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Mosquito adulticidal and repellent activities of botanical extracts against malarial vector, *Anopheles stephensi* Liston (Diptera:Culicidae)

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ABSTRACT

Objective: To determine the adulticidal and repellent activities of different solvent leaf extracts of *Eclipta alba* (*E. alba*) and *Andrographis paniculata* (*A. paniculata*) against malarial vector, *Anopheles stephensi* (*An. stephensi*). **Methods:** Adulticidal efficacy of the crude leaf extracts of *E. alba* and *A. paniculata* with five different solvents like benzene, hexane, ethyl acetate, methanol and chloroform was tested against the five to six day old adult female mosquitoes of *An. stephensi*. The adult mortality was observed after 24 h under the laboratory conditions. The repellent efficacy was determined against *An. stephensi* mosquito species at three concentrations *viz*., 1.0, 2.5 and 5.0 mg/cm^2 under laboratory conditions. **Results:** Among the tested solvents the maximum efficacy was observed in the methanol extract. The LC_{50} and LC_{90} values of *E. alba* and *A. paniculata* against adults of *An. stephensi* were 150.36, 130.19 ppm and 285.22, 244.16 ppm, respectively. No mortality was observed in controls. The *chi*-square values were significant at $P<0.05$ level. Methanol extract of *E. alba* and *A. paniculata* was produce maximum repellency against *An. stephensi*. **Conclusions:** From the results it can be concluded the crude extract of *E. alba* and *A. paniculata* was an excellent potential for controlling *An. stephensi* mosquitoes.

1. Introduction

Mosquitoes are the principal vectors of malaria and other vector borne diseases and contribute to major disease burden in India. Disease transmission can be interrupted by controlling the vectors using various methods. However, the extensive and unbalanced use of chemical insecticides have created problems like enhancing resistance of mosquito population to synthetic insecticides, pollution of environment and adverse effects on the non-target flora and fauna inhabiting the same aquatic habitat[1]. These steadily growing problems, demand an intensive search for new products that are environmentally safe, targetspecific and degradable. Botanical phytochemicals with mosquitocidal potential are now recognized as potent alternative insecticides to replace synthetic insecticides in mosquito control programs due to their excellent larvicidal, ovicidal, adulticidal and repellent properties[2, 3,4].

Extracts or essential oils from plants may be alternative sources of mosquito control agents, since they constitute a rich source of bioactive compounds that are biodegradable into nontoxic products and potentially suitable for use to control mosquitoes. Plant extracts in general have been recognized as an important natural resource of insecticides[5]. A bitter plant, a decoction of *Andrographis paniculata (A. paniculata)* leaves or roots, is used as a vermifuge, against *Dipetalonema reconditum* and *Brugia malayi*[6], and the methanol and ethyl acetate extracts were tested on *Callosobruchus chinensis*[7] and relieve itchy skin and insect bites[8]. *Cocculus hirsutus* is a widely growing plant found in the plains of India in dry localities and is used medicinally by the Indian tribes for a wide range of ailments, including constipation and kidney problems[9]. *Eclipta prostrata* and *Eclipta alba (E. alba)* extracts have been reported to exert diverse biological activity including antiinflammatory, antihemorrhagic, antihyperlipidemic, and antihyperglycemic activities[10].

The leaf extract of *Acalypha indica* with different solvents *viz*, benzene, chloroform, ethyl acetate, and methanol were tested for larvicidal, ovicidal activity, and oviposition attractancy against *Anopheles stephensi* (*An. stephensi*)[2]. Petroleum ether (60-80 ℃) extracts of the leaves of *Vitex negundo* were acted as a promising repellent against *Culex tritaeniorhynchus* (*Cx. tritaeniorhynchus*)[11]. The ethyl acetate extracts of *Hyptis suaveolens* and *Rhododendon tomentosum*, *Hyptis harmaja*, and *Myrica gale* significantly

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reduced biting activity of *Aedes aegypti* (*Ae. aegypti*)[12]. The ethanol extract of *Curcuma aeruginosa, Curcuma aromatica,* and *Curcuma xanthorrhiza* were tested for repellent activity against *Aedes togoi (Ae. togoi), Armigeres subalbatus, Culex quinquefasciatus (Cx. quinquefasciatus)*, and *Culex tritaeniorhynchus (Cx. tritaeniorhynchus)*[13].

Insect repellents are widely used as personal protection against biting arthropods. Personal protection measures, including the use of repellents are important in reducing the risk of contracting disease. The vector borne diseases cause a high level of morbidity and mortality, but they are also responsible for great socio-economical loss. The majority of commercial insect repellent preparations contain the chemical N,N-diethyl-m-toluamide (DEET), first synthesized in 1954[14]. It has been reported that these chemical repellents are not safe for the public use. There has been much research on natural plant extracts both prior to and after the advent of synthetic repellents. Novak[15] emphasized the urgent need of the investigation of phytochemicals as repellents for mosquitoes in his review of nonchemical approach to mosquito control.

Cymbopogon excavatus gave 100% repellency for 2 h, when it was evaluated in the laboratory against *Anopheles arabiensis (An. arabiensis)* and its repellency decreased to 59.3% after 4 h[16]. *Cymbopogon winterianus* oil, mixed with 5% vanillin, gave 100% protection for 6 h against *Aedes aegypti* (*Ae. aegypti), Cx. quinquefasciatus* and *Anopheles dirus (An. dirus),* results compared to those observed with 25% DEET (N,N-diethyl-3-methylbenzanmide)[17]. The oil of *Cymbopogon martinii* provided 100% repellency for 12 h against *Anopheles* mosquitoes in a field test which was carried out by using pairs of volunteers who sat together, one of whom was treated with the oil while the other was not[18]. Oils extracted from the genus *Ocimum* spp. have been traditionally used as repellents[19]. *Ocimum americanum* volatile oil was shown to repel *Ae. aegypti, Ae. dirus* and *Cx. quinquefasciatus*, under cage conditions, for up to 8 h[17]; an ethanolic solution of *Ocimum selloi* oil (10% v/v) seemed to be very effective in repelling *Anopheles braziliensis*[19]; and liquid paraffin solutions of *Ocimum gratissimum* and *Ocimum basilicum* oils exhibited a high bite-protection[20].

Repellency properties of nepetalactone (cyclopentanoid monoterpene) isolated from the catnip plant, *Nepeta cataria*, against 17 species of insects were reported by Eisner[21]. Also, many monoterpenes were reported for their insect repellent properties such as α-pinenen, limonene, terpinolene, citronellol, citronellal, camphor, rotundial, dolichodial, teucrein, and isoborneol[22]. Jantan and Zaki[23] evaluated four essential oils of *Litsea elliptica, Cinnamomum mollissimum, Cymbopogon nardus*, and *Pogostemom cablin*, respectively, for their repellency effect against *Ae. aegypti*. Barnard[24] tested the repellency of five essential oils (Bourbon geranium, cedar wood, clove, peppermint, and thyme) singly applied at different concentrations (5, 10, 25, 50, 75, and 100%) or in combinations against two mosquito species *Ae. aegypti* and *Anopheles albimanus*. Thyme and clove oils were the most effective mosquito repellents. Das *et al.*[25] evaluated the repellent properties of *Zanthoxylum armatum, Zanthoxylum alatum, Curcuma aromatica*, and *An. indica* against mosquitoes in two bases of mustard oil (*Brassica* sp.) and coconut oil (*Cocos* sp.). The methanol extract of the *Foeniculum* fruits has repellent activity against *Ae. aegypti* (L.)[26]. Govindarajan[27] reported that the leaf methanol, benzene, and acetone extracts of *Cassia fistula* were studied for the larvicidal, ovicidal, and repellent activities against *Ae. aegypti*.

Rohani *et al.*[28], has reported the efficacy of few Malaysian essential oils such as *Litsea elliptica*, *Polygonum minus* and *Piper aduncum* as potential mosquito adulticides while Sulaiman *et al.*[29] has reported the essential oils of *Melaleuca cajuputi* and *Cymbopogon nardus* have adulticidal effects on *Aedes* mosquito at high-rise flats in Kuala Lumpur. The compounds, 4 thiophenes, 5-(but-3-ene-1-ynyl)-2, 2′ -bithiophene, 5-(but-3-ene-1-ynyl)-5′ -methyl-2,2′ -bithiophene, 2,2′ ,5′ ,2″ - terthiophene, and 5-methyl-2,2′ ,5′ ,2″ -terthiophene, isolated from floral extract of *Tagetes minuta* were largely responsible for the toxicity exhibited against the adults of *Ae. aegypti* and *Anopheles stephensi (An. stephens)*[30]. In view of the recently increased interest in developing plant origin insecticides as an alternative to chemical insecticide, this study was undertaken to assess the adulticidal and repellent potential of the extracts from the medicinal plants against malarial vector mosquito.

2. Materials and methods

2.1. Plant collection

The Fully developed fresh leaves of *Eclipta alba (E. alba)* and *Andrographis paniculta* (*A. paniculata)* were collected from different regions of Cuddalore District, Tamilnadu, India. It was authenticated by a plant taxonomist from the Department of Botany, Annamalai University. A voucher specimen is deposited at the herbarium of the plant photochemistry division, Annamalai University.

2.2. Extraction

The leaves were washed with tap water, shade dried at room temperature and finely ground. The finely ground plant material (1.0 kg/solvent) was loaded in soxhlet apparatus and was extracted with five different solvents namely benzene, hexane, ethyl acetate, methanol and chloroform individually. The solvent from the extract was removed using a rotary vacuum evaporator to collect the crude extract. The crude residue of these plants varies with the solvents used. The *A. paniculata* and *E. alba* with five different solvents yielded 81.20, 94.85, 79.40, 97.60, 121.30 g and 75.25, 87.30, 70.0, 89.50, 102.80 g of crude residue respectively. Standard stock solutions were prepared at 1% by dissolving the residues in acetone. From this stock solution, different concentrations were prepared and these solutions were used for adulticidal and repellent bioassays.

2.3. Test organisms

The mosquito, *An. stephensi* was 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 one week old chick for blood meal. Mosquitoes were held at (28±2) ℃, 70%-85% relative humidity (RH), with a photo period of 14 h light, 10 h dark.

2.4. Repellent activity

The repellent study was followed by the method of WHO[31]. Three-day-old blood-starved female *An. stephensi* mosquitoes (100) were kept in a net cage (45 cm × 30 cm × 45 cm). The volunteer had no contact with lotions, perfumes, oils or perfumed soaps on the day of the assay. The arms of volunteer, only 25 cm^2 dorsal side of the skin on each arms were exposed and the remaining area covered with rubber gloves. The crude extracts were applied at 1.0, 2.5 and 5.0 mg/cm^2 separately in the exposed area of the forearm. Ethanol was served as the control. *An. stephensi* was tested during the night from 19.00 to 05.00 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. Each test concentration was repeated six times. The volunteer conducted their test of each concentration by inserting the treated and control arm into the 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 following formula.

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

Where Ta is the number of mosquitoes in the control group and Tb is the number of mosquitoes in the treated group.

2.5. Adulticidal activity

Five to six day old sugar-fed adult female mosquitoes were used. The *E. alba* and *A. paniculata* plants leaf extracts were diluted with ethanol to make different concentrations. The diluted plants extracts were impregnated on filter papers (140 × 120 mm). A blank paper consisting of only ethanol was used as control. The papers were left to dry at room temperature to evaporate off the ethanol overnight. Impregnated papers were prepared fresh prior to testing. The bioassay was conducted in an experimental kit consisting of two cylindrical plastic tubes both measuring 125 × 44 mm following the WHO method[32]. One tube served to expose the mosquitoes to the plants extracts and another tube was used to hold the mosquitoes before and after the exposure periods. The impregnated papers were rolled and placed in the exposure tube. Each tube was closed at one end with a 16 mesh size wire screen. Sucrose-fed and blood starved mosquitoes (20) were released into the tube, and the mortality effects of the extracts were observed every 10 min for 3 h exposure period. At the end of 1, 2, and 3h exposure periods, the mosquitoes were placed in the holding tube. Cotton pads soaked in 10% sugar solution with vitamin B complex were placed in the tube during the holding period of 24h. Mortality of the mosquitoes was recorded after 24 h. The above procedure was carried out in triplicate for each plants extracts concentration.

2.6. Statistical analysis

The average adult 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 SPSS 12.0 version software. Results with $P<0.05$ were considered to be statistically significant.

3. Results

The results of the adulticidal activity of hexane, ethyl acetate, benzene, chloroform and methanol extract of *E. alba* and *A. paniculata* against the adult of important vector mosquito *An. stephensi* are presented in Tables 1 and 2. Among five solvent extracts tested, the highest adulticidal activity was observed in methanol extract of *E. alba* and *A. paniculata* against *An. stephensi* with the LC_{50} and LC_{90} values of 150.36, 130.19 ppm and 285.22, 244.16 ppm respectively. The *chi*-square values are significant at $P< 0.05$ level. The 95% confidence limits [LC_{50} (LCL-UCL)] and [LC_{90} (LCL-UCL)] were also calculated. The hexane, ethyl acetate, benzene, chloroform and methanol extract of *E. alba* and *A. paniculata* show significant repellency against *An. stephensi* (Table 3 and 4). In this observation, these two plant crude extracts gave protection against mosquito bites without any allergic reaction to the test person, and also, the repellent activity is dependent on the strength of the plant extracts.

4. 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. In our results showed that crude extract of *A. paniculata* and *E. alba* have significant adulticidal and repellent activity against *An. stephensi* mosquito. The results are comparable with an earlier report by Elango *et al*.[33] who reported that the maximum repellent activity was observed at 500 ppm in methanol extracts of *Aegle marmelos (A. marmelos)* and *Acacia lineata (A. lineate)* and ethyl acetate extract of *Cytisus hirsutus (C. hirsutus)*, and the mean complete protection time ranged from 90 to 120 min with the different extracts tested; no egg hatchability was observed with ethyl acetate extract of *A. marmelos*; methanol extracts *A. marmelos*, *A. lineata*, and *C. hirsutus* were exerted at 1 000 ppm, and the percentage of effective oviposition repellency were 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 were 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. Amer and Mehlhorn[34] have reported that the five most effective oils were those of Litsea (Litsea cubeba), Cajeput (Melaleuca leucadendron), Niaouli (Melaleuca quinquenervia), Violet (Viola odorata), and Catnip (Nepeta cataria), which induced a protection time of 8 h at the maximum and a 100% repellency against *Ae. aegypti, An. stephensi*, and *Cx. quinquefasciatus*. The essential oil of *Zingiber officinalis* (*Z. officinalis*) showed repellent activity at 4.0 mg/cm^2, which provided 100% protection up to 120 min against *Cx. quinquefasciatus*[35]. The seed acetone extract of *Tribulus terrestris* showed 100% repellency in 0, 4, and 6 h and in 1 and 6 h and in 0, 2, and 4 h at 10% concentration against *An. culicifacies, An. stephensi* and *Cx. quinquefasciatus*, respectively[36].

Watanabe *et al*.[37] isolated a new compound, eucamol, and 4-isopropylbenzyl alcohol from *E. camaldulensis*. This new compound was compared with deet and proved to be

highly active against *Ae. aegypti* L.; after 3 h 75% of its repellency remained. Kerosene oil containing 1% neem oil resulted in the deviation of *Aopheles culicifacies* Giles, from living rooms to cattle sheds, followed by a reduced malaria incidence[38]. For personal protection neem oil (1-4%) mixed in coconut oil resulted in a protection of 81-91% from bites of *Anopheles* mosquitoes, when tested in a forested village in India[39]. Dua *et al*.[40] tested neem cream, also a repellent to be applied to the skin, against *Aedes*, *Culex* and *Anopheles* mosquitoes; one application was about 70% effective for 4 h. *Lantana camara* L. flower extract in coconut oil provides 94.5% protection from *Aedes albopictus* and *Ae. aegypti*, with no undesirable adverse effects on human volunteers for 3 months after the application[41].

The seed acetone extract of *Tribulus terrestris* showed strong repellent activity against *An. culicifacies* species, 100% repellency in 1 and 6 h; *An. stephensi* 100% repellency in 0, 4, and 6 h; and *Cx. quinquefasciatus* 100% repellency in 0, 2, and 4 h, at 10% concentration, respectively[42]. Mullai *et al*.[43] have also reported that the 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 against *An. stephensi* with the benzene, petroleum ether, ethyl acetate, and methanol extracts of *Citrullus vulgaris* tested. Govere *et al*.[44] reported that the alcohol plant extract of *Lippia javanica* provided 76.7% protection against *An. arabiensis* after a 4-h period, and *Cymbopogon excavates* and *Pelargonium reniforme* provided 66.7% and 63.3% protection for 3 h, respectively. Karunamoorthi *et al*.[11] have also reported that the leaves of *Echinops* sp. (92.47%), *Ostostegia integrifolia* (90.10%), and *Olea europaea* (79.78%) were also effective and efficient to drive away mosquitoes and the roots of *Silene macroserene* (93.61%), leaves of *Echinops* sp. (92.47%), *Ostostegia integrifolia* (90.10%), and *Olea europaea* (79.78%) were exhibited the significant repellency by direct burning. Tawatsin *et al*.[17] have reported repellent activity against *Ae. aegypti*, *An. dirus* and *Cx. quinquefasciatus* which is due to 5% vanillin which has been added to the essential oil of Curcuma longa.

Selvaraj Pandian *et al*.[45] also studied powdered preparation of the leaves of *Adhatoda vasica* (adhatoda), *Azadirachta indica* (neem) and *Ocimum sanctum* (tulsi), which on burning with charcoal produced smoke which repelled *Armigeres subalbatus* and *Cx. quinquefasciatus* to prevent their biting activity for 6-8 h. Repellent activity of *Ferronia elephantum* (Rutaceae) (*F. elephantum*) leaf extract against *Ae. aegypti*. The total percentage of protection of *F. elephantum* was 45.8% of 1.0 mg/cm^2 and 59.0% at 2.5 mg/cm^2 for 10 h[46]. Isolation of repellent ingredients from *L. camera* (Verbenaceae) flowers and their repellency against *Aedes* mosquitoes. One application of this fraction gave 100% protection for 2 h and may protect 75.8% at

Table 1

Adulticidal activity of different solvent extracts of *E. alba* against *An. stephensi*.

Solvents	Concentration (ppm)	% of mortality±SD	LC_{50} (ppm)(LCL-UCL)	LC_{90} (ppm) (LCL-UCL)	χ^2
Hexane	Control	0.0±0.0	197.88 (161.78-236.39)	361.03 (307.67-459.05)	11.108*
	70	22.7±1.2			
	140	36.2±0.4			
	210	53.6±0.8			
	280	71.2±0.6			
	350	87.9±0.7			
Ethyl acetate	Control	0.0±0.0	184.74 (144.12-226.23)	343.30 (288.39-449.95)	13.839*
	70	26.3±0.6			
	140	39.2±0.4			
	210	58.0±0.9			
	280	73.8±1.1			
	350	91.2±0.9			
Benzene	Control	0.0±0.0	171.55 (126.46-215.40)	323.52 (268.12-436.50)	16.795*
	70	29.8±1.5			
	140	42.2±0.4			
	210	62.7±1.0			
	280	76.9±0.8			
	350	94.3±0.7			
Chloroform	Control	0.0±0.0	156.69 (109.81-200.04)	297.48 (244.83-405.85)	18.729*
	70	32.9±0.9			
	140	45.9±0.4			
	210	68.4±1.1			
	280	81.9±0.6			
	350	97.2±1.0			
Methanol	Control	0.0±0.0	150.36 (96.53-198.88)	285.22 (229.58-412.28)	23.932*
	70	34.9±0.7			
	140	48.0±1.1			
	210	69.4±1.6			
	280	83.0±0.4			
	350	99.6±0.7			

*Significant at $P<0.05$, SD - Standard Deviation ; LCL - Lower Confidence Limits ; UCL - Upper Confidence Limits; χ^2 - *Chi* square.

Table 2
Adulticidal activity of different solvent extracts of *A. paniculata* against *An. stephensi*.

Solvents	Concentration (ppm)	% of mortality±SD	LC_{50} (ppm) (LCL–UCL)	LC_{90} (ppm) (LCL–UCL)	χ^2
Hexane	Control	0.0±0.0	174.20 147.02–203.37	306.12 265.28–376.52	9.528*
	60	19.2±0.4			
	120	32.8±0.8			
	180	52.4±0.2			
	240	68.9±0.4			
	300	90.2±0.7			
Ethyl acetate	Control	0.0±0.0	163.86 135.63–193.02	292.15 252.23–361.43	10.403*
	60	20.2±0.4			
	120	37.2±1.2			
	180	58.4±1.0			
	240	71.9±0.5			
	300	92.0±0.9			
Benzene	Control	0.0±0.0	152.70 118.15–187.18	277.55 233.52–362.54	15.008*
	60	23.9±0.9			
	120	42.2±1.4			
	180	61.0±0.7			
	240	74.2±0.6			
	300	95.6±0.3			
Chloroform	Control	0.0±0.0	142.19 105.12–177.65	262.96 219.04–350.12	16.956*
	60	27.0±0.8			
	120	46.2±1.2			
	180	65.4±0.4			
	240	78.0±1.1			
	300	97.2±0.9			
Methanol	Control	0.0±0.0	130.19 90.77–166.40	244.16 200.94–332.75	19.225*
	60	32.2±1.4			
	120	49.0±1.0			
	180	68.9±0.5			
	240	84.0±0.8			
	300	99.0±0.7			

Table 3
Repellency of different solvent extracts of *E. alba* against *An. stephensi*.

Solvents	Concentration (mg/cm^2)	% of repellency ±SD							
		Time post application of repellent (min.)							
		15	30	60	90	120	150	180	210
Hexane	1.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	68.2±1.4	55.4±1.3	41.3±1.8
	2.5	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	71.7±1.6	62.2±1.8	49.7±2.0
	5.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	73.6±1.4	68.4±1.5
Ethyl acetate	1.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	74.6±1.8	67.0±1.5	51.7±1.7
	2.5	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	79.9±2.0	72.8±1.2	57.6±1.3
	5.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	80.4±2.0	71.9±2.1
Benzene	1.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	70.9±1.2	60.7±1.3	49.6±1.6	36.9±1.4
	2.5	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	74.6±1.5	64.3±1.4	56.7±1.4	43.3±1.5
	5.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	75.3±1.1	66.7±1.7	54.6±1.9
Chloroform	1.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	75.3±2.0	65.9±1.9	56.7±1.2
	2.5	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	81.6±1.9	71.3±2.1	66.9±2.1
	5.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	82.9±1.5	74.6±1.8
Methanol	1.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	79.7±1.6	68.4±2.3	60.3±1.4
	2.5	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	85.6±1.1	76.0±1.5	69.7±2.2
	5.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	87.3±1.7	76.5±1.6

Table 4
Repellency of different solvent extracts of *A. paniculata* against *An. stephensi*.

Solvents	Concentration (mg/cm^2)	% of repellency ±SD							
		Time post application of repellent (min.)							
		30	60	90	120	150	180	210	240
Hexane	1.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	70.9±1.5	61.3±1.6	55.0±1.3
	2.5	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	76.5±1.8	66.7±1.3	58.9±1.7
	5.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	79.3±1.8	71.5±1.9
Ethyl acetate	1.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	76.6±2.0	67.9±2.1	59.5±2.1
	2.5	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	82.2±1.7	70.6±1.6	63.3±1.4
	5.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	82.9±1.4	75.5±1.6
Benzene	1.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	80.6±1.2	71.3±1.9	65.0±1.8
	2.5	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	84.9±1.8	75.6±2.1	67.5±2.1
	5.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	84.7±1.7	79.0±1.5
Chloroform	1.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	82.5±1.6	76.0±1.3	67.7±1.3
	2.5	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	86.2±1.9	80.4±1.8	71.9±1.5
	5.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	88.9±2.0	82.5±2.1
Methanol	1.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	89.6±1.3	81.2±1.6	76.7±1.8
	2.5	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	94.0±1.5	86.9±1.5	79.6±1.3
	5.0	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±1.6	86.0±1.6

7 h[47]. Pushpanathan *et al.*[48] showed that the skin repellent test at 1.0, 2.5, and 5.0 mg/cm^2 concentration of *Cymbopogon citratus* essential oil against the filarial mosquito *Cx. quinquefasciatus* gave 100% protection up to 3.00, 4.00, and 5.00 h, respectively. The total percentage of protection of this essential oil was 49.64% at 1.0 mg/cm^2, 62.19% at 2.5 mg/cm^2, and 74.03% at 5.0 mg/cm^2 for 12 h. About 70 plant extracts were tested for their ability to repel the attacks of blood-sucking arthropods. It was found that a CO_2 extract of the seeds of the Mediterranean plant *Vitex agnus* castus (monks pepper) can be used as a spray to keep away especially *Ixodes ricinus* and *Rhipicephalus sanguineus* ticks from animals and humans for at least 6 h. In addition, mosquitoes, biting flies, and fleas are also repelled for about 6 h[49]. Plant could be an alternative source for mosquito larvicides because they constitute a potential source of bioactive chemicals and generally free from harmful effects. Use of these botanical derivatives in mosquito control instead of synthetic insecticides could reduce the cost and environmental pollution. Further studies on identification of active compounds, toxicity and field trials are needed to recommend the active fraction of these plant extracts for development of eco-friendly chemicals for control of insect vectors.

Conflict of interest statement

We declare that we have no conflict of interest.

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