Repellent activity of *Ageratum houstonianum* Mill. (Asteraceae) leaf extracts against *Anopheles stephensi*, *Aedes aegypti* and *Culex quinquefasciatus* (Diptera: Culicidae)

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1. Introduction

Prevention of man vector mosquito contact is indispensable for protection from mosquito-borne diseases and use of repellents have been advocated for the same. Traditionally plant based products have been used as repellents either as a fumigant or topical applicant. In recent years, new synthetic repellents have been formulated and advocated. However, continuous and indiscriminate use of these synthetic compounds causes adverse effects on the user[1]. There has been a paradigm shift towards botanicals to overcome the problems associated with the use of synthetic compounds. Many plant species have been screened for their repellent and insecticidal property[2-6]. Plant species belonging to the family Asteraceae have been exploited for their medicinal and insecticidal purposes. *Ageratum houstonianum* (*A. houstonianum*) belonging to this family is a medicinal plant and possesses antimicrobial property[7]. Recent studies indicate the plant to possess insecticidal property against adult vector mosquitoes[8]. There are no reports on the repellent property of this plant against mosquitoes. Therefore, in the present study, the repellent activity of *A. houstonianum* crude leaf extracts was evaluated against vector mosquitoes.

2. Materials and methods

2.1. Preparation of plant extract

*A. houstonianum* collected from the foothill regions of Javadhu hills, Tiruvanamalai District, Tamil nadu, India, was taxonomically identified and confirmed at the Department of Plant Biology and Biotechnology, Loyola College, Chennai, Tamilnadu, India. Shade dried and powdered leaves (1 kg) was subjected to sequential 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 the temperatures of 45 °C, 57 °C and 60 °C respectively. The hexane, ethyl acetate and methanol crude extracts thus obtained were lyophilized and stored at 4 °C.
2.2. Bioassay

The tests were carried out against laboratory reared vector mosquitoes free of exposure to insecticides and pathogens. A concentration of 0.01% was chosen. The studies were carried out on laboratory reared albino mice. Before the initiation of the trials, healthy mice of almost equal size, were selected and tagged for identification. The length and diameter of the tail were measured and surface area calculated. Concurrently, the dosages were calculated and experimental solution prepared. During experiments, the mice were each first mosquito bite. Thereafter, observation was made on an hourly basis and mosquitoes that have fed were removed. In the case of An. stephensi and Cx. quinquefasciatus, the study was carried out under natural light from 18.00 to 06.00 h and in Ae. aegypti it was 06.00 to 18.00 h coinciding with the natural feeding time. Hourly air temperature and relative humidity was recorded. Three trials were carried for assessment. Control was run simultaneously and coconut oil (100 g L-1) served as treated control. Per cent protection was calculated on application of the three extracts excluding treated control was as high as 91.7 and 91.5 per cent for An. stephensi and Ae. aegypti in methanol extracts and 85.1 per cent for Cx. quinquefasciatus in hexane extract. The repellent quotient obtained on treatment with hexane, ethyl acetate, methanol extracts and treated control against An. stephensi, Ae. aegypti and Cx. quinquefasciatus was 0.9, 0.7 and 0.7 respectively (Figure 1).

3. Results

No mosquito bites was observed during the first three quarters of night (18.00 to 03.00 h) in hexane, ethyl acetate, methanol and treated control in the case of An. stephensi and Cx. quinquefasciatus and first two quarters of day (06.00 to 12.00 h) in Ae. aegypti. The maximum protection time on application with hexane, ethyl acetate, methanol extracts and treated control was 11.30, 10.30, 10.30 and 9.30 against An. stephensi; 7.30, 7.30, 8.30 and 3.50 against Ae. aegypti and 9.30, 10.30, 11.30 and 9.30 h against Cx. quinquefasciatus respectively. The per cent protection on application of the three extracts are given in Table 1. The order of decreasing protection in the extracts of hexane, ethyl acetate, methanol and treated control was An. stephensi, Ae. aegypti and Cx. quinquefasciatus; An. stephensi, Cx. quinquefasciatus and Aedes aegypti; Ae. aegypti, An. stephensi and Cx. quinquefasciatus; and An. stephensi, Ae. aegypti and Cx. quinquefasciatus respectively. The lowest protection among the three extracts excluding treated control was as high as 91.7 and 81.5 per cent for An. stephensi and Ae. aegypti in methanol extracts and 85.1 per cent for Cx. quinquefasciatus in hexane extract. The repellent quotient obtained on treatment with hexane, ethyl acetate, methanol extracts and treated control against An. stephensi, Ae. aegypti and Cx. quinquefasciatus was 0.9, 0.8 and 0.7; 0.9, 0.8 and 0.8; 0.8, 0.6 and 0.9; 0.7, 0.8 and 0.7 respectively (Figure 1).

Table 1

| Particulars | Vector mosquito species | Hexane | Ethyl acetate | Methanol | Treated control (Coconut oil) | Untreated control |
|-------------|-------------------------|--------|---------------|----------|-----------------------------|------------------|
| Protection time* in hours (Time of first bite) | An. stephensi | 11.30 (05.00–06.00) | 10.30 (04.00–05.00) | 10.30 (04.00–05.00) | 9.30 (03.00–04.00) | 0.25 (18.00–19.00) |
| Per cent protection | An. stephensi | 93.4 (1.3) | 93.4 (1.3) | 91.7 (1.7) | 86.8 (2.7) | 3.4 (19.4) |
| Mean number of fed mosquitoes | Ae. aegypti | 91.5 (1.7) | 88.5 (2.3) | 81.5 (3.7) | 88.5 (2.3) | 0.0 (20.0) |
| Repellency of leaf extracts of A. houstonianum. |

4. Discussion

Phytochemicals such as alkaloids, flavonoids and monoterpenes are known for their mosquito repellent and insecticidal properties[10-12]. A. houstonianum has been reported to possess these biologically active compounds. The repellent efficacy against A. houstonianum however has not been reported. In the present study, results showed potential repellent activity and maximum protection was obtained in hexane and ethyl acetate extract against An. stephensi, hexane extract against Ae. aegypti and methanol extract in the case of Cx. quinquefasciatus. The repellent quotient was 0.9, 0.8 and 0.9 respectively indicating a good
repellent property. However, when the repellent efficacy of coconut oil used as base is considered, the efficacy of the crude extracts indicate poor activity. Coconut oil itself provided 86.8, 88.5 and 85.1 per cent protection against the three vector species studied. Therefore, it is conclusive that the crude extracts of *A. houstonianum* possess poor repellent activity but when used in conjunction with coconut oil, a commonly used domestic product, the mixture provides good protection. The efficacy of coconut oil has also been reported by Ravindran et al.[13]. Further, when considering the protection time, comparatively, the crude extracts yielded better protection in *An. stephensi*, followed by *Cx. quinquefasciatus* and *Ae. aegypti*. As mentioned elsewhere, coconut oil provided almost equivalent protection time in the case of *An. stephensi* and *Cx. quinquefasciatus*. However, in the case of *Ae. aegypti*, coconut oil alone, offered a low protection time of 3 hours and 30 minutes. *Ae. aegypti* mosquitoes are voracious and multiple feeders and this may be the reason for poor protection time and per cent protection.

In a closely related species of the genus *Ageratum* viz., *Ageratum conyzoides*, effective repellent activity of leaves was noticed when used as a fumigant against *Ae. aegypti*. The results showed 100.0 per cent knock down effect within 30 min but 65.0 per cent of the mosquitoes recovered showing its limited use as a repellent[14–16]. The hexane and chloroform leaf extracts of *Eclipta prostrata* and *Tagetes erecta*, plant species belonging to the family Asteraceae, provided 64.0 and 44.0 per cent protection at concentration of 0.0125 per cent against *Culex tritaeniorhynchus* when studied on human volunteers, two hours after application[17,18]. Essential oil of *Ajania tenuifolia* offered 90.4 per cent protection at a dosage of 1.0 per cent, eight hours after application when studied on topical application on mice against *Aedes albopictus*[19].

*A. houstonianum* widely used in traditional medicine has been reported to possess various bioactive compounds that have both medicinal and insecticidal properties. The crude leaf extracts of *A. houstonianum* in combination with coconut oil showed repellent activity against *An. stephensi*, *Ae. aegypti* and *Cx. quinquefasciatus*. Hexane extract showed more repellence against *An. stephensi* followed by methanol extract against *Cx. quinquefasciatus* and ethyl acetate extract against *Ae. aegypti*. Extraction of other bioactive compounds, evaluation of the same and development of effective formulation may be helpful in the addition of yet another phytocompound for mosquito control.

**Conflict of interest statement**

We declare that we have no conflict of interest.

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