringent, thermogenic, febrifuge, antisyphilitic, anthelmintic, galactopurifier, ophthamalic and rejuvenant(20). The extracts of E. acaulis are reported to possess decreasing effect on blood glucose level, reduction in the liver glycogen level and reduction in glycated hemoglobin levels(21). The leaves decoction of E. acaulis is prescribed to treat fever, venereal diseases and its root is used in traditional medicine against tumor,
pneumonia, asthma, migraine, leucorrhoea, snake bite and diarrhea. Leaves are also used as antiabdi[c][22,23]. In this paper, in order to evaluate the ovicidal and larvicidal activities of hexane, chloroform and methanol extracts of four plants, the crude extracts of the roots of the above mentioned plants were tested against the eggs and larvae of *Ae. aegypti* and *Cx. quinquefasciatus* under laboratory conditions.

2. Materials and methods

2.1. Sample collection and preparation of solvent extracts

Root of *R. cordifolia* was purchased from local market, Parrys. Roots of other three plants were collected naturally from the field in Thiruvallur District, India. The plant materials were shade dried in the laboratory for one week and then coarsely powdered. The root powder of each plant (500 g) was sequentially soaked in hexane, chloroform and methanol for 96 h with intermittent shaking. After 96 h, the solution was filtered and the filtrate was concentrated under reduced pressure using rotary vacuum evaporator. All the crude extracts thus obtained were stored in air tight glass vials and Petri dishes.

2.2. Preparation of various concentrations and test mosquitoes

From the crude extract, different concentrations (62.5, 125, 250 and 500 mg/L) were prepared by using acetone. The mosquitoes were reared at (27 ± 2) °C, 75%–85% relative humidity under a photoperiod of 14:10 h as previously reported in our laboratory[24].

2.3. Ovicidal assay

Ovicidal activity was studied following the method of Reegan et al.[25]. Twenty five freshly laid eggs of *Ae. aegypti* and *Cx. quinquefasciatus* were separately exposed to four different concentrations viz., 62.5, 125, 250 and 500 mg/L prepared using acetone. Each concentration was replicated five times. A zadirachtin was used as positive control with the concentration of 10.0 mg/ml for comparison. Control was maintained separately with five replicates and egg mortality was assessed after 120 h post treatment using the following formula:

\[
\text{Percent ovicidal activity} = \frac{\text{Number of unhatched eggs}}{\text{Total number of eggs introduced}} \times 100
\]

2.4. Larvicidal activity

The larvicidal activities of the crude extracts were assessed following the protocol of World Health Organization[26]. The early third instar larvae of *Ae. aegypti* and *Cx. quinquefasciatus* were exposed to the concentrations of 62.5, 125, 250 and 500 mg/L. Five replicates were maintained for every concentration of each extract. Control was maintained separately with five replicates. Larval mortality was recorded after 24 h. Larvae were considered dead when they did not move to the surface of the solution. A zadirachtin was used as positive control with the concentrations of 2.5, 5.0, 7.5 and 10.0 mg/L for comparison.

2.5. Statistical analysis

The mean values and SD were calculated from five replications. The calculated percent ovicidal means were separated by Tukey’s test of multiple comparisons, One-way ANOVA. The larvicidal mortality was corrected by Abbott’s formula[27] and the lethal concentration values of LC50 and LC90 were calculated by using EPA probit analysis program (version 1.5).

3. Results

3.1. Ovicidal activity

The ovicidal activity varied among the different extracts of the plants. The methanol extract of *R. cordifolia* root recorded the highest ovicidal activities of 82.40% and 70.40% against the eggs of *Cx. quinquefasciatus* and *Ae. aegypti*, respectively, at 500 mg/L concentration (Tables 1 and 2). It was followed by hexane extract of *S. peruvina* root which recorded ovicidal activities of 44.80% and 43.20% against the eggs of *Cx. quinquefasciatus* and *Ae. aegypti*, respectively, at 500 mg/L concentration. Further, the hexane extract of *R. cordifolia* root recorded moderate ovicidal activities of 26.40% and 25.60% against the eggs of *Cx. quinquefasciatus* and *Ae. aegypti*, respectively, at 500 mg/L concentration. All the other plant extracts showed low ovicidal activities. The positive control, azadirachtin recorded ovicidal activities of 95.20% and 92.80% against the eggs of *Cx. quinquefasciatus* and *Ae. aegypti*, respectively, at 10.0 mg/L concentration (Tables 1 and 2).

Table 1

| Plant species | Treatment | Concentration (mg/L) | LC50 | LC90 |
|---------------|-----------|----------------------|------|------|
| *G. sylvestre* | Hexane    | 0.00                 |      |      |
|               | Chloroform| 0.80 ± 1.78          | 9.60 ± 1.78 | 18.40 ± 2.19 |
|               | Methanol  | 3.20 ± 1.78          | 22.40 ± 2.19 | 44.80 ± 1.78 |
| *S. peruvina* | Hexane    | 0.00                 |      |      |
|               | Chloroform| 6.40 ± 1.78          | 16.80 ± 2.19 | 39.20 ± 1.78 |
|               | Methanol  | 10.40 ± 2.19         | 23.20 ± 2.19 | 48.80 ± 1.78 |
| *R. cordifolia* | Hexane     | 7.20 ± 2.19           | 10.40 ± 2.19 | 20.80 ± 2.19 |
|                 | Chloroform| 14.40 ± 2.19         | 26.60 ± 2.19 | 51.60 ± 2.19 |
|                 | Methanol  | 25.60 ± 4.56         | 45.60 ± 4.56 | 83.40 ± 4.56 |
| *E. acaulis* | Hexane    | 3.20 ± 2.19          | 12.00 ± 2.19 | 20.00 ± 2.19 |
|               | Chloroform| 6.40 ± 3.34          | 26.00 ± 3.34 | 48.00 ± 3.34 |
|               | Methanol  | 10.40 ± 3.34         | 44.00 ± 3.34 | 78.00 ± 3.34 |
| Control       |           | 0.00                 |      |      |

Data were shown as mean ± SD; Means were separated by Tukey’s test of multiple comparisons, One-way ANOVA. Data with same letters in the column are not significantly different.

Table 2

| Plant species | Treatment | Concentration (mg/L) | LC50 | LC90 |
|---------------|-----------|----------------------|------|------|
| *G. sylvestre* | Hexane    | 0.00                 |      |      |
|               | Chloroform| 0.80 ± 1.78          | 9.60 ± 1.78 | 18.40 ± 2.19 |
|               | Methanol  | 3.20 ± 1.78          | 22.40 ± 2.19 | 44.80 ± 1.78 |
| *S. peruvina* | Hexane    | 0.00                 |      |      |
|               | Chloroform| 6.40 ± 1.78          | 16.80 ± 2.19 | 39.20 ± 1.78 |
|               | Methanol  | 10.40 ± 2.19         | 23.20 ± 2.19 | 48.80 ± 1.78 |
| *R. cordifolia* | Hexane     | 7.20 ± 2.19           | 10.40 ± 2.19 | 20.80 ± 2.19 |
|                 | Chloroform| 14.40 ± 2.19         | 26.60 ± 2.19 | 51.60 ± 2.19 |
|                 | Methanol  | 25.60 ± 4.56         | 45.60 ± 4.56 | 83.40 ± 4.56 |
| *E. acaulis* | Hexane    | 3.20 ± 2.19          | 12.00 ± 2.19 | 20.00 ± 2.19 |
|               | Chloroform| 6.40 ± 3.34          | 26.00 ± 3.34 | 48.00 ± 3.34 |
|               | Methanol  | 10.40 ± 3.34         | 44.00 ± 3.34 | 78.00 ± 3.34 |
| Control       |           | 0.00                 |      |      |

Azadirachtin (100 mg/L) 95.20 ± 1.78

Data were shown as mean ± SD; Means were separated by Tukey’s test of multiple comparisons, One-way ANOVA. Data with same letters in the column are not significantly different.
which recorded LC50 and LC90 values of 106.81, 289.80 mg/L against Cx. quinquefasciatus and 114.13, 434.62 mg/L against Ae. aegypti larvae, respectively. All the other extracts showed only moderate or least larvicidal activities (Tables 3 and 4). Further, it was noted that Cx. quinquefasciatus larvae were more susceptible than Ae. aegypti. The results were compared with positive control azadirachtin, which showed LC50 and LC90 values of 3.00, 6.64 mg/L against Cx. quinquefasciatus and 3.17, 9.67 mg/L against Ae. aegypti larvae, respectively.

4. Discussion

Mosquitoes are nuisance and most dangerous insects, since they transmit pathogens. Vector mosquitoes are well established in tropical and subtropical regions and they also have developed resistance to chemical insecticides. Hence biological control method would be a good approach in mosquito control program.

In the present study, the methanol extract of R. cordifolia recorded the highest ovicidal activities of 82.40% and 70.40% against the eggs of Cx. quinquefasciatus and Ae. aegypti, respectively, at 500 mg/L concentration. Our result is comparable to the report of Elango et al.[28], who had reported that Cocculus hirsutus methanol extract caused 86% ovicidal activity at 500 mg/L concentration against the eggs of Anopheles subpictus. In another study, Marimuthu et al.[29], have reported 100% ovicidal activity at 300 mg/L concentration with methanol extract of Delonix elata against the eggs of Anopheles stephensi (An. stephensi) and Ae. aegypti.

Further, the same methanol extract of R. cordifolia showed good larvicidal activity with LC50 and LC90 values of 95.69, 347.96 mg/L against Cx. quinquefasciatus and 102.23, 350.20 mg/L against Ae. aegypti larvae, respectively. Our results corroborate with the results of Aivazi and Vijayan[30], who had reported LC50 and LC90 values of 116.92, 144.77 mg/L with ethyl acetate extract of Quercus infectoria Gall against the fourth instar larvae of Ae. stephensi. The LC50 values of 177.14 and 513.387 mg/L were reported with methanol extracts of Exophoria irisicalli latex and stem bark, respectively, against the larvae of Cx. quinquefasciatus[31].

Further, our results revealed that Cx. quinquefasciatus larvae were more susceptible than Ae. aegypti. Similar to our results, many investigators have reported varied results among different mosquito species[32] and evaluated the methanolic extracts from fruits and seeds of Solanum xanthocarpum against the larvae of Anopheles culicifacies, An. stephensi, Ae. aegypti and Cx. quinquefasciatus. The LC50 values varied for fruits and seeds with 51.6, 52.2, 118.3, 133.7 mg/L against Cx. quinquefasciatus, 51.6, 52.2, 118.3, 133.7 mg/L against An. stephensi, 51.6, 52.2, 118.3, 133.7 mg/L against Ae. aegypti and Cx. quinquefasciatus, respectively. Similarly, Patil et al.[33] recorded the highest larval mortality with methanol extracts of Plumbago zeylanica root with LC50 value of 167.8 mg/L against Aedes aegypti larvae than An. stephensi, which showed LC50 value of 222.34 mg/L.

Phytochemicals like anthraquinones, alkaloides, glycosides, flavanoids, tannins, saponins, phenols and triterpenoides have been reported earlier from leaves and roots of R. cordifolia[34-36].

In conclusion, the methanol extract of R. cordifolia was the
most potent against the eggs and larvae of Ae. aegypti and Cx. quinquefasciatus. These results suggest that methanol extract of Rubia cordifolia can be probed further for effective mosquito control.

**Conflict of interest statement**

We declare that we have no conflict of interest.

**Acknowledgments**

The authors are thankful to Entomology Research Institute for financial assistance (Grant No. F.58).

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