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Adulticidal activity of *Ageratum houstonianum* Mill. (Asteraceae) leaf extracts against three vector mosquito species (Diptera: Culicidae)

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ABSTRACT

Objective: To determine the intrinsic toxicity of hexane, ethyl acetate and methanol crude extracts of *Ageratum houstonianum* leaves against adult *Anopheles stephensi*, *Aedes aegypti* and *Culex quinquefasciatus* mosquitoes. **Methods:** Bioassay was performed in 2-day-old laboratory reared unfed adult female mosquitoes by topical application at concentrations of 0.01, 0.05, 0.10, 0.25 and 0.50 μ g/mg female adult mosquito. **Results:** *Aedes aegypti* was found to be more susceptible to ethyl acetate and hexane extracts with LD₅₀ value of 0.10 μ g/mg, and both *Anopheles stephensi* and *Culex quinquefasciatus* were susceptible to methanol extract with LD₅₀ values of 0.12 μ g/mg female adult mosquito. **Conclusions:** The results show promising adulticidal activity on topical application and further studies followed by in-depth laboratory and field bioassays are needed to screen, isolate and purify bioactive phytochemical constituents or compounds.

1. Introduction

Mosquitoes are responsible for the transmission of debilitating diseases such as malaria, filaria, Japanese encephalitis, dengue, chikungunya and others[1,2]. Control of mosquitoes particularly vectors is indispensable for the eradication or containment of these diseases. Conventional approach involves use of insecticides against adult and larval stages. The drawbacks associated with long term use of insecticides such as disruption of ecological balance, ill effects on non-target organisms, development of physiological resistance and selection of resistant strains especially among target organisms have prompted search for new alternative insecticides. Plant derived products or compounds are generally considered to be pest specific, readily biodegradable with low bioaccumulation and lack toxicity to higher animals[3,4] and have been screened for bioactivity against vector mosquitoes with many promising

results. Many plants of medical importance have been screened for insecticidal activity against different species of adult mosquitoes[5,6]. *Ageratum houstonianum* Mill. (*A. houstonianum*) of the family Asteraceae is a medicinal plant and possesses antimicrobial properties[7]. The plant is also shown to possess insecticidal property[8–11], but there is no report on its activity against mosquitoes. The plant was therefore screened for its insecticidal property against vector mosquitoes. In the present study, the intrinsic toxicity of the crude leaf extracts against adult vector mosquitoes, viz., *Anopheles stephensi* (*An. stephensi*), *Aedes aegypti* (*Ae. aegypti*) and *Culex quinquefasciatus* (*Cx. quinquefasciatus*) is reported.

2. Materials and methods

2.1. Preparation of plant extract

A. houstonianum was collected from the foothill regions of Javadhu Hills, Tiruvanamalai District, Tamil Nadu, India, which is known for its richness in medicinal plants. Taxonomical identity of the plants was confirmed in the Department of Plant Biology and Biotechnology, Loyola College, Chennai, Tamil Nadu, India. Shade dried and powdered leaves (1 kg) were subjected to sequential

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extraction using 3 L of hexane, ethyl acetate and methanol for a period of 72 h to obtain the crude. Solvent was removed and crude extract was concentrated by rotary vacuum evaporator at temperatures of 45, 57 and 60 °C respectively. The hexane, ethyl acetate and methanol crude extracts thus obtained were lyophilized and stored at 4 °C.

2.2. Rearing and maintenance of vector mosquitoes

Cyclic generations of the three vector species, namely, *An. stephensi*, *Ae. aegypti* and *Cx. quinquefasciatus*, were reared and maintained separately in mosquito cages (61 cm × 61 cm × 61 cm) in an insectary. Mean room temperature of (27±2) °C and a relative humidity of 70%–80% was maintained in the insectary. The adult mosquitoes were fed on 100 g/L glucose solution. For continuous maintenance of mosquito colony, the adult female mosquitoes were blood fed with laboratory reared albino mice. The eggs laid in ovitraps were then transferred to enamel larval trays maintained in the larval rearing chamber. The larvae were fed with larval food (dog biscuits and yeast at a ratio 3:1, w/w). The larvae on becoming pupae were collected, transferred to plastic bowls and kept inside mosquito cage for adult emergence.

2.3. Mosquito bioassay

The intrinsic toxicity test was conducted against the laboratory reared vector mosquitoes free of exposure to insecticides and pathogens following WHO standard protocols^[12] with slight modifications. Non-blood fed 2-day-old adult female mosquitoes were anaesthetized with anaesthetic ether and weighed using an electronic balance. The extract at the desired concentration was prepared by dissolving the crude extracts in acetone, and 0.1 μL of it was applied onto the upper part of the pronotum of each of the immobilized vector mosquito using a micropipette. After treatment, all vector mosquitoes were transferred to plastic bowls, maintained at 25–29 °C and relative humidity of 80%–90%, and provided with 100 g/L glucose solution. At the end of 24 h recovery period, the mosquitoes were scored as dead if they showed no movement. Test doses studied

were 0.01, 0.05, 0.10, 0.25 and 0.50 μg/mg for adult female mosquito. A total of 20 mosquitoes were used for each concentration. Control groups received appropriate dose of acetone only. A total of three trials were undertaken and the results were pooled for analysis.

2.4. Data management and statistical analysis

Mortality was calculated using the following formula:

$$\text{Mortality (\%)} = \text{Number of deaths/Number treated} \times 100. \quad (1)$$

When the control mortality ranged between 5%–20%, the observed mortality was corrected using Abbot's formula^[13]:

$$\text{Mortality (\%)} = (\text{Test mortality} - \text{Control mortality}) / (\text{100} - \text{Control mortality}) \times 100. \quad (2)$$

The lethal dosage for 50% and 95% mortality and the fiducial limits were determined by log-probit analysis using SPSS software version 11.5^[14]. Two way analysis of variance (ANOVA) of mortality followed by Tukey's test was performed to measure differences between test concentrations among extracts and vector species, and chi square analysis to test differences in mortality between vector species at different concentrations.

3. Results

Sequential extraction with hexane, ethyl acetate and methanol yielded 0.84%, 2.90% and 1.21% (w/w) of the starting dry material. The crude extracts were completely soluble in acetone. All vector mosquitoes studied, viz., *An. stephensi*, *Ae. aegypti* and *Cx. quinquefasciatus*, were susceptible to topical application. The susceptibility was dose dependent in all vector species studied. On treatment with increasing concentrations of hexane, ethyl acetate and methanol extracts, the mortality increased by 21.6%, 28.4% and 26.6% in the case of *An. stephensi*; by 21.7%, 26.7% and 25.0% in *Ae. aegypti*; and by 45.0%, 30.0% and 31.6% in *Cx. quinquefasciatus*, respectively (Table 1). No mortality was observed in control. For each extract, two way ANOVA of mortality at different concentrations showed statistically significant difference ($P < 0.05$), confirming dose dependant

Table 1

Adulticidal activity of leaf extracts of *A. houstonianum* against adult female vector mosquitoes.

| Extract | Dose (μg/mg mosquito) | Mortality (%) | | | Chi square value |
|-----------------------|--------------------------|----------------------|--------------------|-----------------------------|------------------|
| | | <i>An. stephensi</i> | <i>Ae. aegypti</i> | <i>Cx. quinquefasciatus</i> | |
| Hexane extract | 0.01 | 31.7±7.7 | 45.0±5.0 | 20.0±8.7 | 8.60* |
| | 0.05 | 36.7±7.7 | 46.7±7.6 | 35.0±8.7 | 2.00 |
| | 0.10 | 43.3±17.6 | 48.3±5.8 | 36.7±7.7 | 1.68 |
| | 0.25 | 46.7±7.7 | 63.3±7.6 | 43.3±12.6 | 5.51 |
| | 0.50 | 53.3±5.8 | 66.7±7.6 | 65.0±8.7 | 2.68 |
| Ethyl acetate extract | 0.01 | 33.3±7.6 | 43.3±10.4 | 31.7±7.6 | 2.07 |
| | 0.05 | 36.7±12.6 | 50.0±5.0 | 36.7±7.6 | 2.94 |
| | 0.10 | 43.3±2.9 | 55.0±10.0 | 38.3±7.6 | 3.54 |
| | 0.25 | 56.7±7.6 | 56.7±2.9 | 46.7±7.6 | 1.61 |
| | 0.50 | 61.7±10.4 | 70.0±5.0 | 61.7±2.9 | 1.21 |
| Methanol extract | 0.01 | 36.7±7.6 | 43.3±10.4 | 36.7±7.6 | 0.75 |
| | 0.05 | 43.3±2.9 | 48.3±12.6 | 40.0±10.0 | 0.86 |
| | 0.10 | 53.3±7.6 | 48.3±7.6 | 50.0±5.0 | 0.31 |
| | 0.25 | 56.7±10.4 | 50.0±15.0 | 58.3±7.6 | 0.94 |
| | 0.50 | 63.3±10.4 | 68.3±7.6 | 68.3±7.6 | 0.45 |

The data are expressed as mean ± S.D. No mortality in control was observed. *Significance at $P < 0.05$ level.

Table 2LD₅₀ and LD₉₅ values of leaf extracts of *A. houstonianum* against adult female vector mosquitoes.

| Extract | <i>An. stephensi</i> | | <i>Ae. aegypti</i> | | <i>Cx. quinquefasciatus</i> | |
|-----------------------|----------------------|------------------|--------------------|------------------|-----------------------------|------------------|
| | LD ₅₀ | LD ₉₅ | LD ₅₀ | LD ₉₅ | LD ₅₀ | LD ₉₅ |
| Hexane extract | 0.18 (0.12–0.45) | 0.64 (0.40–1.95) | 0.10 (0.05–0.20) | 0.48 (0.31–1.16) | 0.16 (0.12–0.24) | 0.46 (0.34–0.75) |
| Ethyl acetate extract | 0.14 (0.09–0.24) | 0.50 (0.34–1.00) | 0.10 (0.04–0.19) | 0.47 (0.31–1.17) | 0.16 (0.11–0.26) | 0.52 (0.37–0.97) |
| Methanol extract | 0.12 (0.07–0.24) | 0.51 (0.33–1.29) | 0.12 (0.06–0.25) | 0.51 (0.32–1.44) | 0.12 (0.07–0.19) | 0.44 (0.31–0.86) |

Units are expressed as μ g/mg female adult mosquito. Figures in parenthesis represent confidence intervals.

toxicity. *An. stephensi* and *Cx. quinquefasciatus* were relatively more susceptible to treatment with methanol (LD₅₀ = 0.12 μ g/mg), whereas *Ae. aegypti* to ethyl acetate and hexane extracts (LD₅₀ = 0.10 μ g/mg in both extracts) (Table 2). Chi square analysis for comparison of mortality between vector species ranged between 0.31 and 8.60 and the mortality of adult female mosquitoes was found to be statistically significant only in hexane extract at a dose of 0.01 μ g/mg ($P = 0.01358$), indicating no significant difference in mortality between the vector species.

4. Discussion

A. houstonianum, commonly referred to as floss flower, is widely distributed in tropical and subtropical regions of the world. Its usefulness in the treatment of various ailments is well known. The insecticidal property of the crude extract of this plant species against vector mosquitoes is not known. There are however reports indicating effective mosquitocidal property of closely related species like *Ageratum conyzoides*. Nevertheless, the intrinsic toxicity against adult vector mosquitoes as determined by topical application has not been studied in both species though few reports are available in respect of other plants species. Choochote et al. worked on the intrinsic toxicity of ethanolic extract of the whole plant of *Piper longum*, *Piper ribesoides* and *Piper sarmentosum* against *Ae. aegypti*. They reported the activity to be comparatively high in *Piper sarmentosum* followed by *Piper ribesoides* and *Piper longum* with LD₅₀ values of 0.14, 0.15 and 0.26 μ g/mg female adult mosquito^[15]. When compared to the results of the present study, the LD₅₀ values of hexane, ethyl acetate and methanol crude extracts were 0.10, 0.10 and 0.12 μ g/mg female adult mosquito against *Ae. aegypti*, indicating greater toxicity of crude extract of *A. houstonianum*. Dose dependent mortality was observed and the toxicity was almost similar in all the three vector species. *Ae. aegypti* was more susceptible to the hexane and ethyl acetate extracts, whereas *An. stephensi* and *Cx. quinquefasciatus* to the methanol crude extract. The results show promising adulticidal activity on topical application and requires further studies on isolation, purification and characterization of bioactive phytochemical constituents. Further, development of aerosolized formulation for effective delivery as aerial toxicants will be helpful in effective management of adult vector mosquitoes.

Conflict of interest statement

We declare that we have no conflict of interest.

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