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REPELLENT PROPERTIES OF *AGERATINA ADENOPHORA* AGAINST DENGUE VECTOR MOSQUITO, *AEDES AEGYPTI* LINN. (DIPTERA: CULICIDAE)

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ABSTRACT

Mosquitoes are the vectors of a number of human and zoonotic disease pathogens affecting human and animal hosts, including those that cause malaria, filariasis, Japanese encephalitis (JE), and dengue and yellow fevers. In view of the fact that mosquitoes develop genetic resistance to synthetic insecticides and even to bio pesticides such as *Bacillus sphaericus* the application of easily degradable botanicals for the control of mosquitoes is recommended. The present study determines the repellent activity of *Ageratina adenophora* (*A. adenophora*) (Spreng.) King & H. Rob. (Family: Asteraceae) extract against the dengue vector, *Aedes aegypti* (*Ae. aegypti*). The repellent efficacy was determined against *Ae. aegypti* mosquito species at three concentrations viz., 1.0, 2.5 and 5.0 mg/cm^2 under the laboratory conditions. The methanol extract of *A. adenophora* found to be more repellency than the other extracts. A higher concentration of 5.0 mg/cm^2 provided 100% protection up to 120 minutes, the lower concentration of 2.5 and 1.0 mg/cm^2 provided 100 % protection up to 90 and 60 minutes, respectively. The results clearly show that repellent activity was dose dependent. From the results it can be concluded the crude methanol extract of *A. adenophora* was an excellent potential for controlling the dengue vector mosquito *Ae. aegypti*.

Key words: Ageratina adenophora, repellent activity, dengue vector, *Aedes aegypti.*

INTRODUCTION

Aedes aegypti (Diptera: Culicidae) is an arbovirus vector responsible for yellow fever in central and south America and in West Africa. It is also the vector for dengue hemorrhagic fever (DHF), endemic to south –East Asia, the pacific Islands, Africa and the Americas. It is estimated that 2.5 billion people are currently at risk for dengue fever (DF), DHF, and dengue shock syndrome (DSS). The size and spread of the dengue pandemic, the unpredictability of the epidemic occurrences and the circulation of virulent and non- virulent strains make DHF/DSS a model for emerging infectious disease. Despite of this challenge, the development of dengue virus vaccines is still a long way to be of any use due to several obstacles. Chemical insecticides have continued to be commonly used for controlling mosquitoes on the control of mosquitoes, either by killing or repelling them. However, the appearance of mosquito resistance to conventional insecticides, together with public concern about the safety and

availability of the insecticides have prompted the necessity to search for alternative insecticides that would be environmentally acceptable and less costly. Therefore, in recent years the use of environmentally friendly and easily biodegradable natural insecticides of plant origin has received renewed importance for malaria and other disease control. Interest in this field is based on the fact lead to the accumulation of chemical residues in flora, fauna, soil and entire environment in general (Sivakumar *et al.,* 2011).

Plants may be a source of alternative agents for control of mosquitoes, because they are rich in bioactive chemicals. They are active against a limited number of species including specific target insects, and are bio-degradable (Niraimathi *et al.,* 2010). Govindarajan *et al*. (2011) reported that the ovicidal and repellent activities of methanol leaf extract of *Ervatamia coronaria* and *Caesalpinia pulcherrima* against *Cx. quinquefasiatus, Ae. aegypti* and *An. stephensi.* Govindarajan and Sivakumar (2012) studied that the repellent activity of hexane, ethyl acetate, benzene, chloroform and methanol extract of repellent properties of *Cardiospermum halicacabum* against *Cx. quinquefasiatus, Ae. aegypti* and *An. stephensi.* The larvicidal and ovicidal efficacy of different extracts of *Andrographis paniculata* against *Cx. quinquefasiatus,* and *Ae. aegypti* (Govindarajan, 2011).

The larvicidal and ovicidal activity of benzene, hexane, ethylacetate, methanol and chloroform leaf extract of *Eclipta alba* against dengue vector, *Ae. aegypti* (Govindarajan and Karuppannan, 2011). Chemical composition and larvicidal activity of leaf essential oil from *Clausena anisata* against *Cx. quinquefasiatus, Ae. aegypti* and *An. stephensi* (Govindarajan, 2010 *a*).

Ageratina adenophora is a perennial herbaceous exotic shrub which may grow up to 1 or 2 m height. It has opposite trowel-shaped serrated leaves that are 6-10 cm long by 3-6 cm in width. The small compound flowers occur in late spring and summer, and are found in clusters at the end of branches. Each flower head is up to 0.5cm in diameter and creamy white in color. They are followed by a small brown seed with a white feathery parachute. The mosquito repellent properties of *A. adenophora* have not yet reported. Therefore, the present study was carried out to determine the repellent efficacy of *A. adenophora* leaves extract against dengue vector mosquito, *Aedes aegypti.* (Diptera: Culicidae).

MATERIALS AND METHODS

Plant collection

Fully developed leaves of *A. adenophora* were collected from hilly regions of the Nilgiris District, Tamil Nadu, India. It was authenticated by a plant taxonomist from the Department of Botany, Annamalai University. A voucher specimen is deposited at the herbarium of Plant Phytochemistry Division, Department of Zoology, Annamalai University, India.

Extraction

The fully developed fresh leaves of the plant *A. adenophora* were collected and washed with tap water, shade dried at room temperature. The dried leaves were powdered with the help of electrical blender. The powdered leaf material (1.0 kg) was then subjected to extraction in various solvents *viz.,* hexane, benzene, chloroform, ethyl acetate and methanol (3.0 L) using soxhlet extraction apparatus for 8 hours individually. The extract was filtered through a Buchner funnel with Whatman number 1 filter paper. The filtrate was evaporated to dryness under reduced pressure using rotary vacuum evaporator. The residue was then made in to a 1% stock solution with ethanol. From these stock solutions, the various range of concentration (1.0, 2.5 and 5.0 mg/cm^2) were prepared by dissolving the residues in ethanol.

Mosquito

Ae. aegypti were reared in the Vector Control Laboratory, Department of Zoology, Annamalai University. The larvae were fed on dog biscuits and yeast powder in the 3:1 ratio. Adults were provided with 10% sucrose solution and membrane feeding on goat blood. Mosquitoes were held at 28 ± 2°C, 70– 85% relative humidity (RH), with a photo period of 12 h light: 12 h dark.

Repellent activity

The repellency of the *A. adenophora* plant crude extracts against *Ae. aegypti* was evaluated by using the percentage of protection in relation to dose method (World Health Organization, 2009). Three days old blood starved female *Ae.*

aegypti mosquitoes (100) were kept on a net cage (45cm×30cm×45cm). Two cages with hungry mosquitoes for test and control were kept aside. The volunteer had no contact with lotions, perfumes, oils or perfumed soaps on the day of the assay. The arms of the volunteer skin washed and cleaned with ethanol and ethanol served as control. After air drying, the arms of the volunteer, only 25 cm^2 dorsal side of the skin on each arm was exposed and the remaining area covered by rubber gloves. The different concentrations of crude extracts were applied. *Ae. aegypti* was tasted during the day time from 07.00 to 17.00 h. The control and treated arm were introduced simultaneously into the mosquito cage, and gently tapping the sides on the experimental cages, the mosquitoes were activated. The volunteer conducted their test of each concentration by inserting the treated and control arm into cages at a same time for one full minute for every 5 min. The mosquitoes that land on the hand were recorded and then shaken off before it imbibes any blood. The percentage of repellency was calculated by the formula.

$$\% \text{ repellency} = [(Ta - Tb) / Ta] \times 100$$

Where *T*a is the number of mosquitoes in the control group, and *T*b is the number of mosquitoes in the treated group.

3. Results

The results from the skin repellent activity of *A. adenophora* leaf extract against *Ae. aegypti* is given in Table 1. The repellent activity was very high at the initial stage of exposure. Increase in the exposure period showed reduction in repellent activity and it depends upon the concentration of the extract and density of mosquito. The methanol extract of *A. adenophora* found to be more repellency than the other extracts. A higher concentration of 5.0 mg/cm^2 provided 100% protection up to 120 minutes, the lower concentration of 2.5 and 1.0 mg/cm^2 provided 100 % protection up to 90 and 60 minutes, respectively. The results clearly show that repellent activity was dose dependent.

Table 1. Repellency of different solvent leaf extracts of *A. adenophora* against *Ae. aegypti*.

Solvents	Concentration (mg/cm^2)	Repellency% ±SD							
		Time of post application (minutes)							
		15	30	60	90	120	150	180	210
Methanol	1.0	100±0.0	100±0.0	100±0.0	92.6±1.6	78.5±1.0	66.2±1.2	53.2±1.8	41.3±1.2
	2.5	100±0.0	100±0.0	100±0.0	100±0.0	94.2±1.6	80.0±1.6	69.3±1.0	53.0±1.6
	5.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	96.0±1.4	80.6±1.2	63.8±1.4
Ethyl acetate	1.0	100±0.0	100±0.0	93.6±1.4	80.2±1.2	66.3±1.2	54.8±1.6	40.5±1.0	31.6±1.0
	2.5	100±0.0	100±0.0	100±0.0	95.8±1.4	78.6±1.4	63.5±1.6	50.8±1.8	36.2±1.6
	5.0	100±0.0	100±0.0	100±0.0	100±0.0	96.0±1.2	80.7±1.0	65.3±1.2	44.2±1.2
Chloroform	1.0	100±0.0	100±0.0	90.4±1.2	78.5±1.8	65.4±1.6	50.3±1.6	38.4±1.8	28.2±1.4
	2.5	100±0.0	100±0.0	100±0.0	90.7±1.4	72.4±1.2	60.1±1.4	46.6±1.2	34.3±1.2
	5.0	100±0.0	100±0.0	100±0.0	100±0.0	92.3±1.6	77.2±1.6	61.0±1.8	42.2±1.8
Benzene	1.0	100±0.0	100±0.0	88.2±1.6	75.2±1.2	60.1±1.4	47.6±1.0	31.6±1.2	22.0±1.6
	2.5	100±0.0	100±0.0	100±0.0	86.6±1.5	68.4±2.1	54.7±1.5	42.1±1.0	32.1±1.2
	5.0	100±0.0	100±0.0	100±0.0	96.8±1.8	87.6±1.4	69.4±1.0	54.7±2.1	38.6±1.8
Hexane	1.0	100±0.0	100±0.0	82.7±1.4	71.2±1.0	58.8±1.4	42.4±1.2	27.8±1.8	20.3±1.4
	2.5	100±0.0	100±0.0	100±0.0	82.7±1.2	66.2±1.4	48.6±1.2	36.8±1.0	28.3±1.2
	5.0	100±0.0	100±0.0	100±0.0	93.6±1.0	85.2±1.2	66.4±1.4	48.6±1.2	33.9±1.2

DISCUSSION

Different parts of plants contain a complex of chemicals with unique biological activity which is thought to be due to toxins and secondary metabolites. Which act as attractants or deterrents our result showed that the crude extract of *A. adenophora* have significant repellent activity. This result is also comparable to earlier reports of Pushpanathan *et al.* (2008) who observed the essential oil of *Zingiber officinalis* was evaluated for larvicidal and repellent activity against the filarial mosquito *Cx. quinquefasiatus* with the LC_{50} value was 50.78ppm, skin repellent test at 1.0, 2.0, 3.0 and 4.0 mg/cm^2 concentration of *Z. officinalis* gave 100% protection up to 15,30,60, and 120 min. Mandal (2011) reported that repellent activity of *Eucalyptus* and *Azadirachta indica* seed oil against the filarial mosquito *Cx. quinquefasiatus.* The test oils showed excellent repellent action against *Cx. quinquefasiatus.* The *A. indica* seed oil provided 90.26% and 88.83% protection and the *Eucalyptus* oil 93.37% and 92.04% at concentrations 50% and 100% (v/v), respectively, with the protection time up to 240 min. there was no bite within 120 min. and 180 min., respectively, due to the action of *Eucalyptus* and *A. indica* seed oil, and thus 100% protection from the bite of *Cx. quinquefasiatus* mosquito was achieved. Govindarajan (2010) evaluated the larvicidal activity of crude extract of *Sida acuta* against three important mosquitoes with LC_{50} values ranging between 38 to 48 mg/l; the crude extract had strong repellent action against three species of mosquitoes as it provided 100% protection against *An. stephensi* for 180 min. followed by *Ae. aegypti* (150) min. and *Cx. quinquefasiatus* (120) min. The crude extract of *Ervatamia coronaria* and *Caesalpinia pulcherrima* exerted zero hatchability (100% mortality) at 250, 200 and 150 and 375, 300 and 225 ppm for *Cx. quinquefasiatus*, *Ae. aegypti* and *An. stephensi,* respectively. The methanol extract of *E. coronaria* found to be more repellent than *C. pulcherrima*.

Conclusion

The findings of the present investigation revealed that the leaf extract of *A. adenophora* possess remarkable repellent activity against the mosquito *Ae. aegypti*. This is the first report on the mosquito repellent activity of the methanol extract of *A. adenophora* plant. Further investigations are needed to elucidate this activity against a wide range of mosquito species and also the active ingredient(s) of the extract responsible for repellent activity in *Ae. aegypti* should be identified and utilized, if possible, in preparing a commercial product/ formulation to be used as a mosquitocidal.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest associated with this article.

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