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Mosquitocidal activity of indigenous plants of Western Ghats, *Achras sapota* Linn. (Sapotaceae) and *Cassia auriculata* L. (Fabaceae) against a common malarial vector, *Anopheles stephensi* Liston (Culicidae: Diptera)

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PEER REVIEW

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Comments

In general, this manuscript is written well with scope and it will be useful for scientific society. It will motivate many scientific society to conduct their research in this field. Since it is needed to control the mosquito, this kind of work should be encouraged to save our green planet.
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ABSTRACT

Objective: To evaluate the mosquito larvicidal, ovicidal, pupicidal and repellent activities of hexane, diethyl ether, dichloromethane, acetone and methanol extracts of Indian medicinal plants, *Achras sapota* (*A. sapota*) and *Cassia auriculata* (*C. auriculata*) at different concentrations against *Anopheles stephensi* (*An. stephensi*), a malarial vector.

Methods: Twenty five early third instar larvae of *An. stephensi* were exposed to various concentrations (30-210 mg/L) of plants extracts and were assayed in the laboratory by using the protocol of WHO 2005; then after 24 h LC_{50} values of the *A. sapota* and *C. auriculata* leaf extract was determined by probit analysis. The ovicidal activity was tested with the extracts ranging from 50-350 mg/L. The pupicidal activity was recorded after 24 h of exposure to the extract. The repellent efficacy was determined against mosquito species at two different concentrations 1.5 and 3.0 mg/cm^2 under laboratory conditions.

Results: Among the five different extracts tested against the *An. stephensi*, methanol extract of *A. sapota* proved to be an more effective solvent extract in almost all the parameters studied than *C. auriculata*.

Conclusions: It is inferred that the leaf extract of *A. sapota* and *C. auriculata* could be used in vector control programme.

KEYWORDS

Achras sapota, *Cassia auriculata*, *Anopheles stephensi*, Larvicidal activity, Ovicidal activity, Pupicidal activity, Repellent activity

1. Introduction

Malaria is transmitted by the mosquitoes belonging to the genus *Anopheles*. Malaria infections are recorded to be 300-500 million new cases every year in the world, and 2 million people suffer from malaria annually in Indian subcontinent alone. This species normally breeds in paddy fields and rain water storages[1]. Man has suffered from the activities of mosquito since time immemorial. It is believed that mosquitoes rank as the most important insect pest for human. They had influenced and still influence man's selection of living and working sites. The mosquitoes scourge human with their vicious biting and continuous singing, but more seriously they transmit

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malaria, filaria, Japanese encephalitis and dengue fever to human beings. These diseases devastate Indian economy every year. Worldwide, mosquitoes transmit diseases to more than 700 000 000 people annually and are responsible for 1 death for every 17 people currently alive. Malaria results from an infection by a protozoan carried by mosquitoes, and according to reports from the World Health Organization (WHO), it causes as many as 3 000 000 deaths annually. Mosquitoes also transmit the arboviruses responsible for yellow fever, dengue haemorrhagic fever, epidemic polyarthritis, and several forms of encephalitis. Mosquitoes are ubiquitous, belonging to the Order Diptera, Suborder Nematocera and Family Culicidae. More than 3 100 species of mosquitoes belonging to 34 genera have been recorded and arranged under three sub-families, namely, Anophelinae, Culicinae and Toxorhynchitinae. Thus in the struggle between man and insects vectors, man has used his knowledge to bring under control his tiny enemies which have harmed him even before the dawn of civilization. Mosquito control is the task of managing the population of mosquitoes to reduce their damage to human health, economics and enjoyment of mosquito-ridden areas. Due to the dreadful effect of synthetic chemicals, safer vector control methods are needed. Integrated pest management in planning, developing and accepting ecofriendly technologies for application aimed at controlling insects on sustainable basis. Integrated pest management amounts to minimizing the use of conventional chemicals by using other means of pest control as much as possible. Bacteria, protozoan, viruses, fungi and fishes are the recommended agents in National Malaria Eradication Programme[2].

Over the past five decades, more than 2 000 plant species belonging to different families and genera have been reported to contain toxic principles, which are effective against insects. Among the well-represented plant pesticides, pyrethrum obtained from *Chrysanthemum cinerariaefolium* is mainly used as a domestic insecticide because of its non-toxicity to human beings and warm-blooded animals. The naturally occurring pesticides thus appear to play a prominent role in the development of future commercial pesticides not only for agricultural crop productivity but also for the safety of environment and public health. Biologically active plants showed a great potential efficiency as larvicides. The use of botanical derivatives in mosquito control, especially for mosquito larvae, as an alternative to synthetic insecticides is more eco-friendly in insect control. Biopesticides from various plants have a long history. Even in Neolithic times (7000 BC) farmers in their own way have prepared pesticides from various plant extracts. It is reported that more than 600 plant species in the world can control harmful insects and pests. Interest in the development of botanical insecticides started in the early 1930s and was sustained through the late 1950s. However, interest in botanical pesticides was revived during recent years because of some drawbacks in the synthetic insecticides, including lack of selectivity, impact on the environment and the emergence and spread of pest resistance. Currently, isolation, identification and development of natural products are under the focus of numerous research programmes around the world. So far only few insecticides of plant origin have been available in the market. Recently a number of plants were evaluated for their action against mosquito larvae[3-10]. Recent studies on insect-plant interactions have revealed that the plants possess many subtle interfaces that interfere with the growth, development and behaviour of insects. Many of the defensive components of plants lack toxicity to higher animals. Historically, the secondary chemical compounds of plants provided the lead for over 25% of the prescription drugs used in human medicine and some of these pharmacologically active plants have also provided lead for natural insecticides[11].

In India, there are various plants known for their insecticidal property and popular as pesticides. The most important and popular plant is neem, which has international recognition. Almost every part of this wonder plant gives various bitter compounds (many of which are sulphur compounds) having insecticidal properties. Smokes of the gums of various trees are used as mosquito repellents and insecticides. Tulsi, a sacred basil *Ocimum* sp. is known to have various insecticidal properties. Extracts of tobacco leaves has been traditionally known as pesticide and insecticide in India and elsewhere. Indian farmers have been using various plant extracts as insecticides and pesticides. The use of certain plant extracts for insect control has several appealing features such as easily biodegradable than the synthetic insecticides, less hazardous, the plants offer rich storehouse of chemicals of diverse biological activities. The discovery rate of new insecticides from synthetic sources has declined in recent years and the natural pesticides has gained more attantion in the discovery of new insecticides. Indian subcontinent is a vast repository of medicinal plants that are used in traditional medical treatments[12-15]. Many Westerners have long regarded the Indian systems of medicine as a rich source of knowledge. The various indigenous systems such as Siddha, Ayurveda, Unani and Allopathy use several plant species for ailments. Though around 20 000 medicinal plants have been recorded in India, traditional communities are using only 800 plants for curing different diseases. WHO has shown a great interest in documenting the use of medicinal plants used by tribals from different parts of the world[16,17]. Many developing countries have intensified their efforts in documenting the ethnomedical data on medicinal plants. Biologically active compounds from natural sources have always attracted scientists working on infectious diseases. Therefore, the

present study was carried out to document the mosquito larvicidal, ovicidal, pupicidal and repellent potentials of hexane, diethyl ether, dichloromethane, acetone and methanol extracts of Indian medicinal plants *Achras sapota* (*A. sapota*) and *Cassia auriculata* (*C. auriculata*) with different concentrations against *Anopheles stephensi* (*An. stephensi*) larvae and eggs.

2. Materials and methods

2.1.Collection and processing of plants

Several hundred medicinal plant species from the Indian subcontinent have been identified, and their usage have been documented in the ethnobotanical literature. These literature guided the selection of plants for the present study. Plant sampling was carried out during the growing season and fully developed leaves of the *A. sapota* and *C. auriculata* were collected in and around Yercaud hill station (11.7794° N, 78.2034° E), Salem Districts of the Tamilnadu, India. At the time of collection, the pressed voucher herbarium specimens were prepared for each species and identified with the help of plant taxonomist, Department of Zoology, Govt. Arts College (Autonomous), Nandanam, Chennai-35, whenever possible, flowering or fruiting specimens were collected to facilitate taxonomic identification. Bulk samples were air-dried in the shade and after drying, each sample was ground into a fine powder.

2.2. Extraction method

The dried leaves (1 kg) were powdered mechanically using commercial electrical stainless steel blender and extracted sequentially with hexane, diethyl ether, dichloromethane, acetone and methanol (500 mL, Ranchem) in a Soxhlet apparatus separately until exhaustion. The extract was concentrated under reduced pressure 22-26 mmHg at 45 °C by Rota Vapour and the residue obtained was stored at 4 °C.

2.3. Mosquito rearing

Eggs of *An. stephensi* were collected from Indian Council of Medical Research Centre, Virudachalam. The egg rafts were then brought to the laboratory. The eggs were placed in enamel trays (30 cm×24 cm×5 cm), each containing 2 L of tap water and kept at room temperature [(28±2) °C] with a photoperiod of 16:8 h (L:D) for larval hatching. The larvae of each mosquito species were maintained in separate trays under the same laboratory conditions and fed with a powdered feed containing a mixture of dog biscuit and baker's yeast (3:1 ratio). The trays with pupae of each mosquito species were maintained in separate mosquito cages at (26±2) °C and relative humidity of (85±3)% under a photoperiod of 16:8 h (L:D) for adult emergence. Cotton soaked in 10% aqueous sucrose solution in a Petri dish to feed adult mosquitoes was also placed in each mosquito cage. An immobilized young chick was placed for 3 h inside the cage in order to provide blood meal especially for female mosquitoes. A plastic tray (11 cm×10 cm×4 cm) filled with tap water with a lining of partially immersed filter paper was then placed inside each cage to enable the female mosquitoes to lay their eggs. The eggs obtained from the laboratory-reared mosquitoes were immediately used for toxicity assays or allowed to hatch out under the controlled laboratory conditions described above. Only the newly hatched specific instars of larvae or the pupae of selected mosquitoes were used in all bioassays.

2.4. Larvicidal activity

The larvicidal activity of plant crude extract was assessed by using the standard method as prescribed by WHO[18]. From the stock solution, seven different test concentrations 30, 60, 90, 120, 150, 180 and 210 mg/L of each plant extracts were prepared and they were tested against the freshly moulted (0-6 h) third instar larvae of *An. stephensi* individually, dimethylsulfoxide (DMSO) (emulsifier) in water served as a control. The larvae of this mosquito species (25) were introduced in 500 mL plastic cups containing 250 mL of aqueous medium (249 mL of dechlorinated water+1 mL of emulsifier) and the required amount of plant extract was added. The larval mortality was observed and recorded after 24 h of treatment. For each experiment, five replicates were maintained at a time. The percentage mortality was calculated by using Abbott's formula[19]. The LC_{50}, LC_{90}, 95% confidence limit of lower confidence limit and upper confidence limit, slope, regression and *Chi*-square values were calculated by using Probit analysis with Statistical Package for Social Sciences 17.0 Version in MS-Excel, 2007.

2.5. Ovicidal activity

The method of Su and Mulla was slightly modified and used to test the ovicidal activity[20]. The various concentrations (50, 100, 150, 200, 250, 300 and 350 mg/L) were prepared from the stock solution as stated in the previous experiments. Before treatment, the eggs of *An. stephensi* were counted individually with the help of hand lens. Freshly hatched eggs (0-6 h) of this mosquito species (100) were exposed to each concentration of leaf extract until they hatched or died. Eggs exposed to DMSO in water served as control. After treatment, the eggs from each concentration were individually transferred to distilled water cups for hatching assessment after counting the eggs under a microscope. Each test was replicated five times. The ovicidal activity was assessed 48 h post treatment by the following

formula.

$$\%OA=\frac{\%EHC-\%EHT}{\%EHC}\times 100$$

Where, %OA=percent of ovicidal activity; %EHC=percent of eggs hatched in control; %EHT=percent of eggs hatched in treatment.

2.6. Pupicidal activity

Batches of thirty pupae were introduced into 500 mL of the test medium containing particular concentration (125 and 250 mg/L) of the crude extracts in a plastic cups in five replications. In control, the same number of pupae was maintained in 500 mL of dechlorinated water containing appropriate volume of DMSO. All containers were maintained at room temperature [(28±2) °C] with naturally prevailing photoperiod (12:12 h/L:D) in the laboratory. Any pupa was considered to be dead if did not move when prodded repeatedly with a soft brush. Mortality of each pupa was recorded after 24 h of exposure to the extract following the Abbott formula[19].

$$\text{Mortality }(\%)=\frac{\%MT-\%MC}{100-\%MC}\times 100$$

Where , %MT=% pupal mortality in treatment and %MC=% pupal mortality in control.

2.7. Repellent activity

The repellent study was conducted following the methods of WHO[21]. The blood-starved female *An. stephensi* mosquito (100) (2-4 days old) was kept in a net cage (45 cm×45 cm× 40 cm). The volunteer had no contact with lotions, perfumes or perfumed soaps on the day of the assay. The arms of the test person were cleaned with isopropanol. After air drying the arms, only 25 cm^2 of the dorsal side of the skin on each arm was exposed, the remaining area being covered by rubber gloves. The plants extract was dissolved in isopropanol and isopropanol served as control. The selected plants leaf extract at 1.5 and 3.0 mg/cm^2 concentration was applied. The control and treated arms were introduced simultaneously into the cage. The numbers of bites were counted over 5 min every 60 min, *An. stephensi* was tested during the night time from 19:00 h to 7:00 h. The experiment was conducted five times. It was observed that there was no skin irritation from the plant extract.

2.8. Determination of lethal concentrations

Lethal concentration (LC_{50}) represents the concentration of the test material that caused 50% mortality of the test (target and non-target) organisms within the specified period of exposure, and it was determined by exposing various developmental stages of the mosquitoes to different concentrations of the extract. Based on the mortality of the test organisms recorded in these bioassays, LC_{50} and LC_{90} was calculated along with their fiducial limits at 95% confidence level by probit analysis using Statistical Package for Social Sciences 17.0 software. Results with $P<0.05$ were considered to be statistically significant.

3. Results

3.1. Larvicidal activity

The *An. stephensi* larvae and eggs were treated with different concentrations of *A. sapota* and *C. auriculata* hexane, diethyl ether, dichloromethane, acetone and methanol extracts. The larval mortality was calculated after 24 h exposure period and shown in table 1. The LC_{50} and LC_{90} values of hexane, diethyl ether, dichloromethane, acetone and methanol extract of *A. sapota* against *An. stephensi* are 54.82, 48.85, 45.37, 39.82, 39.54 and 123.14, 118.48, 118.35, 98.64 and 98.53 mg/L, respectively. The LC_{50} and LC_{90} values of hexane, diethyl ether, dichloromethane, acetone and methanol extract of *C. auriculata* are 97.44, 89.47, 86.33, 78.44, 74.82 and 207.93, 198.33, 198.42, 194.52 and 190.26 mg/L, respectively.

Table 1

Larvicidal activity *A. sapota* and *C. auriculata* different extracts against *An. stephensi*.

Name of the solvent	LC_{50} (mg/L)	95% Confidence Limits (mg/L)		LC_{90} (mg/L)	Slope	Regression	*Chi*-square*
		LCL	UCL				
A. sapota							
Hexane	54.82	43.83	64.39	123.14	3.0421274	$y=2.3856x+2.3268$	13.937*
Diethylether	48.85	38.36	56.44	118.48	3.2741662	$y=2.2516x+1.5291$	12.203*
Dichloromethane	45.37	37.62	54.52	118.35	3.4072632	$y=1.1428x+2.2634$	11.429*
Acetone	39.82	32.64	46.29	98.64	4.1584624	$y=2.6712x+2.7324$	12.852*
Methanol	39.54	30.85	48.32	98.53	4.1584624	$y=1.3814x+2.4513$	12.290*
C. auriculata							
Hexane	97.44	76.83	118.35	207.93	3.3647350	$y=3.6742x+3.6185$	11.482*
Diethylether	89.47	64.62	99.52	198.33	3.2403000	$y=1.7634x+2.5274$	11.228*
Dichloromethane	86.33	62.82	98.64	198.42	3.2172654	$y=2.5938x+3.2863$	13.365*
Acetone	78.44	57.19	93.83	194.52	3.1838462	$y=2.3286x+4.3285$	12.501*
Methanol	74.82	52.64	91.73	190.26	3.1838462	$y=1.2316x+4.5962$	13.294*

Each value was expressed as mean±SD, representing mean of five values. *Significantly different at $P<0.05$ (MANOVA; LSD-Tukey's Test). LCL-Lower confidence limit; UCL-Upper confidence limit.

3.2. Ovicidal activity

Different solvent extracts were tested against the freshly laid eggs of *An. stephensi*. Hexane extract of *A. sapota* showed 22.8% egg mortality whereas 21.2% egg mortality was noted at 50 mg/L of *C. auriculata* extract. Similarly at 100 mg/L 36.4% and 38.4% egg mortality were observed in *A. sapota* and *C. auriculata* respectively. Besides, 55.3% and 70.2% ovicidal activities were noted at 150 and 200 mg/L of *A. sapota* and 45.8% and 64.4% at 150 and 200 mg/L of the

hexane extract of *C. auriculata*. At 250 mg/L of *A. sapota* and *C. auriculata* extracts, 89.2% and 75.2% ovicidal activity was recorded against the eggs of *An. stephensi* respectively. Though, at higher concentrations *i.e.* 300 and 350 mg/L, the recorded ovicidal activities did not show much variations but they shown significant activity than other concentrations tested against the eggs of *An. stephensi* in *A. sapota* extract and greater variations in *C. auriculata* extract (82.6% and 86.2%).

Diethylether extract of *A. sapota* showed 29.2% ovicidal activity whereas 27.6% egg mortality was noted at 50 mg/L of *C. auriculata* extract. Similarly at 100 mg/L, 42.6% and 41.8% egg mortality were observed in *A. sapota* and *C. auriculata* extract respectively. Besides, 64.8% and 75.6% ovicidal activities were noted at 150 and 200 mg/L of the diethyl ether extract of *A. sapota* and 55.6% and 69.8% at 150 and 200 mg/L of the diethyl ether extract of *C. auriculata*. At 250 mg/L of *A. sapota* and *C. auriculata* extract, 91.3% and 80.4% ovicidal activity was recorded against the eggs of *An. stephensi* respectively. Though, at higher concentrations *i.e.* 300 and 350 mg/L, the recorded ovicidal activities did not show much variations but they shown significant activity than other concentrations tested against the eggs of *An. stephensi* in *A. sapota* and greater variations in *C. auriculata* (84.6% and 88.4%).

Dichloromethane extract of *A. sapota* showed 33.6% egg mortality whereas 36.4% egg mortality was noted at 50 mg/L of *C. auriculata* extract. Similarly at 100 mg/L 46.4% and 45.6% egg mortality were observed in *A. sapota* and *C. auriculata* respectively. Besides, 66.4% and 88.6% ovicidal activities were noted at 150 and 200 mg/L of *A. sapota* extract and 68.4% and 72.6% of *C. auriculata* extract. At 250 mg/L, 94.6% and 84.6% ovicidal activity was recorded against the eggs of *An. stephensi* in *A. sapota* and *C. auriculata* respectively. Though, at higher concentrations *i.e.* 300 and 350 mg/L, the recorded ovicidal activities did not show much variations but they show significant activity than other concentrations tested against the eggs of *An. stephensi* in *A. sapota* and greater variations in *C. auriculata* (88.4% and 93.6%).

Acetone extract of *A. sapota* showed 48.2% egg mortality whereas 38.8% egg mortality was noted at 50 mg/L of *C. auriculata*. Similarly at 100 mg/L 53.2% and 52.8% egg mortality were observed in *A. sapota* and *C. auriculata* respectively. Besides, 75.2% and 91.4% ovicidal activities were noted at 150 and 200 mg/L of *A. sapota* and 72.8% and 81.4% of *C. auriculata* extract. At 250 mg/L 97.8% and 86.6% ovicidal activity was recorded against the eggs of *An. stephensi* in *A. sapota* and *C. auriculata* respectively. Though, at higher concentrations *i.e.*, 300 and 350 mg/L, the recorded ovicidal activities were did show much variations but they shown significant activity than other concentrations tested against the eggs of *An. stephensi* in *A. sapota* and greater variations in *C. auriculata* (91.5% and 95.6%).

Methanol extract of *A. sapota* showed 51.8% egg mortality whereas 42.4% egg mortality was noted against 50 mg/L of *C. auriculata*. Similarly at 100 mg/L 59.8 and 59.6% egg mortality were observed in *A. sapota* and *C. auriculata* respectively. Besides, 79.8% and 93.6% ovicidal activities were noted at 150 and 200 mg/L of *A. sapota* and 73.6% and 86.8% of *C. auriculata*. At 250 mg/L 98.6% and 91.4% ovicidal activity was recorded against the eggs of *An. stephensi* in *A. sapota* and *C. auriculata* respectively. Though, at higher concentrations *i.e.* 300 and 350 mg/L, the recorded ovicidal activities did not show much variations but they shown significant activity than other concentrations tested against the eggs of *An. stephensi* in *A. sapota* and greater variations in *C. auriculata* (94.8% and 97.4%).

3.3. Pupicidal activity

Pupal mortality of 71.4% with the adult emergence of 28.6% and 88.4% pupal mortality with the adult emergence of 11.6% were noted at 125 and 250 mg/L concentration of hexane extract of *A. sapota*. Hexane extract of *C. auriculata* showed 74.6% and 89.4% pupal mortality with 25.4% and 10.6% adult emergence at 125 and 250 mg/L concentrations respectively.

Pupal mortality of 74.6% with the adult emergence of 25.4% and 90.6% pupal mortality with the adult emergence of 9.4% were noted at 125 and 250 mg/L concentration of diethyl ether extract of *A. sapota*. Diethylether extract of *C. auriculata* showed 82.6% and 94.0% pupal mortality with 17.4% and 6.0% adult emergence at 125 and 250 mg/L concentrations respectively.

Pupal mortality of 82.6% with the adult emergence of 17.4% and 92.6% pupal mortality with the adult emergence of 7.4% were noted at 125 and 250 mg/L concentration of dichloromethane extract of *A. sapota*. Dichloromethane extract of *C. auriculata* showed 75.6% and 85.4% pupal mortality with 24.4% and 14.6% adult emergence at 125 and 250 mg/L concentrations respectively. Pupal mortality of 84.0% with the adult emergence of 16.0% and 85.4% pupal mortality with the adult emergence of 14.6% were noted at 125 and 250 mg/L concentration of acetone extract of *A. sapota*. Acetone extract of *C. auriculata* showed 88.0% and 97.4% pupal mortality with 12.0% and 2.6% adult emergence at 125 and 250 mg/L concentrations respectively.

Pupal mortality of 89.4% with the adult emergence of 10.6% and 100% pupal mortality with the adult emergence of 0%

Table 2
Repellent activity of *A. sapota* and *C. auriculata* different extracts against *An. stephensi*.

Name of the solvent	Concentration (mg/cm^2)	60 min	120 min	180 min	240 min	300 min	360 min	420 min	480 min
A. sapota									
Hexane	1.5	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	89.8±3.9	80.3±2.4	73.2±4.6
	3.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	96.4±2.4	91.1±3.5	83.4±4.2
Diethylether	1.5	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	84.6±4.6	72.3±4.8
	3.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	95.4±6.3	88.4±6.2
Dichloromethane	1.5	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	85.2±4.2	81.6±4.5
	3.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	96.6±4.7	85.4±4.7
Acetone	1.5	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	91.4±6.2	78.7±2.4
	3.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	96.5±4.4	84.8±6.9
Methanol	1.5	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	88.6±4.4
	3.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	96.4±8.2
C. auriculata									
Hexane	1.5	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	86.3±4.8	81.8±3.9	72.8±2.4
	3.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	93.1±7.4	87.4±8.4	81.4±6.8
Diethylether	1.5	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	83.8±8.6	78.5±4.4
	3.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	93.6±4.9	91.4±2.9
Dichloromethane	1.5	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	84.6±4.6	72.3±6.4
	3.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	95.8±6.3	78.4±8.6
Acetone	1.5	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	84.8±4.4	76.8±4.5
	3.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	96.4±8.8	89.6±6.7
Methanol	1.5	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	91.6±8.4
	3.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	98.4±8.2

Values represent mean±SD of five replications.

were noted at 125 and 250 mg/L concentration of methanol extract of *A. sapota*. Methanol extract of *C. auriculata* showed 91.4% and 98.0% pupal mortality with 8.6% and 2.0% adult emergence at 125 and 250 mg/L concentrations respectively. Whereas, no pupal mortality was recorded in control batches and they shown 100% adult emergence.

3.4. Repellent activity

As shown in Table 2, methanol extracts of both plants were found to be the most effective for repellent activity and a higher concentration of the same extracts 3.0 mg/cm^2 provide 100% protection up to 420 min. The chi-square values are significant at $P\leqslant0.05$ and the tested plants crude extracts have exerted promising larvicidal, ovicidal, pupicidal and repellent activity against selected vector mosquito.

4. Discussion

In recent years, there seems to be an increasing legislative restrictions concerning the use of pesticides; safe but efficient alternate must be developed for the least toxic but most efficient means of integrated vector control, especially during emergency situations. Vector control is facing a threat due to the emergence of resistance in vector mosquitoes to conventional synthetic insecticides, calling for either countermeasures or development of newer insecticides. Earlier, several workers reported spectrum of activity not only on mosquitoes but also on several other kinds of invertebrate organisms[22]. Essential oil of *Rosmarinus officinalis* has been reported for their biological activities against *Ceratitis capitata*[23]. Zhu *et al*.[24] reported that amyris oil possesses the inhibitory effect with LC_{50} values in 24 h of 58 μg/mL for *Aedes aegypti (Ae. aegypti)*, 78 μg/mL for *Aedes albopictus*, and 77 μg/mL for *Culex pipiens pallens*. Rahuman *et al*.[25] have reported that the LC_{50} value of petroleum ether extracts of *Jatropha curcas* and *Pedilanthus tithymaloides* were 8.79 and 55.26 mg/L, respectively against *Ae. aegypti*. Kannathasan *et al*.[26] have reported that the methanol leaf extracts of *Vitex negundo, Vitex trifolia, Vitex peduncularis*, and *Vitex altissima* were used for larvicidal assay with LC_{50} value of 212.57, 41.41, 76.28, and 128.04 mg/L respectively against the early fourth instar larvae of *Culex quinquefasciatus (Cx. quinquefasciatus)*. Finally, Mohan and Ramaswamy[27] evaluated the efficacy of *Ageratina adenophora* against *Culex* and found that it showed an LC_{50} of 227.19 mg/L after 24 h of treatment. Senthilkumar *et al*.[28] have also reported that the larvicidal and adulticidal activities of ethanolic and water mixture (50:50) of plant extracts of *Eucalyptus globules, Cymbopogon citratus, Artemisia annua, Justicia gendarussa, Myristica fragrans, Annona squamosa* and *Centella asiatica* were tested against *An. stephensi*, and the most effective activity between 80% and 100% was observed in all extracts. Dua *et al*.[29] determined that LD_{50} values of the essential oil of *Lantana camara*

leaves were 0.06, 0.05, 0.05, 0.05 and 0.06 mg/cm^2 while LD_{90} values were 0.10, 0.10, 0.09, 0.09 and 0.10 mg/cm^2 against *Ae. aegypti, Cx. quinquefasciatus, Anopheles culicifacies, Anopheles fluviatilis and An. stephensi* respectively. Knock down time (KDT_{50} and KDT_{90}) of the essential oil were 20, 18, 15, 12, 14 min 35, 28 25, 18 and 23 min against *Ae. aegypti, Cx. quinquefasciatus, Anopheles culicifacies, Anopheles fluviatilis* and *An. stephensi*, respectively on 0.208 mg/cm^2 impregnated paper. Murugesan and Muthusamy[30] reported that bioassays with an ethanolic extract of *Melia azedarach* were performed on the larval stages of *An. stephensi*, *Cx. quinquefasciatus* and *Ae. aegypti*. The LC_{50} and 90% lethal concentration of root extract of *Valeriana jatamansi* against adult *An. stephensi, Anopheles culicifacies, Ae. aegypti, Aedes albopictus* and *Cx. quinquefasciatus* were 0.14, 0.16, 0.09, 0.08, and 0.17 and 0.24, 0.34, 0.25, 0.21, and 0.28 mg/cm^2, respectively[31].

Hossain *et al.*[32] reported that the mortality rate was higher in 50 mg/L doses of methanolic extracts of both *Dregea volubilis* and *Bombax malabaricum* against *Cx. quinquefaciatus*. The corresponding LC_{50}values were 56.97 mg/L and 48.85 mg/L. Abdalla *et al.*[33] has also reported that the *Calotropis procera* extracts against *Cx. quinquefasciatus* caused high, moderate and low larval mortality in the larvicidal experiment against 3rd instar larvae. It was found that, LC_{50}–LC_{90} values calculated were 273.53–783.43, 366.44–1018.59 and 454.99–1224.62 mg/L for 2nd, 3rd and 4th larval instars, respectively, of *Anopheles arabiensis* and 187.93–433.51, 218.27–538.27 and 264.85–769.13 mg/L for 2nd, 3rd and 4th larval instars, respectively, of *Cx. quinquefasciatus*. Eliningaya *et al.*[34] have reported the mortality of *Cx. quinquefasciatus* ranged from 0.5% to 96.75% while for *Anopheles gambiae* it ranged from 13.75% to 97.91%. The LC_{50} and LC_{95} value in the laboratory was similar for both species while in the semi-field they were different from each other. Mullai *et al.*[35] reported that the cucurbitaceous plant *Citrullus vulgaris* was tested for ovicidal and repellent activities against *An. stephensi*. For ovicidal activity, 100% mortality was observed at 250 mg/L of benzene extract and the other extracts exerted 100% mortality at 300 mg/L. Skin repellent test at 1.0, 2.5 and 5.0 mg/cm^2 concentration gave the mean complete protection time ranged from 119.17 to 387.83 min with the four different extracts tested. These results could encourage the search for new active natural compounds offering an alternative to synthetic repellents and insecticides from other medicinal plants. The results of this study may contribute to increase the opportunity for natural control of various medically important pests by botanical pesticides. Since these are often active against specific target insects, less expensive, easily biodegradable to non-toxic products and potentially suitable for use in mosquito control program[36], they could lead to development of new possible safer insect control agents.

An attempt has been made to evaluate the role of different extracts of *A. sapota* and *C. auriculata* for their larvicidal, ovicidal, repellent and pupicidal activities against various life stages of *An. stephensi*. The results reported in this study provide the possibility for further investigations of the efficacy of larvicidal activity, ovicidal activity, repellent activity and pupicidal activities of natural plant extracts as a potential agent for combating malarial vector mosquito *An. stephensi*.

Conflict of interest statement

The authors have no conflict of interest.

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Comments

Background

Mosquitoes transmit the arboviruses responsible for yellow fever, dengue haemorrhagic fever, epidemic polyarthritis, and several forms of encephalitis. Mosquito control is the task of managing the population of mosquitoes to reduce their damage to human health, economics and enjoyment of mosquito-ridden areas. Due to the dreadful effect of synthetic chemicals, safer vector control methods is needed. Hence many researchers forcus on the use of botanical derivatives in mosquito control especially for mosquito larvae, as an alternative to synthetic insecticides.

Research frontiers

This manuscript stated the negative impacts of chemical pesticides and also emphasized the importance of the phyto-compounds and their role in nature to save the environment. Thus, no doubt, this kind of research must be encouraged to mitigate the environmental pollution by chemical pesticides. The plant derived compounds could be possible utilized in the vector control programme in the near future.

Related reports

The materials and methods adapted in the present study are reproducible and are universally accepted by several authors and WHO. The concentration fixed in the present research are admissible to the non target organisms like aquatic organisms.

Innovations and breakthroughs

One of the important innovative approach in the present investigation is that *A. sapota* extracted with methanol showed good results against the selected mosquito. Thus it paves the way for further exploration of possible utilization of the selected plant against other mosquito species.

Applications

A. sapota and *C. auriculata* extracts showed promising larvicidal, ovicidal, repellent and pupicidal activities against various life stages of *An. stephensi*. The results reported in this study provide the possibility for further investigations of the efficacy of larvicidal activity, ovicidal activity, repellent activity and pupicidal activities of natural plant extracts as a potential agent for controlling malarial vector mosquito *An. stephensi*.

Peer review

In general, this manuscript is written well with scope and it will be useful for scientific society. It will motivate many scientific society to conduct their research in this field. Since it is needed to control the mosquito, this kind of work should be encouraged to save our green planet.

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