



Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Disease

journal homepage: www.elsevier.com/locate/apjtd



Document heading doi: 10.1016/S2222-1808(12)60104-2 © 2012 by the Asian Pacific Journal of Tropical Disease. All rights reserved.

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

Samuel Tennyson<sup>1\*</sup>, John Ravindran<sup>2</sup>, Alex Eapen<sup>2</sup>, John William<sup>3</sup><sup>1</sup>Department of Zoology, Madras Christian College, Chennai 600 059, Tamilnadu, India<sup>2</sup>National Institute of Malaria Research (ICMR) Field Unit, NIE Campus, 2nd Main Road, TNHB Ayapakkam, Chennai 600 077, Tamilnadu, India<sup>3</sup>School of Entomology and Centre for Natural Resources Management (SECNARM), P.G. & Research Department of Advanced Zoology and Biotechnology, Loyola College, Chennai 600 034, Tamilnadu, India

### ARTICLE INFO

#### Article history:

Received 24 August 2012

Received in revised form 8 August 2012

Accepted 12 September 2012

Available online 28 December 2012

#### Keywords:

*Ageratum houstonianum*

Leaf extracts

Repellent activity

*Anopheles stephensi**Aedes aegypti**Culex quinquefasciatus*.

### ABSTRACT

**Objective:** To determine the repellent activity of hexane, ethyl acetate and methanol crude extracts of *Ageratum houstonianum* (*A. houstonianum*) leaves against adult *Anopheles stephensi* (*An. stephensi*), *Aedes aegypti* (*Ae. aegypti*) and *Culex quinquefasciatus* (*Cx. quinquefasciatus*) mosquitoes. **Methods:** Bioassay on laboratory reared Swiss albino mice by topical application at a fixed concentration of 0.01 per cent with coconut oil as a base. **Results:** Crude leaf extracts of *A. houstonianum* in combination with coconut oil repelled vector mosquitoes. Maximum protection for a period of 11.30 h was obtained against *Cx. quinquefasciatus* and *An. stephensi* when treated with methanol and hexane extracts followed by 8.30 h against *Ae. aegypti* in methanol extract. Amongst the three extracts, methanol extract gave the maximum protection of 95.0% against *Cx. quinquefasciatus*. Hexane and ethyl acetate extracts gave a maximum of 93.4% protection against *An. stephensi*. **Conclusions:** The crude extracts of *A. houstonianum* leaves in combination with coconut oil showed repellent activity with repellent quotient ranging from 0.6 to 0.9.

## 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 repellents causes adverse effects on the user<sup>[1]</sup>. 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<sup>[2–6]</sup>. 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<sup>[7]</sup>. Recent studies indicate the plant to possess insecticidal property against adult vector mosquitoes<sup>[8]</sup>. 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 taxonomical 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.

\*Corresponding author: Dr. Samuel Tennyson, Assistant Professor, Department of Zoology, Madras Christian College, Chennai 600 059, Tamilnadu, India.  
Tel: +91 9884116135  
E-mail: samtennyson@gmail.com

### 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 held in a mice holder which were completely covered and made inaccessible to the mosquitoes except for the tail region. Known quantity of the plant extracts mixed with 100 µL of coconut oil was applied evenly on the tail. After application, the tail was taped to a wooden strip measuring 15.0 cm in length and 4.0 cm in width at the base and tip in order to immobilize the tail. The mice holder along with the mice was kept in a 1 feet (1.0×1.0×1.0) mosquito cage. Twenty healthy 48 h starved female mosquitoes were released in each cage. Continuous observation was carried until the 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 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 µL) served as treated control. Per cent protection and Repellent quotient (RQ)<sup>[9]</sup> were calculated using the following formula:

$$\text{Per cent protection} = \frac{\text{NC} - \text{NT}}{\text{NC}} \times 100$$

Where

NC: Number of fed mosquitoes in control;  
 NT: Number of fed mosquitoes in treated;

$$\text{Repellent quotient (RQ)} = \frac{\text{NR} - \text{NUR}}{\text{NR} + \text{NUR}}$$

Where

NR: Number of mosquitoes repelled;  
 NUR: Number of mosquitoes unrepelled;  
 Repellent quotient returns a value of 1.0 for complete repellency and 0 for no effect.

### 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 3.30 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).

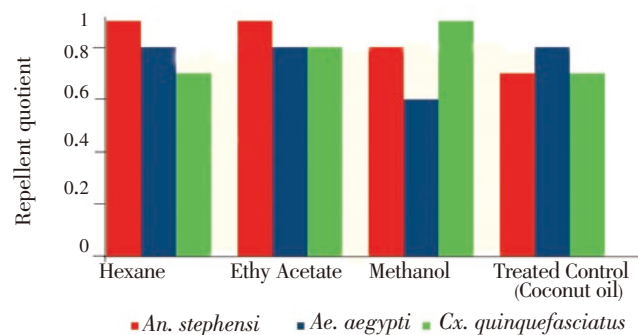


Figure 1. Repellency of leaf extracts of *A. houstonianum*.

### 4. Discussion

Phytochemicals such as alkaloids, flavonoids and monoterpenes are known for their mosquito repellent and insecticidal properties<sup>[10–12]</sup>. *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 the 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

Table 1

Repellent activity of *A. houstonianum* leaf extracts against vector mosquitoes.

Particulars	Vector mosquito species	Hexane	Ethyl acetate	Methanol	Treated control (Coconut oil)	Untreated control
Protection time* in hours (Time of first bite)	<i>An. stephensi</i>	11.30 (05.00–06.00)	10.30 (04.00– 05.00)	10.30 (04.00–05.00)	9.30 (03.00–04.00)	0.35 (18.00–19.00)
	<i>Ae. aegypti</i>	7.30 (13.00–14.00)	7.30 (13.00–14.00)	8.30 (14.00–15.00)	3.30 (09.00–10.00)	0.00.10 (06.00–07.00)
	<i>Cx. quinquefasciatus</i>	9.30 (03.00–04.00)	10.30 (04.00–05.00)	11.30 (05.00–06.00)	9.30 (03.00–04.00)	0.25 (18.00–19.00)
Per cent protection (Mean number of fed mosquitoes)	<i>An. stephensi</i>	93.4 (1.3)	93.4 (1.3)	91.7 (1.7)	86.8 (2.7)	3.4 (19.4)
	<i>Ae. aegypti</i>	91.5 (1.7)	88.5 (2.3)	81.5 (3.7)	88.5 (2.3)	0.0 (20.0)
	<i>Cx. quinquefasciatus</i>	85.1 (3.0)	91.7 (1.7)	95.0 (1.0)	85.1 (3.0)	3.4 (19.3)

n=3; \*for protection time, the median for each duration is represented.

repellent property. However, when the repellent efficacy of coconut oil used as base is considered, the efficacy of the crude extracts mentioned previously 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.<sup>[13]</sup>. 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<sup>[14–16]</sup>. 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<sup>[17,18]</sup>. 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*<sup>[19]</sup>.

*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 phytochemical for mosquito control.

### Conflict of interest statement

We declare that we have no conflict of interest.

### Acknowledgements

The authors are thankful to the staff members of National Institute of Malaria Research (ICMR), Field Unit, Chennai, Tamilnadu, India for their assistance and facilities provided.

### References

- [1] Mandal S. Repellent activity of *Eucalyptus* and *Azadirachta indica* seed oil against the filarial mosquito *Culex quinquefasciatus* Say (Diptera: Culicidae) in India. *Asian Pac J Trop Biomed* 2011; **1**(2): S109–S112.
- [2] Shaalan EAS, Canyonb D, Younesc MWF, Wahaba HA, Mansoura AH. A review of botanical phytochemicals with mosquitocidal potential. *Environ Int* 2005; **31**: 1149–1166.
- [3] Sakthivadivel M, Daniel T. Evaluation of certain insecticidal plants for the control of vector mosquitoes viz., *Culex quinquefasciatus*, *Anopheles stephensi* and *Aedes aegypti*. *Appl Entomol Zool* 2008; **43**(1): 57–63.
- [4] Kamaraj C, Rahuman AA, Bagavan A, Elango G, Zahir AA, Santhoshkumar T. Larvicidal and repellent activity of medicinal plant extracts from Eastern Ghats of South India against malaria and filariasis vectors. *Asian Pac J Trop Med* 2011; **4**(9): 698–705.
- [5] Govindarajan M, Sivakumar R. Mosquito adulticidal and repellent activities of botanical extracts against the malarial vector, *Anopheles stephensi* Liston (Diptera: Culicidae). *Asian Pac J Trop Med* 2011; **4**(12): 941–947.
- [6] Samuel T, Ravindran KJ, Arivoli S. Screening of plant extracts for ovicidal activity against *Culex quinquefasciatus* Say (Diptera: Culicidae). *Appl Bot* 2011a; **40**: 5456–5460.
- [7] Samuel T, Balaraju K, Kyungseok P, Raja AK, Ravindran KJ, Eapen A, et al. *In vitro* antimicrobial activity of *Ageratum houstonianum* Mill. (Asteraceae). *Food Sci* 2011b; **35**: 2897–2900.
- [8] Ravindran J, Samuel T, Alex E, William J. Adulticidal activity of *Ageratum houstonianum* Mill. (Asteraceae) leaf extracts against three vector mosquito species (Diptera: Culicidae). *Asian Pac J Trop Dis* 2012; **2**(3): 177–179.
- [9] Williams CR, Smith BPC, Best SM, Tyler MJ. Mosquito repellents in frog skin. *Biol Lett* 2006; **2**(2): 242–245.
- [10] Trongtokit Y, Rongsriyam Y, Komalamisra N, Apiwatnasoru CH. Comparative repellency of 38 essential oils against mosquito bites. *Phytother Res* 2005; **19**: 303–309.
- [11] Jaenson TG, Palsson P, Karlson AB. Evaluation of extracts and oils of mosquito (Diptera: Culicidae) repellent plants from Sweden and Guinea Bissau. *J Med Entomol* 2006; **43**: 113–119.
- [12] Kiran S, Devi P. Evaluation of mosquitocidal activity of essential oil and sesquiterpenes from leaves of *Chloroxylon swietenia*. *Parasitol Res* 2007; **101**: 413–418.
- [13] Ravindran J, Eapen A, Kar I. Evaluation of repellent action of neem oil against the filarial vector, *Culex quinquefasciatus* (Diptera: Culicidae). *Ind J Malariol* 2002; **39**: 13–17.
- [14] Kazembe TC, Nkomo S. Mosquitocides of *Ageratum conyzoides*, *Boscia salicifolia* and *Grewia monticola*. *Ind J Trad Knowledge* 2010; **9**(2): 394–397.
- [15] Prabhu K, Murugan K, Nareshkumar A, Ramasubramanian N, Bragadeeswaran S. Larvicidal and repellent potential of *Moringa oleifera* against malarial vector, *Anopheles stephensi* Liston (Insecta: Diptera: Culicidae). *Asian Pac J Trop Biomed* 2011; **1**(2): 124–129.
- [16] Govindarajan M, Mathivanan T, Elumalai K, Krishnappa K, Anandan A. Ovicidal and repellent activities of botanical extracts against *Culex quinquefasciatus*, *Aedes aegypti* and *Anopheles stephensi* (Diptera: Culicidae). *Asian Pac J Trop Biomed* 2011; **1**(1): 43–48.
- [17] Elango G, Rahuman AA, Bagwan A, Kamraj C, Zahir AA, Rajakumar G, et al. Efficacy of botanical extracts against Japanese Encephalitis vector, *Culex tritaeniorhynchus*. *Parasitol Res* 2010; **106**: 481–492.
- [18] Kovendan K, Murugan K, Vincent S, Barnard DR. Mosquito larvicidal properties of *Orthosiphon thymiflorus* (Roth) Slessen. (Family: Labiatae) against mosquito vectors, *Anopheles stephensi*, *Culex quinquefasciatus* and *Aedes aegypti* (Diptera: Culicidae). *Asian Pac J Trop Med* 2012; **5**(4): 299–305.
- [19] Yang P, Ma Y. Repellent effect of plant essential oils against *Aedes albopictus*. *J Vector Ecol* 2005; **30**(2): 231–234.