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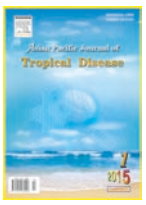

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Larvicidal activity of *Ageratum houstonianum* Mill. (Asteraceae) leaf extracts against *Anopheles stephensi*, *Aedes aegypti* and *Culex quinquefasciatus* (Diptera: Culicidae)

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ABSTRACT

Objective: To evaluate the larvicidal activity of *Ageratum houstonianum* (*A. houstonianum*) crude leaf extracts against the immatures of vector mosquitoes.
Methods: Bioassays were performed in the laboratory with hexane, ethyl acetate and methanol crude leaf extracts of *A. houstonianum* at concentrations of 62.5, 125, 250, 500, 1000, 2000, 4000 and 8000 mg/L against the third instar larvae of *Anopheles stephensi*, *Aedes aegypti* and *Culex quinquefasciatus*.
Results: Poor larvicidal activity was observed. The lowest LC_{50} value was noted in ethyl acetate extract against all three vector mosquito species studied and was 3377.84, 1952.12 and 3558.32 mg/L respectively after 24 h. The effect of toxicity was also manifested in a shorter period when compared to the other extracts *viz*., hexane and methanol. In *Anopheles stephensi*, more than 80% mortality was however observed at higher concentrations, after 24 h exposure in all the three extracts. In *Aedes aegypti* and *Culex quinquefasciatus*, this was observed by 3 and 24 h respectively in ethyl acetate extract.
Conclusions: Screening of other parts of *A. houstonianum* with other solvents from different places for its larvicidal activity is recommended.

1. Introduction

Vector mosquito control is indispensible for the control of mosquito-borne diseases. Numerous insecticides have been synthesized as tools against vector mosquitoes. For the past few decades, the problem of vector resistance to insecticides has developed in many vector-borne disease endemic areas in the world. Therefore, new compounds to overcome the problem are being searched for. Plants and their compounds have been intensively screened for their insecticidal property[1-4]. Plants belonging to the family Asteraceae have been extensively screened for their mosquitocidal property[5-9]. The genus *Ageratum* belonging to this family has been reported to possess insecticidal compounds[10] including precocenes[11]. The plant *Ageratum houstonianum* (*A. houstonianum*) is found widely distributed in many parts of India and Asian countries[12]. The present authors have reported on the adulticidal[13], repellent[14], oviposition[15] and ovicidal[16] activity of the crude leaf extract of *A. houstonianum* against vector mosquitoes. The larvicidal activity of *A. houstonianum* crude leaf extracts against *Anopheles stephensi* (*An. stephensi*), *Aedes aegypti* (*Ae. aegypti*) and *Culex quinquefasciatus* (*Cx. quinquefasciatus*), the vector of malaria, dengue and filariasis is reported here.

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2. Material and methods

2.1. Preparation of crude extract

A. houstonianum was collected from the foothill regions of

Javadhu hills, Tiruvanamalai district (Latitude 12°32'46" N Longitude 79°08'30" E), Tamil Nadu, India. Taxonomical identity of the plant was confirmed at the Department of Plant Biology and Biotechnology, Loyola College, Chennai, Tamil Nadu, India. Hexane, ethyl acetate and methanolic crude leaf extracts obtained by sequential extraction method reported elsewhere were stored at 4 ℃[13].

2.2. Larvicidal bioassay

Standard World Health Organization[17] protocol with minor modifications was adopted for the study. Larvicidal activity at test concentrations of 62.5, 125, 250, 500, 1000, 2000, 4000 and 8000 mg/L of the crude extract was assessed. The required test concentrations and quantity of test solution was prepared by serially diluting one per cent stock solution of the crude extract. Three replicates for each concentration and a control were run during each trial. Controls were run simultaneously. Experiments were carried out in plastic bowls (diameter: 12.0 cm; depth/height: 6.0 cm) of 500 mL capacity containing 250 mL of test solution. Tween 80 (0.01 mL) dissolved in distilled water served as treated control. Distilled water served as untreated control. Mosquito immature particularly third instar larvae were obtained from laboratory colonized mosquitoes of F_1 generation. Twenty healthy larvae were released in each bowl and mortality was observed 3, 6, 9, 12, 24, 48 and 72 h after treatment. Immatures were fed with larval feed (dog biscuit and yeast 3:1) during the experiment. The larvae were scored dead when they did not show any movement. A total of three trials were carried out. LC_{50} values were calculated using probit analysis[18] for 24 h only. When the control mortality ranged from 5%-20%, the observed percentage mortality was corrected by Abbott's[19] formula. Mean per cent mortality of the three crude extracts was calculated to determine which of the extracts caused profound mortality using the following formula.

$$\text{Mean per cent mortality}=\frac{\text{Mean mortality observed in an extract}}{\text{Sum mean mortality of all extracts}}\times 100$$

Two-way ANOVA was carried out (24 h) to determine the difference in larval mortality between concentrations.

3. Results

Hexane, ethyl acetate and methanol crude leaf extracts of *A. houstonianum* caused larval mortality in 24 h among all the three vector species studied except the methanol extract against *Ae. aegypti*. One hundred per cent mortality was obtained only against *Ae. aegypti* on exposure to ethyl acetate extract at the maximum concentration of 8000 mg/L. No mortality was observed in treated and untreated controls during any of the trials (Table 1).

Mortality was first observed at different hours in different concentrations. In *An. stephensi*, the immature mortality was first observed in the sixth hour at all concentrations in hexane extract, in the third hour at concentrations of 500 to 8000 in ethyl acetate and in the sixth hour at the highest concentration of 8000 mg/L in methanol extract. In *Ae. aegypti*, mortality was first observed in the third hour in hexane extract at concentrations ranging from 250 to 8000 mg/L. In ethyl acetate extract, it was at the third hour in concentration ranging from 500 to 8000 mg/L. No mortality was observed in methanol extract. In the case of *Cx. quinquefasciatus*, it was first observed at the third hour in all concentrations against all extracts tested. It is expected that at higher concentrations the toxic effect will be manifested within a short period on exposure. This was however different in the present study. The concentration at which 80% and more mortality and the time at which this was obtained are presented in Table 2. Figure 1 shows the range of mortality obtained at different concentrations and times.

Table 2

Concentration and time at which 80% and more larval mortality caused by crude leaf extracts of *A. houstonianum.*

Solvents	Vector mosquito species		
	An. stephensi	*Ae. aegypti*	*Cx. quinquefasciatus*
Hexane	8000 (48)	Nil*	4000 (72)
Ethyl acetate	2000 (48)	4000 (3)	2000 (72)
Methanol	8000 (48)	Nil*	4000 (48)

*Mortality of 80% and above not obtained. Values denote concentration in mg/L and those in parenthesis denote time in hours.

Table 1

Larvicidal activity of *A. houstonianum* leaf extracts against vector mosquitoes at 24 hours.

Particulars		*An. stephensi*			*Ae. aegypti*			*Cx. quinquefasciatus*		
		Hexane	Ethyl acetate	Methanol	Hexane	Ethyl acetate	Methanol	Hexane	Ethyl acetate	Methanol
Concentration (mg/L)	UC	0.0 ± 0.0 (0.0)	0.0 ± 0.0 (0.0)	0.0 ± 0.0 (0.0)	0.0 ± 0.0 (0.0)	0.0 ± 0.0 (0.0)	0.0 ± 0.0 (0.0)	0.0 ± 0.0 (0.0)	0.0 ± 0.0 (0.0)	0.0 ± 0.0 (0.0)
	TC	0.0 ± 0.0 (0.0)	0.0 ± 0.0 (0.0)	0.0 ± 0.0 (0.0)	0.0 ± 0.0 (0.0)	0.0 ± 0.0 (0.0)	0.0 ± 0.0 (0.0)	0.0 ± 0.0 (0.0)	0.0 ± 0.0 (0.0)	0.0 ± 0.0 (0.0)
	62.5	2.4 ± 2.1 (12.0)	0.3 ± 0.6 (1.5)	0.0 ± 0.0 (0.0)	0.0 ± 0.0 (0.0)	0.2 ± 0.2 (1.5)	0.0 ± 0.0 (0.0)	2.9 ± 2.8 (14.5)	2.0 ± 2.4 (10.0)	2.6 ± 3.8 (13.0)
	125	3.0 ± 2.6 (15.0)	1.1 ± 1.4 (5.5)	0.0 ± 0.0 (0.0)	0.9 ± 0.8 (4.5)	1.1 ± 0.9 (5.5)	0.0 ± 0.0 (0.0)	2.3 ± 1.2 (11.5)	2.5 ± 2.0 (12.5)	2.3 ± 2.3 (11.5)
	250	3.5 ± 3.5 (17.5)	1.2 ± 1.3 (6.0)	0.0 ± 0.0 (0.0)	1.9 ± 1.6 (9.5)	1.5 ± 1.3 (7.5)	0.0 ± 0.0 (0.0)	2.1 ± 1.3 (10.5)	3.0 ± 1.5 (15.0)	2.3 ± 2.2 (11.5)
	500	3.9 ± 3.7 (19.5)	6.2 ± 3.4 (31.0)	0.0 ± 0.0 (0.0)	2.0 ± 1.7 (10.0)	2.5 ± 2.3 (12.5)	0.0 ± 0.0 (0.0)	2.1 ± 1.7 (10.5)	3.3 ± 1.0 (16.5)	3.3 ± 3.2 (16.5)
	1000	4.2 ± 4.0 (21.0)	11.0 ± 2.4 (55.0)	0.0 ± 0.0 (0.0)	2.9 ± 2.5 (14.5)	5.4 ± 4.7 (27.0)	0.0 ± 0.0 (0.0)	3.1 ± 3.3 (15.5)	3.8 ± 4.7 (19.0)	4.1 ± 2.2 (20.5)
	2000	5.2 ± 3.9 (26.0)	11.3 ± 2.0 (56.5)	0.1 ± 0.2 (0.5)	4.8 ± 4.2 (24.0)	11.1 ± 6.5 (55.5)	0.0 ± 0.0 (0.0)	4.3 ± 3.8 (21.5)	6.9 ± 3.2 (34.5)	5.5 ± 4.0 (27.5)
	4000	6.2 ± 3.9 (31.0)	14.2 ± 1.4 (71.0)	4.3 ± 6.4 (21.5)	6.0 ± 5.2 (30.0)	18.6 ± 1.4 (93.0)	0.0 ± 0.0 (0.0)	7.9 ± 7.2 (39.5)	14.1 ± 4.9 (70.5)	9.0 ± 2.6 (45.0)
	8000	9.4 ± 1.9 (47.0)	15.4 ± 2.9 (77.0)	12.3 ± 5.2 (61.5)	7.1 ± 6.2 (35.5)	20.0 ± 0.0 (100.0)	0.0 ± 0.0 (0.0)	11.6 ± 8.9 (58.0)	17.4 ± 2.9 (87.0)	13.9 ± 5.2 (69.5)
F value		2.729*	34.010*	7.006*	20.167	181.187*	NA	2.281	13.495*	6.130*
LC_{50} (mg/L)		7911.88	3377.84	6941.06	8889.13	1952.12	NA	6299.35	3558.32	5189.83

Data are expressed as Mean ±SD, n = 3. UC: Untreated control; TC: Treated control; *Significant at P<0.05 level; NA: Not applicable; Values in parenthesis denote per cent larval mortality

Amongst the extracts, mortality varied. In *An. stephensi*, at concentrations of 62.5, 125 and 250 mg/L, the mean per cent mortality was high in hexane and was 88.9%, 73.2% and 74.5% respectively. At 500, 1000, 2000, 4000 and 8000 mg/L, it was high in ethyl acetate with 61.4%, 72.4%, 68.1%, 57.5% and 41.5% respectively. Activity was comparatively low in methanol extract. In *Ae. aegypti*, mean per cent was high in ethyl acetate extract in all concentrations and was 100.0%, 55.0%, 55.5%, 65.1%, 69.8%, 75.6% and 73.8% respectively except at 250 mg/L in hexane extract which was 55.9%. In the case of methanol extract, no larval mortality was obtained in any of the concentrations. In the case of *Cx. quinquefasciatus*, ethyl acetate extract showed highest mean per cent mortality in all concentrations and was 35.2%, 40.5%, 37.9%, 41.1%, 45.5%, and 40.6% respectively except in hexane extract in 125 mg/L which was 38.7% and in methanol in 1000 mg/L which was 37.3%. Among the extracts, the lowest LC_{50} values of 3377.84, 1952.12 and 3558.32 mg/L was observed against *An. stephensi*, *Ae. aegypti* and *Cx. quinquefasciatus* respectively in ethyl acetate extract (Table 1).

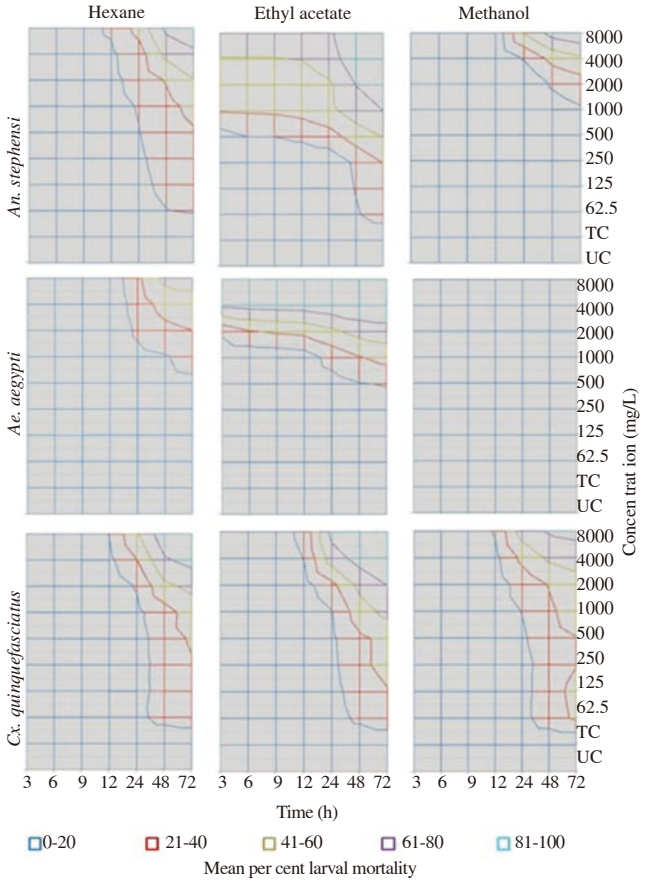

Figure 1. Larvicidal activity of *A. houstonianum* crude leaf extracts against vector mosquitoes at different hours.
No larval mortality was observed in methanol extract against *Ae. aegypti.*

4. Discussion

Testing of plant extracts for the control of vector mosquitoes has been carried out during the earlier part of the nineteenth century[20]. Since then, there has been an interest in screening of plants for identifying the activity not only against the larval stages but also against all other stages of mosquitoes including eggs. Vector resistance to conventional insecticides has intensified the search for new phytocompounds and tools for mosquito control. Various plant species *viz.*, *Tagetes minuta*, *Eclipta prostrata*[21] and *Artemisia annua*[22] belonging to the family Asteraceae showed high level of potential larvicidal activity.

Plant extracts that causes high level of mortality at reduced concentration and those which can cause effective mortality within a short span of time can be considered to possess potential phytotoxicity. In the present study, *A. houstonianum* crude leaf extracts showed poor larvicidal activity. The LC_{50} value for 24 h observation was very high indicating poor activity. Among the extracts, ethyl acetate showed comparatively better activity. The LC_{50} value of ethyl acetate was 2.34 and 2.05 times lesser than hexane and methanol extracts against *An. stephensi*, and 1.77 and 1.45 against *Cx. quinquefasciatus*. In *Ae. aegypti*, the LC_{50} value was 4.55 times lesser than hexane. There was no mortality observed on treatment with methanol extract. The activity was however poor and well below 10 mg/L which has been the recommended LC_{50} value to be considered for further evaluation as a larvicide[23]. The toxic effects resulting in the mortality manifested differently on exposure to the extracts at different times. However, more than 80% mortality was observed only at higher concentrations. Comparatively the ethyl acetate extracts showed early toxicity resulting in mortality in a short duration of time on exposure to the extracts against *An. stephensi* and *Ae. aegypti* only. Methanol extracts showed delayed mortality against *An. stephensi* and *Cx. quinquefasciatus* and no mortality against *Ae. aegypti* (Figure 1).

The larvicidal activity, in *A. houstonianum*, when compared to its nearest species *viz.*, *Ageratum conyzoides* (*A. conyzoides*) varied. The LC_{50} values of the crude methanol, petroleum ether and carbon tetrachloride leaf extracts of *A. conyzoides* against *Cx. quinquefasciatus* was 5 105.0, 425.6 and 3 139.3 ppm respectively[24] and was found to show comparatively better activity than *A. houstonianum*. Sakthivadivel and Daniel[7] has also reported LC_{50} values of > 200 ppm against *An. stephensi*, *Ae. aegypti* and *Cx. quinquefasciatus* on exposure to petroleum ether leaf extracts of *A. conyzoides*. Plants are reported to possess alkaloids that are potential insecticidal compounds[25]. Free alkaloids are lipophilic and are better extracted in lipophilic solvents[26], herein ethyl acetate which has a polarity index of 4.1[2]. Comparatively, ethyl acetate crude leaf extract showed better result. However, the activity was poor indicating the poor content of the active component in the leaves.

A. houstonianum has been reported to possess insecticidal phytocompounds *viz.*, pyrrolizidine alkaloids, monoterpenes and chromenes[11] which was expected to cause mortality against the immature vector mosquitoes. The absence of potential mortality needs investigations. It is known that phytocompounds responsible for the larvicidal activity varies geographically, seasonally, the parts

of the plant used, the solvent and the method of extraction used[23]. Screening for larvicidal activity is therefore required keeping in view of the above mentioned factors in order to draw any further valid conclusions on the potential larvicidal property of the plant.

Conflict of interest statement

We declare that we have no conflict of interest.

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