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Larvicidal and repellent activity of medicinal plant extracts from Eastern Ghats of South India against malaria and filariasis vectors

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ABSTRACT

Objective: To evaluate the larvicidal and repellent activities of ethyl acetate and methanol extracts of *Acacia concinna* (*A. concinna*), *Cassia siamea* (*C. siamea*), *Coriandrum sativum* (*C. sativum*),*Cuminum cyminum* (*C. cyminum*), *Lantana camara* (*L. camara*), *Nelumbo nucifera* (*N. nucifera*) *Phyllanthus amarus* (*P. amarus*), *Piper nigrum* (*P. nigrum*) and *Trachyspermum ammi* (*T. ammi*) against *Anopheles stephensi* (*An. stephensi*) and *Culex quinquefasciatus* (*Cx. quinquefasciatus*). **Methods:** The larvicidal activity of medicinal plant extracts were tested against early fourth-instar larvae of malaria and filariasis vectors. The mortality was observed 24 h and 48 h after treatment, data were subjected to probit analysis to determine the lethal concentrations (LC_{50} and LC_{90}) to kill 50 and 90 per cent of the treated larvae of the tested species. The repellent efficacy was determined against two mosquito species at five concentrations (31.25, 62.50, 125.00, 250.00, and 500.00 ppm) under the laboratory conditions. **Results:** All plant extracts showed moderate effects after 24 h and 48 h of exposure; however, the highest activity was observed after 24 h in the leaf methanol extract of *N. nucifera*, seed ethyl acetate and methanol extract of *P. nigrum* against the larvae of *An. stephensi* (LC_{50} = 34.76, 24.54 and 30.20 ppm) and against *Cx. quinquefasciatus* (LC_{50} = 37.49, 43.94 and 57.39 ppm), respectively. The toxic effect of leaf methanol extract of *C. siamea*, seed methanol extract of *C. cyminum*, leaf ethyl acetate extract of *N. nucifera*, leaf ethyl acetate and methanol extract of *P. amarus* and seed methanol extract of *T. ammi* were showed 100% mortality against *An. stephensi* and *Cx. quinquefasciatus* after 48 h exposer. The maximum repellent activity was observed at 500 ppm in methanol extracts of *N. nucifera*, ethyl acetate and methanol extract of *P. nigrum* and methanol extract of *T. ammi* and the mean complete protection time ranged from 30 to 150 min with the different extracts tested. **Conclusions:** These results suggest that the leaf and seed extracts of *C. siamea, N. nucifera, P. amarus*, *P. nigrum* and *T. ammi* have the potential to be used as an ideal ecofriendly approach for the control of the *An. stephensi* and *Cx. quinquefasciatus*.

1. Introduction

Mosquitoes are the important and major blood- sucking vectors and it transmits parasites and pathogens which cause devastating impact on human beings. It is estimated that every year at least 500 million people in the world suffer from one or the other tropical diseases that include malaria, lymphatic filariasis, schistosomiasis, dengue, trypanosomiasis and leishmaniasis. One to two million deaths are reported annually due to malaria worldwide. Lymphatic filariasis affects at least 120 million people in 73 countries in Africa, India, Southeast Asia, and Pacific Islands. These diseases not only cause high levels of morbidity and mortality, but also inflict great economic loss and social disruption on developing countries such as India, China, etc. India alone contributes around 40% of global filariasis burden and the estimated annual economic loss is about 720 crore[1].

Anopheles stephensi (*An. stephensi*) Liston is the primary vector of malaria in India and other West Asian countries. Malaria remains one of the most prevalent diseases in the tropical world. With 200 million to 450 million infections annually worldwide, it causes up to 2.7 million deaths[2]. *Culex quinquefasciatus* (*Cx. quinquefasciatus*) Say acts

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as a vector for filariasis in India. Human filariasis is a major public health hazard and remains a challenging socioeconomic problem in many of the tropical countries[3]. Lymphatic filariasis caused by *Wuchereria bancrofti* (*W. bancrofti*) and transmitted by mosquito *Cx. quinquefasciatus* is found to be more endemic in the Indian subcontinent. It is reported that *Cx. quinquefasciatus* infects more than 100 million individuals worldwide annually[4]. The disease remains endemic in more than 100 developing tropical countries, and its control is a major goal for improved worldwide health.

In recent years synthetic insecticides in mosquito control resulted in environmental hazards through persistence and accumulation of non-biodegradable chemicals in ecosystem, biological magnification through the food chains, development of insecticide resistance among vector species and toxic effect in human health and non target organisms. These developments demand renewed alternative insecticidal agents with high bio control potentiality but cause little or no harmful effect to environment and human health. One possible strategy is the rational localisation of bioactive products from phytochemicals by systematically exploring the global floral biodiversity. There is an ever-increasing demand for plant-based insecticides as they are nontoxic, easily available at affordable prices, biodegradable and show broadspectrum targetspecific activities against different species of vector mosquitoes. Furthermore, unlike conventional commercial insecticides that are based on single active ingredient, plantderived insecticides comprise botanical blends of secondary metabolites which act concertedly on both behavioural and physiological processes. Thus, echances of pests developing resistance to such substances are meagre.

The adulticidal, repellent, and larvicidal activity of crude hexane, ethyl acetate, and methanol extracts of *Cassia angustifolia* (Fabaceae) (*C. angustifola*) were moderate effective against adult and early fourth instar larvae of *Culex gelidus* (*Cx. gelidus*)and *Cx. quinquefasciatus*[5]. Aqueous and organic extracts of *Acacia polyacantha* (*A. polyacantha*) showed the strongest anthelmintic activity against *Caenorhabditis elegans*[6], betulin and the methanolic extract of *Acacia mellifera* (*A. mellifera*) were most effective against *Plasmodium berghei*, and only bark extract produced considerable antimalarial activity[7]. The leaf extract of *Cassia siamea* (*C. siamea*) showed powerful antimalarial activity against *Plasmodium falciparum* (*P. falciparum*) *in vitro* as well as *in vivo* against *Plasmodium berghei* (*P. berghei*)[8]. Hexane, chloroform, ethyl acetate, acetone and methanol of leaf and flower extracts of *Cassia auriculata* (*C. auriculata*) were good effect against fourth instar larvae of malaria vector, *An. stephensi* and lymphatic filariasis vector, *Cx. quinquefasciatus*[9]. The methanol, benzene and acetone leaf extract of *Cassia fistula* had larvicidal, ovicidal and repellent activity against *Aedes aegypt* (*Ae. aegypti*)[10]. The leaf ethanolic extract of *C. obtusifolia* had larvicidal and oviposition deterrence effects against *An. stephensi*[11]. The acetone and petroleum ether extracts of *Coriandrum sativum* (*C. sativum*) were moderate effective against *Ae. aegypti*[12], aqueous extract of *C. sativum* was investigated against *Ae. fluviatilis*[13]. The essential oil of *Oenanthe pimpinelloides* (*O. pimpinelloides*) (Apiaceae) was evaluated against *Cx. pipiens*[14], the aqueous extract of *Daucus carota* (*D. carota*) had good effect against fourth instar larvae of *Cx. annulirostris*[15].

The essential oil of *Cuminum myrrha* (*C. myrrha*) was most effective against early fourth stage larvae of *Ae. aegypti* and *Cx. pipiens* pallens[16]. Essential oil extracted from cumin seeds *C. cyminum* had acaricidal activity against tick larvae of *Rhipicephalus* (*Boophilus*) *microplus* (*R. Boophilus* microplus)[17]. The essential oil isolated from the leaves of *Lantana camara* (*L. camara*) were moderate effective against mosquito vectors, *Ae. aegypti, Cx. quinquefasciatus, An. culicifacies, An. fluvialitis* and *An. stephensi*[18]. The flower extracts of *L. camara* had good repellent effect against *Ae. albopictus* and *Ae. aegypti*[19]. The hexane, chloroform, ethyl acetate, acetone, methanol, and aqueous leaf extracts of *Nelumbo nucifera* (*N. nucifera*) were effective against fourth instar larvae of *An. subpictus* and *Cx. quinquefasciatus*[20].

Elethyl acetate, butanol, and petroleum ether leaf extracts of *Phyllanthus amarus* (*P. amarus*) were tested against the early fourth instar larvae of *Ae. aegypti* and *Cx. quinquefasciatus*[21], and hexane, chloroform, ethyl acetate, acetone and methanol leaf extracts of *P. emblica* had adulticidal and larvicidal effect against adult cattle tick *Haemaphysalis bispinosa* (*H. bispinosa*), sheep fluke *Paramphistomum cervi* (*P. cervi*), fourth instar larvae of malaria vector, *An. subpictus* and Japanese encephalitis vector, *Cx. tritaeniorhynchus*[22]. The ethanolic extracts of dried fruits of *Piper longum* (*P. longum*) and *P. nigrum*, were tested against the different instars of *Ae. aegypti*[23]. The larvicidal activity of aqueous and ethanolic extracts of *P. nigrum* were more active against early fourth instar larvae of *Cx. quinquefasciatus*[24] and the ethanol extract of *P. nigrum* were tested against *Ae. aegypti*[25]. The essential oil of seeds of *Trachyspermum ammi* (*T. ammi*) showed promising results for larvicidal, oviposition-deterrent, vapor toxicity, and repellent activity against malarial vector, *An. stephensi*[26].

The purpose of the present investigation was to explore the larvicidal and repellent activity of ethyl acetate and methanol extracts from leaf, seed and stem of nine plant species *A. concinna, C. siamea, C. sativum, C. cyminum, L. camara, N. nucifera, P. amarus, P. nigrum* and *T. ammi,* against the malarial vector *An. stephensi* and lymphatic filariasis vector *Cx. quinquefasciatus* larvae in a search for effective and affordable natural products to be used in the control of vectors.

2. Materials and methods

2.1. Plant collection

The seeds of *A. concinna, C. sativum, C. cyminum*, leaf of *C. siamea* , *L. camara, N. nucifera,* leaf and stem of *P. amarus*, seeds of *P. nigrum* and *T. ammi* were selected on the basis of aromatic smell, bitter taste, and ethnopharmacological and ethnobotanical literature surveys. The plant materials were collected from Javadhu Hills (78°35′ and 79°35′ East longitude and 12°24′ and

12°55′ North latitude with an area of 2 405 square km), Tiruvannamalai district and Dharmapuri district, Tamil Nadu, India in July 2010 and the taxonomic identification was made by Dr. Hema C, Department of Botany, Arignar Anna Govt. Arts College for Women, Walajapet, Vellore, India. The voucher specimen was numbered and kept in our research laboratory for further reference.

2.2. Insect rearing

An. stephensi and *Cx. quinquefasciatus* larvae were collected from stagnant water area of Melvisharam and identified in Zonal Entomological Research Centre, Vellore Tamil Nadu, to start the colony, and larvae were kept in plastic and enamel trays containing tap water. They were maintained and reared in the laboratory as per the method of Kamaraj *et al*[27].

2.3. Preparation of plant extracts

The dried leaf (800 g), and seed (700 g) and stem (250 g) were powdered mechanically using commercial electrical stainless steel blender and extracted successively with ethyl acetate (3 000 mL, Qualigens), and methanol (3 800 mL, Qualigens) in a soxhlet apparatus (boiling point range 60-80 ℃) for 8 h. The extracts were filtered and concentrated under reduced pressure. The residue obtained was stored at 4 ℃. The residues were, and then made in to a 1% stock solution with acetone (stock solution). From the stock solution, 500-1.563 ppm was prepared with dechlorinated tap water. Polysorbate 80 (Qualigens) was used as an emulsifier at the concentration of 0.05% in the final test solution.

2.4. Larvicidal bioassay

During preliminary screening with the laboratory trial, the larvae of *An. stephensi* and *Cx. quinquefasciatus* were collected from the insect-rearing cage and identified in Zonal Entomological Research Centre, Vellore. One gram of crude extract was first dissolved in 100 mL of acetone (stock solution). From the stock solution, 500 ppm was prepared with dechlorinated tap water. Polysorbate 80 (Qualigens) was used as an emulsifier at the concentration of 0.05% in the final test solution. The larvicidal activity was assessed by the procedure of WHO[28] with some modification and as per the method of Rahuman *et al*[29]. For bioassay test, larvae were taken in five batches of 20 in 249 mL of water and 1.0 mL of the desired plant extract concentration. The control was set up with acetone, polysorbate 80, and dechlorinated tap water. The numbers of dead larvae were counted after 24 h of exposure, and the percentage of mortality was reported from the average of five replicates. The experimental media in which 100% mortality of larvae occurs alone were selected for dose-response bioassay.

2.5. Repellent activity

An. stephensi and *Cx. quinquefasciatus* mosquitoes were collected from the insect-rearing cage for the testing of repellent activities. The stock solutions of the extracts were diluted with respective solvent, Polysorbate 80 and distilled water to obtain test solutions of 31.25, 62.50, 125.00, 250.00 and 500.00 ppm. For repellent experiment, 50 laboratory reared, blood-starved adult female mosquitoes that were between 3 and 10 days old were placed into separate laboratory cages (45 cm × 45 cm × 40 cm). Before each test, the forearm and hand of a human subject were washed with unscented neutral soap, thoroughly rinsed, and allowed to dry 10 min before extracts application. The different plant extracts being tested were applied from the elbow to the fingertips. The arm was left undisturbed. An arm treated with respective acetone and Polysorbate 80 served as control. The control and treated arms were introduced simultaneously into the cage. The numbers of bites were counted over 5 min, every 30 min, from 18：00 h to 06：00 . Protection time was recorded as the time elapsed between repellent application and the observation period immediately preceding that in which a confirmed bite was obtained. If no bites were confirmed at 150 min, tests were discontinued and protection time was recorded as 150 min. An attempt of the mosquito to insert its stylets was considered a bite. No mosquito attempted to bite the control arm during the observation period. That trial was discarded, and the test was repeated with a new batch of mosquitoes to ensure that lack of bites was due to repellence and not to mosquitoes not being predisposed to get a blood meal at the time. All experiments were conducted five times in separate cages and in each replicate different volunteer were used to nullify any effect of skin differences on repellency. It was observed that there was no skin irritation from the plant extract. The percentage protection was calculated by using the following formula[30-32].

Protection = (No. of bites received by control arm - No. of bites received by treated arm) (No. of bites received by control arm) × 100.

2.6. Dose-response bioassay

From the stock solution, different concentrations ranging from 1.563 to 500 ppm were prepared for larvicidal and repellent activity. Based on the preliminary screening results, crude leaf, seed and stem ethyl acetate and methanol extracts of *A. concinna, C. siamea, C. sativum, C. cyminum, L. camara, N. nucifera* , *P. amarus* , *P. nigrum* and *T. ammi* were subjected to dose-response bioassay for larvicidal activity against the larvae of *An. stephensi* and *Cx. quinquefasciatus*. Numbers of dead larvae were counted after 24 h and 48 h of exposure, and the percentage of mortality was reported from the average of five replicates. However, at the end of 24 h and 48 h, selected test samples turned out to be equal in their toxic potential.

2.7. Statistical analysis

The average larval mortality data were subjected to probit analysis for calculating LC_{50}, LC_{90} and other statistics at 95% fiducial limits of upper confidence limit and lower confidence limit, and chi-square values were calculated using the software developed by Reddy *et al*[33]. Results with P<0.05 were considered to be statistically significant.

3. Results

The screening is a better means of evaluation of the potential larvicidal activity of plants popularly used for this purpose. The effect of the leaf, seed and stem ethyl acetate and methanol extracts of *A. concinna, C. siamea, C. sativum, C. cyminum, L. camara, N. nucifera*, *P. amarus*, *P. nigrum* and *T. ammi* were tested at 500 ppm and showed activity against the fourth-instar larvae of *An. stephensi* and *Cx. quinquefasciatus* (Table 1). All plant extracts showed moderate larvicidal effects after 24 h; however, leaf methanol extract of *N. nucifera*, seed ethyl acetate and methanol extract of *P. nigrum* against the larvae of *An. stephensi* (LC_{50} = 34.76, 24.54 and 30.20 ppm) and against *Cx. quinquefasciatus* (LC_{50} = 37.49, 43.94 and 57.39 ppm) (Table 2), leaf methanol extract of *C. siamea*, seed methanol extract of *C. cyminum*, leaf ethyl acetate extract of *N. nucifera*, leaf ethyl acetate and methanol extract of *P. amarus* and seed methanol extract of *T. ammi* were showed 100% mortality against *An. stephensi* (LC_{50}=53.94, 39.23, 47.85, 41.99, 37.91, 52.48, 44.99, 64.55 ppm); and against *Cx. quinquefasciatus* (LC_{50} =46.61, 44.37, 45.16, 54.85, 48.76, 62.39, 50.85 and 37.49 ppm, respectively). Chi-square value was significant at $P<0.05$ level (Table 3).

The tested plant extracts have exerted promising repellent activities against *An. stephensi* and *Cx. quinquefasciatus*. In the present study, we observed 150-min protection at 500 ppm in leaf and seed methanol extracts of *C. siamea, N. nucifera, P. amarus, P. nigrum* and *T. ammi* against *An. stephensi* and *Cx. quinquefasciatus*. Results from the

Table 1
Larvicidal activity of different extracts against fourth instar larvae of *An. stephensi* and *Cx. quinquefasciatus* at 500 ppm (%).

Botanical name/Family	Parts used	Solvents	Species	Mortality	
				24 h	48 h
A. concinna (Willd.) DC. Var/ Fabaceae	Seed	Ethyl cetate	*An. stephensi*	20.60±1.12	46.40±1.62
			Cx. quinquefasciatus	32.40±1.68	60.20±1.04
		Methanol	*An. stephensi*	28.00±1.04	42.00±1.24
			Cx. quinquefasciatus	42.20±1.20	86.80±1.06
C. siamea Lam / Leguminosae	Leaf	Ethyl acetate	*An. stephensi*	48.20±2.04	64.20±1.21
			Cx. quinquefasciatus	32.40±2.60	56.10±1.46
		Methanol	*An. stephensi*	82.30±1.67	100.00±0.00
			Cx. quinquefasciatus	56.40±1.24	100.00±0.00
C. sativum L./ Apiaceae	Seed	Ethyl acetate	*An. stephensi*	26.20±1.08	52.60±1.58
			Cx. quinquefasciatus	20.00±1.24	46.20±1.62
		Methanol	*An. stephensi*	38.40±46.2	68.00±1.06
			Cx. quinquefasciatus	46.20±2.28	72.80±2.82
C. cyminum L./ Apiaceae	Seed	Ethyl acetate	*An. stephensi*	74.20±2.95	*92.60±2.62*
			Cx. quinquefasciatus	64.80±1.62	82.00±1.28
		Methanol	*An. stephensi*	87.20±1.92	100.00±0.00
			Cx. quinquefasciatus	75.60±2.48	100.00±0.00
L. camara L./ Verbenaceae	Leaf	Ethyl acetate	An. stephensi	42.60±2.50	86.20±2.24
			Cx. quinquefasciatus	31.40±2.02	68.40±2.94
		Methanol	*An. stephensi*	48.00±1.62	93.60±2.12
			Cx. quinquefasciatus	46.30±2.42	89.00±1.84
N. nucifera Gaertn./ Nymphaeaceae	Leaf	Ethyl acetate	*An. stephensi*	72.60±2.31	100.00±0.00
			Cx. quinquefasciatus	68.50±1.62	100.00±0.00
		Methanol	*An. stephensi*	100.00±0.00	100.00±0.00
			Cx. quinquefasciatus	100.00±0.00	100.00±0.00
P. amarus L./ Phyllanthaceae	Leaf	Ethyl acetate	*An. stephensi*	86.30±1.60	100.00±0.00
			Cx. quinquefasciatus	72.60±1.68	100.00±0.00
		Methanol	*An. stephensi*	92.00±1.46	100.00±0.00
			Cx. quinquefasciatus	80.20±1.82	100.00±0.00
	Stem	Ethyl acetate	*An. stephensi*	32.00±2.86	56.40±1.58
			Cx. quinquefasciatus	28.40±2.62	48.50±1.61
		Methanol	*An. stephensi*	42.50±1.86	75.60±2.49
			Cx. quinquefasciatus	34.60±2.18	67.20±1.04
P. nigrum L./ Piperaceae	Seed	Ethyl acetate	*An. stephensi*	100.00±0.00	100.00±0.00
			Cx. quinquefasciatus	100.00±0.00	100.00±0.00
		Methanol	*An. stephensi*	100.00±0.00	100.00±0.00
			Cx. quinquefasciatus	100.00±0.00	100.00±0.00
T. ammi L./ Apiaceae	Seed	Ethyl acetate	*An. stephensi*	56.00±1.68	92.80±1.29
			Cx. quinquefasciatus	43.70±2.42	87.30±2.04
		Methanol	*An. stephensi*	68.20±1.86	100.00±0.00
			Cx. quinquefasciatus	53.80±2.48	100.00±0.00

Control—Nil mortality;a Mean value of three replicates.

Table 2

LC_{50} and LC_{90} value of different solvent crude extracts against *An. stephensi* and *Cx. quinquefasciatus* for 24 h (ppm).

Plant species	Parts used	Solvents	Species	LC_{50} Mean±SE	LC_{50} UCL– LCL	LC_{90} Mean±SE	LC_{90} UCL– LCL	χ^2(df = 4)
N .nucifera	Leaf	Ethyl acetate	*An. stephensi*	34.76±2.49	39.66–29.86	172.78±21.12	214.19–131.37	9.48
			Cx. quinquefasciatus	37.49±2.66	43.19–32.78	176.69±20.70	217.28–136.10	10.15
P. nigrum	Seed	Ethyl acetate	*An..stephensi*	24.54±1.69	27.84–21.23	108.03±12.02	131.58–84.48	4.25
			Cx. quinquefasciatus	43.94±3.18	50.18–37.71	216.88±25.39	266.65–167.11	3.97
		Methanol	*An..stephensi*	30.20±2.21	34.54–25.87	156.05±19.51	194.29–117.81	11.36
			Cx. quinquefasciatus	57.39±4.12	65.47–49.32	284.18±35.35	353.47–214.89	10.82

Control – Nil mortality. Significant at $P< 0.05$ level; LC_{50} – Lethal concentration that kills 50% of the exposed larvae, LC_{90} – Lethal concentration that kills 90% of the exposed larvae, UCL=Upper confidence Limit; LCL=Lower confidence Limit, χ^2 –Chi–square, *df* –degree of freedom.

Table 3

LC_{50} and LC_{90} value of different solvent crude extracts against *An. stephensi* and *Cx. quinquefasciatus* for 48 h(ppm).

Plant species	Parts used	Solvents	Species	LC_{50} Mean±SE	LC_{50} UCL– LCL	LC_{90} Mean±SE	LC_{90} UCL– LCL	χ^2 (*df* = 4)
C. siamea	Leaf	Methanol	*An. stephensi*	53.94±4.32	62.41–45.47	350.95±50.09	449.13–252.77	12.19
			Cx. quinquefasciatus	46.61±3.39	53.15–40.07	223.38±26.05	274.43–172.33	3.84
C. cyminum	Seed	Methanol	*An. stephensi*	39.23±2.74	44.59–33.87	176.35±20.06	215.68–137.03	4.68
			Cx. quinquefasciatus	44.37±3.57	56.37–42.38	249.67±30.81	310.05–189.29	9.80
N. nucifera	Leaf	Methanol	*An. stephensi*	47.85±3.83	55.36–42.38	307.21±42.61	390.74–223.69	9.51
P. amarus	Leaf	Ethyl acetate	*Cx. quinquefasciatus*	45.16±3.33	51.69–38.63	239.31±31.36	300.77–177.85	13.34
			An. stephensi	41.99±0.88	48.72–26.27	132.80±17.93	128.34–57.26	12.32
			Cx. quinquefasciatus	54.85±1.04	62.89–16.81	168.62±18.89	175.09–110.14	9.78
	Leaf	Methanol	*An. stephensi*	37.91±1.31	2.49–18.33	94.11±11.90	117.94–70.78	8.83
			Cx. quinquefasciatus	48.76±2.49	59.66–29.86	172.78±21.12	214.19–131.37	9.51
P. nigrum	Seed	Ethyl acetate	*An. stephensi*	52.48±3.87	61.08–45.93	270.66±34.06	337.42–203.89	11.60
			Cx. quinquefasciatus	62.39±4.12	65.47–49.32	284.18±35.35	353.47–214.89	10.82
	Seed	Methanol	*An. stephensi*	44.99±0.88	53.72–23.27	126.80±12.93	94.34–67.26	12.32
			Cx. quinquefasciatus	50.85±1.04	56.89–32.81	68.62±7.89	84.09–59.14	4.78
T. ammi	Seed	Methanol	*An. stephensi*	64.55±4.69	73.75–55.36	196.79±13.45	245.16–162.42	13.63
			Cx. quinquefasciatus	37.49±2.66	43.19–32.78	176.69±20.70	217.28–136.10	10.15

Control – Nil mortality. Significant at $P< 0.05$ level; LC_{50} – Lethal concentration that kills 50% of the exposed larvae, LC_{90} – Lethal concentration that kills 90% of the exposed larvae, UCL=Upper confidence Limit; LCL=Lower confidence Limit, χ^2 –Chi–square, *df* –degree of freedom.

Table 4

Repellent activity of different plant extracts against *An. stephensi* and *Cx. quinquefasciatus* at 500 ppm (%).

Plant species	Parts used	Species	Ethyl acetate 30 min	Ethyl acetate 60 min	Ethyl acetate 90 min	Ethyl acetate 120 min	Ethyl acetate 150min	Methanol 30 min	Methanol 60 min	Methanol 90 min	Methanol 120 min	Methanol 150 min
A. concinna	Seed	*An. stephensi*	58.00±2.24	35.00±1.62	26.00±1.87	12.00±2.46	8.00±2.02	42.00±1.14	30.00±2.40	18.00±4.25	10.00±2.64	8±2.26
		Cx. quinquefasciatus	46.00±1.20	28.00±1.64	18.00±1.72	6.00±1.00	2.00±1.82	36.00±1.32	22.00±1.00	10.00±2.82	6.00±1.00	4.00±1.68
C. siamea	Leaf	*An. stephensi*	98.00±2.64	62.00±2.84	26.00±2.11	14.00±2.00	8.00±2.49	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00
		Cx. quinquefasciatus	69.00±2.11	32.00±2.13	12.00±1.43	10.00±1.26	4.00±1.32	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00
C. sativum	Seed	*An. stephensi*	75.00±1.42	31.00±2.76	21.00±3.21	16.00±2.80	12.00±1.62	74.00±2.71	68.00±2.63	42.00±3.24	30.00±2.64	22.00±2.54
		Cx. quinquefasciatus	68.00±1.38	37.00±1.80	15.00±1.11	12.00±3.64	10.00±1.72	71.00±1.82	52.00±3.21	36.00±2.24	24.00±2.48	18.00±3.00
C. cyminum	Seed	*An. stephensi*	92.00±1.76	54.00±2.38	14.00±1.22	10.00±1.89	8.00±3.28	100.00±0.00	90.00±2.11	56.00±2.11	26.00±2.11	14.00±1.84
		Cx. quinquefasciatus	98.00±1.41	65.00±3.41	22.00±3.71	14.00±2.08	10.00±3.86	100.00±0.00	92.00±1.82	62.00±2.72	34.00±1.68	23.00±1.76
L. camara	Leaf	*An. stephensi*	98.00±1.14	62.00±1.72	36.00±2.76	28.00±1.64	14.00±3.42	82.00±2.48	56.00±2.12	18.00±1.24	14.00±2.11	10.00±3.48
		Cx. quinquefasciatus	93.00±1.71	72.00±1.26	42.00±2.60	24.00±1.80	16.00±2.62	86.00±3.41	78.00±1.11	35.00±1.82	21.00±1.68	12.00±2.64
N. nucifera	Leaf	*An. stephensi*	100.00±0.00	100.00±0.00	100.00±0.00	92.00±1.82	80.00±2.43	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00	98.00±0.24
		Cx. quinquefasciatus	100.00±0.00	100.00±0.00	100.00±0.00	98.00±1.42	86.00±1.42	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00	92.00±2.62
P.amarus	Leaf	*An. stephensi*	100.00±0.00	100.00±0.00	92.00±2.00	68.00±2.96	46.00±2.84	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00
		Cx. quinquefasciatus	100.00±0.00	100.00±0.00	85.00±1.18	58.00±3.52	32.00±3.92	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00
	Stem	*An. stephensi*	64.00±1.72	36.00±2.26	27.00±2.11	16.00±2.80	12.00±2.46	82.00±2.46	62.00±1.72	54.00±2.38	31.00±2.76	21.00±3.21
		Cx. quinquefasciatus	76.00±1.26	48.00±2.60	34.00±1.68	12.00±3.64	6.00±1.00	88.00±1.62	74.00±1.26	65.00±3.41	37.00±1.80	15.00±1.06
P.nigrum	Seed	*An. stephensi*	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00
		Cx. quinquefasciatus	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00
T. ammi	Seed	*An. stephensi*	92.00±1.60	80.00±2.46	62.00±1.72	48.00±2.96	27.00±2.11	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00
		Cx. quinquefasciatus	98.00±1.46	86.00±1.62	74.00±1.26	52.00±3.52	34.00±1.68	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00

Mean value of five replicates.

skin repellent activity of leaf ethyl acetate and methanol extracts of *A. concinna*, *C. siamea*, *C. sativum*, *C. cyminum*, *L. camara*, *N. nucifera* , *P. amarus* , *P. nigrum* and *T. ammi* against blood-starved adult female of *An. stephensi* and *Cx. quinquefasciatus* are given in Table 4. It showed that the percentage protection was in relation to dose and time (minutes). The highest concentrations of 500 ppm provided over 150 and 90 min protection in methanol extracts of *C. siamea*, *N. nucifera*, *P. amarus*, *P. nigrum and T. ammi* against *An. stephensi* and *Cx. quinquefasciatus* bites, respectively. Similarly, at 500 ppm, it provided over 30 min protection in methanol extract of *C. cyminum*, 30 to 60 min of protection in leaf ethyl acetate extract of *N. nucifera*, and *P. amarus* and 120 min of protection was observed in *N. nucifera* and *P. amarus* against *An. stephensi* and *Cx. quinquefasciatus*. Lower concentrations provided 30 to 60 min of protection. The control provided only (3.80±0.72) min of protection.

4. Discussion

The obtained results revealed the larvicidal effect of ten plants corresponding to different botanical families on *An. stephensi* and *Cx. quinquefasciatus*. The highest larval mortality was found in leaf acetone and methanol of *Canna indica* (*C. indica*) (LC_{50}= 29.62 and 40.77 ppm; LC_{90} =148.55 and 165.00 ppm) against second instar larvae (LC_{50} = 121.88 and 69.76 ppm; LC_{90} = 624.35 and 304.27 ppm) and against fourth- instar larvae of methanol and petroleum ether extracts of *Ipomoea carnea* (*I. carnea*) (LC_{50} = 41.82 and 39.32 ppm; LC_{90} = 423.76 and 176.39 ppm) against second instar larvae (LC_{50} = 163.81 and 41.75 ppm; LC_{90}= 627.38 and 162.63 ppm) and against fourth instar larvae of *Cx. quinquefasciatus*, respectively[34].

Larvicidal activity of the acetone, chloroform, ethyl acetate, hexane and methanol leaf extracts of *Ocimum canum* (*O. canum*), *O. sanctum* and *Rhinacanthus nasutus* (*R. nasutus*) were studied against fourth instar larvae of *Ae. aegypti* and *Cx. quinquefasciatus*, the larval mortality was observed after 24 h of exposure, were the highest larval mortality was found in methanol extract of *O. canum* and *R. nasutus* and acetone extract of *O. sanctum* against the larvae of *Ae. aegypti (*LC_{50}=99.42, 94.43 and 81.56 ppm) and against *Cx. quinquefasciatus* (LC_{50}=44.54, 73.40 and 38.30 ppm), respectively[35], the larvicidal activity of acetone, chloroform, ethyl acetate, hexane and methanol peel, leaf and flower extracts of *Citrus sinensis* (*C. sinensis)*, *O. canum, O. sanctum* and *R. nasutus* were tested against malaria vector *An. stephensi* and the highest larval mortality was found in peel methanol extract of *C. sinensis*, leaf and flower ethyl acetate extracts of *O. canum* against the larvae of *An. stephensi* (LC_{50}= 95.74, 101.53, 28.96, LC_{90} = 303.20, 492.43 and 168.05 ppm), respectively[36]. Larvicidal activity of crude hexane, ethyl acetate, petroleum ether, acetone, and methanol extracts of the leaf of *Citrullus colocynthis* (*C. colocynthis*), *Coccinia indica* (*C. indica*), *Cucumis sativus* (*C. sativus*), *Momordica charantia (M. charantia*), and *Trichosanthes anguina* (*T. anguina*) were tested against the early fourth instar larvae of *Ae. aegypti* and *Cx. quinquefasciatus* and the larval mortality was observed after 24 h of exposure; the highest larval mortality was found in petroleum ether extract of *C. colocynthis*, methanol extracts of *C. indica, C. sativus, M. charantia*, and acetone extract of *T. anguina* against the larvae of *Ae. aegypti* (LC_{50}=74.57, 309.46, 492.73, 199.14, and 554.20 ppm) and against *Cx.* quinquefasciatus (LC_{50}= 88.24, 377.69, 623.80, 207.61, and 842.34 ppm), respectively[37].

The crude leaf ethyl acetate, acetone, and methanol extracts of *Aegle marmelos*, *Andrographis lineata* (*A. lineata*), *A. paniculata, Cocculus hirsutus* (*C. hirsutus*), *Eclipta prostrata* (*E. prostrata*), and *Tagetes erecta* (*T. erecta*) on repellent activity against *Cx. tritaeniorhynchus*, the maximum repellent activity was observed at 500 ppm in methanol extracts of *A. marmelos*, ethyl acetate extracts of *A. lineata*, *C. hirsutus*, and *E. prostrata* and the mean complete protection time ranged from 120 to 150 min with the different extracts tested[38]. Autran *et al*[39] have reported that the essential oil from leaves and stems of *P. marginatum* exhibited an oviposition deterrent effect against *Ae. aegypti* at 50 and 100 ppm in that significantly lower numbers of eggs (<50%) were laid in glass vessels containing the test solutions compared with the control solution. The acetone, ethyl acetate, and methanol leaf extracts of *A. marmelos*, *A. lineata*, and *C. hirsutus* tested for oviposition-deterrent, ovicidal, and repellent activities against *An. subpictus*. The percentage of effective oviposition repellency of 92.60, 93.04, 95.20, 88.26, 92.80, 94.01, 95.77, 96.93, and 92.54 at 500 ppm and the lowest repellency of 47.14, 58.00, 56.52, 64.93, 71.09, 66.42, 50.62, 57.62, and 65.73 at 31.25 ppm in acetone, ethyl acetate, and methanol extracts of *A. marmelos, A. lineata*, and *C. hirsutus*, respectively[32]. Venkatachalam and Jebanesan[40] have also reported that the repellent activity of methanol extract of *Ferronia elephantum* (*F. elephantum*) leaves against *Ae. aegypti* activity at 1.0 and 2.5 mg/cm^2 concentrations gave 100% protection up to (2.14±0.16) h and (4.00±0.24) h, respectively, and the total percentage protection was 45.8% at 1.0 mg/cm^2 and 59.0% at 2.5 mg/cm^2 for 10 h. The aqueous extract of *R. nasutus* showed LC_{50} values of 5.124 and 9.681 mg/L against *Cx. quinquefasciatus* and *Ae. aegypti*, respectively[41].

Earlier authors reported that the petroleum ether extract of *R. nasutus* possessed larvicidal effects with LC_{50} values between 3.9 and 11.5 mg/L and *Derris elliptica* (*D. elliptica*) showed LC_{50} values between 11.2 and 18.84 mg/L against *Ae. aegypti, Cx. quinquefasciatus, An. dirus*, and *Mansonia uniformis* (*M. uniformis*)[42]. The adulticidal, repellent, and larvicidal activity of crude hexane, ethyl acetate, and methanol extracts of *Aristolochia indica* (*A. indica*), *C. angustifolia*, *Diospyros melanoxylon* (*D. melanoxylon*), *Dolichos biflorus* (*D. biflorus)*, *Gymnema sylvestre* (*G. sylvestre*), *Justicia procumbens* (*J. procumbens*), *Mimosa pudica* (*M. pudica*), and *Zingiber zerumbet* (*Z. zerumbet*) were tested against adult and early fourth instar larvae of *Cx. gelidus* and *Cx. quinquefasciatus*, the effective adult mortality was observed in methanol extract of *A. indica*, ethyl acetate extract of *D. biflorus*, and ethyl acetate and hexane extract of *Z. zerumbet* against *Cx. gelidus* and *Cx. ouinquefasciatus* (LD_{50}=37.75, 78.56, 129.44, 86.13, 80.06, 112.42, 53.83,and 46.61; LD_{90}=166.83, 379.14, 521.50, 289.83, 328.18, 455.72, 181.15, and 354.50 ppm, respectively), complete protections for 150 min were found in hexane and methanol extract of *A. indica* and *Z. zerumbet* at 1 000 ppm against mosquito bites, the highest larval mortality was found in the hexane extract of *Z. zerumbet*, ethyl acetate extract of *D. biflorus*, and methanol extracts of *A. indica* against *C. gelidus* (LC_{50}=26.48, 33.02, and 12.47 ppm; LC_{90}=127.73, 128.79, and 62.33 ppm) and against *Cx. quinquefasciatus* (LC_{50}=69.18, 34.76, and 25.60 ppm; LC_{90}= 324.40, 172.78, and 105.52 ppm), respectively, after 24 h[5].

Chowdhury *et al*[43] have reported that the chloroform and methanol extracts of mature leaves of *Solanum villosum* (*S. villosum*) showed the LC_{50} value for all instars between

24.20 and 33.73 ppm after 24 h and between 23.47 and 30.63 ppm after 48 h of exposure period against *An. subpictus*. The larvicidal activity of acetone, chloroform, ethyl acetate, hexane, and methanol dried leaf, flower, and seed extracts of *Achyranthes aspera*(*A. aspera*), *Anisomeles malabarica* (*A. malabarica*), *Gloriosa superb* (*G. superba*), *Psidium guajava* (*P. guajava*), *Ricinus communis* (*R. communis*), and *S. trilobatum*, tested against fourth instar larvae of *An. subpictus* and *Cx. tritaeniorhynchus* were the highest larval mortality was observed leaf ethyl acetate extract of *A. aspera*, leaf chloroform extract of *A. malabarica*, flower methanol of *G. superba*, and leaf methanol extract of *R. communis* against the larvae of *A. subpictus* (LC_{50}=48.83,135.36, 106.77, and 102.71 ppm; LC_{90}=225.36, 527.24, 471.90, and 483.04 ppm); and leaf ethyl acetate extract of *A. aspera*, leaf chloroform extract of *A. malabarica*, flower methanol extract of *G. superba*, and leaf methanol extract of *R. communis* against the larvae of *Cx. tritaeniorhynchus* (LC_{50}=68.27, 95.98, 59.51, and 93.94 ppm; LC_{90}=306.88,393.83, 278.99, and 413.27 ppm), respectively[44-51].

The hexane fraction of *Kaempferia galangal* (*K. galangal*) was found to exhibit the highest larvicidal effect with the LC_{50} of 42.33 ppm against *Cx. quinquefasciatus* and possessed repellency against *Cx. tritaeniorhynchus*[52]. The acetone, chloroform, ethyl acetate, hexane and methanol extracts of peel and leaf extracts of *Citrus sinensis*, *O. canum*, *O. sanctum* and *R. nasutus* were tested against fourth -instar larvae of malaria vector, *An. subpictus*, Japanese encephalitis vector, *Cx. tritaeniorhynchus* were showed the highest mortality in peel chloroform extract of *C. sinensis*, leaf ethyl acetate extracts of *O. canum* and *O. sanctum* and leaf chloroform extract of *R. nasutus* against the larvae of *An. subpictus* (LC_{50}=58.25,88.15, 21.67 and 40.46 ppm; LC_{90}=298.31, 528.70, 98.34 and 267.20 ppm), peel methanol extract of *C. sinensis*, leaf methanol extract of *O. canum*, ethyl acetate extracts of *O. sanctum* and *R. nasutus* against the larvae of *Cx. tritaeniorhynchus* (LC_{50}=38.15, 72.40, 109.12 and 39.32 ppm; LC_{90}=184.67, 268.93, 646.62 and 176.39 ppm) respectively[53].

In conclusion, an attempt has been made to evaluate the role of medicinal plant extracts' larvicidal and repellent bioassay against *An. stephensi* and *Cx. quinquefasciatus* activity. The results reported in this study open the possibility for further investigations of the efficacy of larvicidal and repellent properties of natural product extracts. The isolation and purification of crude extract of leaf methanol extracts of *A. concinna, N. nucifera* and *P. amarus* and seed methanol extract of *P. nigrum* and *T. ammi* are in progress.

Conflict of interest statement

We declare that we have no conflict of interest.

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