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

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PEER REVIEW

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Comments

The authors have evaluated the impact of the ovicidal activity of crude hexane, ethyl acetate and methanol leaf extracts of *A. houstonianum* for their toxicity against the eggs of three important vector mosquitoes, *viz.*, *An. stephensi*, *Ae. aegypti* and *Cx. quinquefasciatus*. The activity of ethyl acetate extract was more effective. This is a first ovicidal investigation of *A. houstonianum* crude leaf extracts against vector mosquitoes and a new methodology (single tubing method) has been used for carrying out the ovicidal activity.
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ABSTRACT

Objective: To study the ovicidal activity of *Ageratum houstonianum* (*A. houstonianum*) leaf extracts against the eggs of vector mosquitoes and to develop additional tools for the control of mosquito-borne diseases.

Methods: The ovicidal activity of crude hexane, ethyl acetate and methanol leaf extracts of *A. houstonianum* were assayed for their toxicity against the eggs of three important vector mosquitoes, *viz.*, *Anopheles stephensi*, *Aedes aegypti* and *Culex quinquefasciatus* at concentrations of 2.5, 5.0, 10.0 and 20.0 mg/L of the crude extract.

Results: All extracts showed activity. The minimum concentration at which maximum egg mortality rate of 80% and above obtained was 10.0 mg/L in the case of methanol and ethyl acetate against *Anopheles stephensi* and *Aedes aegypti* respectively and 5.0 mg/L in ethyl acetate extract against *Culex quinquefasciatus*. One hundred per cent egg mortality was obtained only in ethyl acetate extract at 20.0 mg/L against *Aedes aegypti*.

Conclusions: The crude leaf extracts of *A. houstonianum* did not exhibit potential ovicidal activity against the vector species studied. Among the crude leaf extracts tested, the activity of ethyl acetate extract was more effective. More research on the screening of phytochemicals as a potential ovicidal agent is warranted to add more tools in the control of mosquitoes.

KEYWORDS

Ageratum houstonianum, Crude leaf extracts, *Aedes aegypti*, *Anopheles stephensi*, *Culex quinquefasciatus*, Ovicidal activity

1. Introduction

Control of vector mosquitoes is relentlessly carried out to reduce mosquito-borne disease burden in many countries in the world. Various tools and strategies targeting immature and adult mosquitoes are employed for effective control. There are, however, no tools available for large scale control of vector mosquitoes at the embryonic stage. In recent years, more attention has been given to screen plants for their phytocompounds that can cause disruption

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in development of embryo in eggs laid by mosquitoes. Many researchers have reported plant extracts to possess ovicidal activity against mosquitoes[1-9]. Plants belonging to the Asteraceae family also showed ovicidal activity[10,11]. *Ageratum houstonianum* (*A. houstonianum*) belonging to this family is reported to be widely distributed in India, Central America, Europe[12] and South America[13]. This plant has been screened for mosquitocidal properties[14-16]. In the present study, the ovicidal activity of hexane, ethyl acetate and methanol crude leaf extracts of this plant on the eggs of *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. Taxonomical identity of the plants 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 °C[14].

2.2. Ovicidal bioassay

The ovicidal activity of the plant extracts was assessed against the freshly laid eggs of three vector species *viz.*, *An. stephensi*, *Ae. aegypti* and *Cx. quinquefasciatus*. Laboratory maintained gravid female vector mosquitoes were used for the study. For this experiment, single tubing method was adopted. Here, the gravid female mosquitoes were held in plastic bowls (diameter: 12.0 cm; depth/height: 6.0 cm) of 500 mL capacity with 100 mL of prepared experimental solutions inside the bowls for egg laying. Filter paper strips measuring 10.0 cm in length and 2.0 cm in width was placed at the inner sides of the bowls adjoining the surface of the experimental solutions to facilitate egg laying. In the case of experiments with *Cx. quinquefasciatus*, a wooden stick measuring 2.0 mm in thickness was placed diagonally inside the bowl as a support for the egg laying mosquitoes. The bowls were then individually covered with a transparent muslin cloth. The experimental mosquitoes were maintained at a room temperature of (27±2) °C and a relative humidity of about 75%-85%. The adult mosquitoes were observed daily for laying of eggs. The adult mosquitoes were removed after egg laying and the numbers of eggs laid were counted under a dissection microscope. The experiments were carried out at concentrations of 2.5, 5.0, 10.0 and 20.0 mg/L of the crude extract (0.25%, 0.50%, 1.00% and 2.00%). Tween 80 (0.1 mL) dissolved in distilled water served as treated control. Distilled water was used as untreated control. Three replicates for each concentration was maintained. A total of three trials were carried out. The number of eggs hatched and unhatched was counted after 4 d in *An. stephensi* and *Cx. quinquefasciatus* and after 10 d in the case of *Ae. aegypti*. The ovicidal activity was assessed in terms of egg mortality rate (EMR) using the formula given below. Two way ANOVA followed by Tukey's test was performed to determine the difference in EMR between concentrations.

$$\text{EMR (\%)} = \frac{\text{Number of eggs unhatched}}{\text{Number of eggs laid}} \times 100$$

3. Results

In *An. stephensi*, the mean number of eggs laid in all concentrations ranged from 43.8 to 107.0; 51.9 to 78.3 and 30.6 to 76.6 in hexane, ethyl acetate and methanol respectively. The mean number of eggs hatched in these extracts at 2.5, 5.0, 10.0 and 20.0 mg/L concentration was 33.6, 29.0, 14.7, 2.5; 27.9, 15.4, 20.3, 1.6; 34.0, 21.0, 13.8, 0.6 respectively. The minimum and maximum EMR was found in methanol extract at 2.5 and 20.0 mg/L respectively. Two way ANOVA of EMR in different concentrations was found to be statistically significant at $P<0.05$ level in all the extracts. In the case of *Ae. aegypti*, the mean number of eggs laid ranged from 36.9 to 63.4; 29.7 to 48.8 and 48.1 to 69.2 and the mean number of eggs hatched was 26.8, 18.6, 9.5 and 0.8; 20.2, 15.4, 8.8 and 0.0; 13.4, 15.9, 10.3 and 0.6 respectively. Minimum EMR was observed in hexane at 2.5 mg/L and maximum in ethyl acetate at 20.0 mg/L. Two way ANOVA of EMR in different concentrations was however not found to be statistically significant in all the extracts. In *Cx. quinquefasciatus*, one egg raft containing 150 individual eggs was laid in each of the test concentrations. The mean number of eggs hatched was 73.3, 53.1, 43.1 and 17.0 in hexane; 52.5, 29.9, 19.8 and 5.5 in ethyl acetate; 72.0, 44.2, 29.5 and 5.7 in methanol respectively. EMR was found to be minimum in hexane at 2.5 mg/L and maximum in ethyl acetate extract at 20.0 mg/L. Two way ANOVA of EMR in different concentrations was statistically significant at $P<0.05$ level in all extracts (Table 1). Among the extracts, the lowest concentration wherein highest mortality of 80% and above (EMR) observed was at 10.0, 10.0 and 5.0 mg/L in methanol, ethyl acetate and ethyl acetate extracts against *An. stephensi*, *Ae. aegypti* and *Cx.*

Table 1
Ovicidal activity of *A. houstonianum* leaf extracts against vector mosquitoes.

Vector mosquito species	Solvents	Egg mortality rate (Mean±SD) at different concentrations					
		Untreated control	Treated control	2.5 mg/L	5.0 mg/L	10.0 mg/L	20.0 mg/L
An. stephensi	Hexane	3.1±1.8[a]	5.9±4.2[a]	68.9±24.9[bc]	57.4±18.3[b]	65.9±11.4[bc]	95.3±3.7[c]
	Ethyl acetate			63.8±13.3[b]	72.1±12.4[bc]	71.2±10.7[bc]	93.5±10.3[c]
	Methanol			55.4±23.3[b]	69.0±10.1[b]	82.0±6.0[bc]	97.1±3.7[c]
Ae. aegypti	Hexane	2.3±0.8[a]	3.9±1.0[a]	57.8±10.7[*]	67.9±15.4[*]	75.0±12.7[*]	98.2±1.7[*]
	Ethyl acetate			58.3±4.6[*]	63.0±9.7[*]	82.4±10.9[*]	100.0±0.0[*]
	Methanol			76.3±3.4[*]	76.5±4.4[*]	84.4±4.9[*]	99.2±1.5[*]
Cx. quinquefasciatus	Hexane	7.2±1.1[a]	8.5±1.6[a]	51.1±12.4[b]	64.6±1.8[c]	71.2±1.5[c]	88.7±1.5[d]
	Ethyl acetate			65.0±2.6[b]	80.1±3.8[c]	86.8±1.1[d]	96.3±2.4[c]
	Methanol			52.0±15.1[b]	70.6±17.6[bc]	80.3±16.0[bc]	96.2±3.4[c]

*Not significant; Different alphabet superscripts in same row of different extracts show significant difference at $P<0.05$ level by two way ANOVA followed by Tukey's test.

quinquefasciatus respectively (Figure 1).

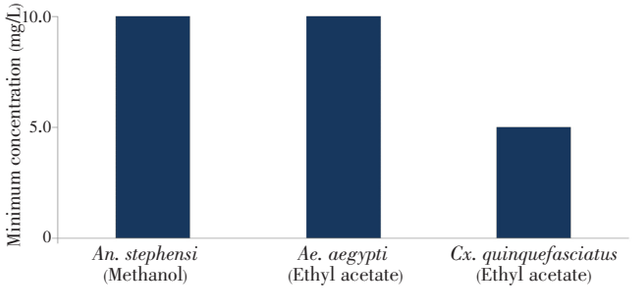

Figure 1. Minimum concentration of extracts exhibiting maximum EMR of 80% and above against vector mosquitoes.

4. Discussion

Phytotoxic compounds interfere in the process of embryogenesis and cause mortality among the embryo. The minimum lethal effective concentration which indicate 100.0% egg mortality or unhatchability was at concentration of 20.0 mg/L in respect of ethyl acetate extract against *Ae. aegypti*. In no other species/extracts 100.0% mortality or absolute EMR was obtained. At the same concentration, irrespective of ethyl acetate extract against *Ae. aegypti*, the EMR ranged between 88.7% to 99.2% against the vector species studied. The lowest concentration wherein highest mortality of 80% and above observed was at 5.0 mg/L in ethyl acetate leaf extract against *Cx. quinquefasciatus*. The potential ovicidal activity at a concentration of 5.0 mg/L and above indicates poor ovicidal activity of the different extracts of *A. houstonianum* leaves. Comparatively, the ovicidal activity was more in *Ae. aegypti*. Nevertheless, not much difference exists in the ovicidal activity among the different extracts and vector mosquitoes studied.

Though reports are not available on *A. houstonianum* or other *Ageratum* species, the ovicidal activity of other plants belonging to Asteraceae family are available but relatively few. Elango *et al*[17] reported that the hexane leaf extract of *Tagetes erecta* exerted 100.0% egg mortality at 0.1% and more than 50% egg mortality was observed at 0.012%. Hexane and chloroform leaf extracts of *Eclipta prostrata* provided more than 50.0% egg mortality at 0.025% against the egg rafts of *Culex tritaeniorhynchus*. Samidurai *et al*[18] reported 100.0% egg morality in *Pemphis acidula* methanol and acetone leaf extracts at concentrations of 0.035% and 0.045% against the egg rafts of *Cx. quinquefasciatus* and at 0.045% and 0.05% against the eggs of *Ae. aegypti*.

Ovicidal activity of plant extracts has been reported to be affected by different factors particularly the age of the egg, the concentration and the exposure period. The age of the egg has been found to influence the ovicidal activity of compounds. Exposure of freshly laid eggs to phytotoxins has been found to cause higher mortality rates. In the present study, freshly laid eggs were exposed to various concentrations of extracts. As reported, the exposure of the eggs to the phytotoxins/extracts at the time of oviposition affects embryogenesis was a likely event, but did not cause effective mortality as observed from the results. Rajkumar and Jebanesan[19] have reported flavonoid compounds from *Poncirus trifoliata* to be effective as an ovicide in the early stage of egg development of *Ae. aegypti*. Similarly, Govindarajan *et al*[20] also observed on the same against the leaf extract of *Acalypha indica* on the eggs of *An. stephensi*. In another study, Govindarajan *et al*[21] on comparing the ovicidal activity of *Cassia fistula* methanolic leaf extract against the egg rafts of *Cx. quinquefasciatus* and eggs of *An. stephensi* showed that younger age group of eggs showed maximum mortality rate when compared to the older age group. Usta *et al*[22] reported that phytocompounds such as flavonoids acts as an effective ovicide when treated at the early stages of egg development and higher concentration of these compounds cause maximum egg mortality.

Higher concentrations always yielded better mortality rates and this was observed in the present study and similar results were reported by Govindarajan *et al*[21]. Broadbent and Pree[23] reported that when eggs were directly exposed to higher concentrations of the compounds, more chemicals entered the egg shell, which affected the embryogenesis.

Exposure time also has a crucial role in causing toxicity[24]. Longer exposure periods also facilitate increased penetration of the compounds into the egg shells, thus increasing their effectiveness[2]. Shorter duration of treatment was decisively inferior to longer exposure to insecticides at the egg stage[25]. Smith and Salkeld[26] reported differences in susceptibility to ovicides to occur due to differential rates of uptake, penetration through the chorion, conversion to active inhibitor, detoxification and failure of the toxicant to reach the target. Grosscurt[27] observed that the efficiency to act on the embryo inside the egg shell depends on an efficient penetration of the insecticide, which in turn is influenced by the exposure period. The eggs of mosquitoes are found to be much more tolerance to the action of insecticides compared to larval stages. Insect eggs are covered with a shell, which differs biochemically from the integument of the larvae, and the difference in penetration of the insecticide through the egg shell, and the larval integument is reflected in the observed toxicity differences[25].

Studies on the ovicidal activity of plant extracts have received more attention since plant extracts can be used against eggs of vector mosquitoes, especially of those, that lay eggs in baited ovitraps. Used along with an oviposition attractant and a larvicidal agent such as an insect growth regulator compound, this combination may contribute extensively to assist in surveillance coupled with intervention.

In conclusion, the crude leaf extracts of *A. houstonianum* did not exhibit potential ovicidal activity against the vector species studied. Among the crude leaf extracts tested, the activity of ethyl acetate extract was more effective. More research on the screening of phytochemicals as a potential ovicidal agent is warranted to add more tools in the control of mosquitoes.

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

This research paper describes the ovicidal activity of *A. houstonianum* leaf extracts against *An. stephensi*, *Ae. aegypti* and *Cx. quinquefasciatus*. In countries such as India where the prevelance of mosquito-borne disease are high the work is relevant in helping to find a solution to this problem.

Research frontiers

The paper discusses the ovicidal activity of crude hexane, ethyl acetate and methanol leaf extracts of *A. houstonianum* for their toxicity against the eggs of three important vector mosquitoes, *viz.*, *An. stephensi*, *Ae. aegypti* and *Cx. quinquefasciatus*.

Related reports

With regard to the materials and methods part, a new methodology (single tubing method) was adopted to carry out ovicidal bioassay against the eggs of the vector mosquito species studied.

Innovations & breakthroughs

This paper reports on the use of single tubing methodology which is found to be a new technique used for ovicidal bioassay, where the gravid female mosquitoes were held in plastic bowls of prepared experimental solutions for egg laying.

Applications

The experimental results indicate that the ethyl acetate leaf extract of the plant-when used along with an oviposition attractant and a larvicidal agent such as an insect growth regulator compound, may contribute extensively to assist in surveillance coupled with intervention and could also be used as an effective source of natural pest control to help reduce the problems associated with vector-borne diseases.

Peer review

The authors have evaluated the impact of the ovicidal activity of crude hexane, ethyl acetate and methanol leaf extracts of *A. houstonianum* for their toxicity against the eggs of three important vector mosquitoes, *viz.*, *An. stephensi*, *Ae. aegypti* and *Cx. quinquefasciatus*. The activity of ethyl acetate extract was more effective. This is a first ovicidal investigation of *A. houstonianum* crude leaf extracts against vector mosquitoes and a new methodology (single tubing method) has been used for carrying out the ovicidal activity.

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