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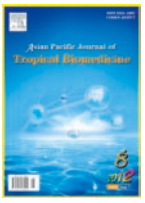

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Repellent properties of *Cardiospermum halicacabum* Linn. (Family: Sapindaceae) plant leaf extracts against three important vector mosquitoes

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ABSTRACT

Objective: To determine repellent activity of hexane, ethyl acetate, benzene, chloroform and methanol extract of *Cardiospermum halicacabum* (*C. halicacabum*) against *Culex quinquefasciatus* (*Cx. quinquefasciatus*), *Aedes aegypti* (*Ae. aegypti*) and *Anopheles stephensi* (*An. stephensi*). **Methods:** Evaluation was carried out in a net cage (45 cm×30 cm×25 cm) containing 100 blood starved female mosquitoes of three mosquito species and were assayed in the laboratory condition by using the protocol of WHO 2005; The plant leaf crude extracts of *C. halicacabum* was applied at 1.0, 2.5, and 5.0 mg/cm^2 separately in the exposed area of the fore arm. Only ethanol served as control. **Results:** In this observation, the plant crude extracts gave protection against mosquito bites without any allergic reaction to the test person, and also, the repellent activity was dependent on the strength of the plant extracts. The tested plant crude extracts had exerted promising repellent against all the three mosquitoes. **Conclusions:** From the results it can be concluded the crude extract of *C. halicacabum* was potential for controlling *Cx. quinquefasciatus*, *Ae. aegypti* and *An. stephensi* mosquitoes.

1. Introduction

Mosquitoes are vectors of several diseases affecting humans and domestic animals worldwide. Mosquitoes are the major vector for the transmission of malaria, dengue fever, yellow fever, filariasis, schistosomiasis, Japanese encephalitis (JE) *etc*, causing millions of deaths every year[1]. Mosquitoes also cause allergic responses in humans that include local skin and systemic reactions such as angioedema[2]. Despite ongoing efforts to control the disease, malaria still remains a serious public health problem in about 90 countries worldwide. *Aedes aegypti* (*Ae. aegypti*) is generally known as a vector for an arbovirus responsible for dengue fever, which is endemic to Southeast Asia, the Pacific island area, Africa, and the Americas. This mosquito is also the vector of yellow fever in Central and South America and West Africa. Dengue fever has become an important public health problem as the number of reported cases continues to increase, especially with more severe forms of the disease, dengue hemorrhagic fever, and dengue shock syndrome, or with unusual manifestations such as central nervous system involvement. *Aedes* mosquitoes are responsible for the spread of serious human diseases such as dengue and chikunguniya. Dengue is prevalent in more than 100 countries and threatens the health of approximately 2.5 billion people. Around 80 million people are infected annually at an attack rate of 4% worldwide [3]. *Culex quinquefasciatus* (*Cx. quinquefasciatus*) (Say.) acts 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. Lymphatic filariasis caused by *Wuchereria 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].

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. The disease remains endemic in more than 100 developing

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tropical countries, and its control is a major goal for improved worldwide health. On a global scale, malaria causes 300-500 million cases and results in 1.5-3 million deaths annually. In India, malaria is one of the most important causes of direct or indirect infant, child, and adult mortality. About 2 million confirmed malaria cases and 1 000 deaths are reported annually, although 15 million cases and 20 000 deaths are estimated by WHO South East Asia Regional Office. India contributes 77% of the total malaria in Southeast Asia[5-10]. Phytochemical and microbes which is used as insecticides for killing larvae, adult or as mosquitoes repellents for protection against mosquito bites[11-16]. Several phytochemicals extracted from various botanical sources have been reported to have detrimental effects on mosquitoes [17-21].

Several alternative mosquito repellents contain some plant oils such as penny royal, citronella, eucalyptus, soybean, or peppermint as putative active ingredients[17]. Essential oil of *Cinnamomum zeylanicum* showed oviposition-deterrent and repellent activities, and the essential oils of *Zingiber officinale* and *Rosmarinus officinalis* also showed both ovicidal and repellent activities against *An. stephensi*, *Ae. aegypti*, and *Cx. quinquefasciatus*[18]. Govindarajan[19] reported that the leaf methanol, benzene, and acetone extracts of *Cassia fistula* were studied for the larvicidal, ovicidal, and repellent activities against *Ae. aegypti*. Traditional application methods such as thermal expulsion and direct burning of mosquito repellent plants (*Corymba citriodora*, *Ocimum suave*, among others) have shown to decrease the number of *Anopheles* mosquitoes entering a house[20]. *Cymbopogon* plant have been traditionally used to repel mosquitoes in jungle regions such as the Bolivian Amazon[21]. Many extracts and essential oils isolated from these plants have been tested against different kinds of arthropods. *Cymbopogon excavatus* gave 100% repellency for 2 h, when it was evaluated in the laboratory against *Anopheles arabiensis* and its repellency decreased to 59.3% after 4 h[22]. *Cymbopogon winterianus* oil, mixed with 5% vanillin, gave 100% protection for 6 h against *Ae. aegypti*, *Cx. quinquefasciatus* and *Anopheles dirus*, results compared to those observed with 25% DEET (N,N-diethyl-3-methylbenzanmide)[23].

Trongtokit *et al*[24] have assessed repellent activity of 38 Thai essential oils and found that an effective time of repellency strongly depended on the concentrations, experiment designs, and mosquito species. Govindarajan *et al*[25] evaluated the ovicidal and repellent activities of methanol leaf extract of *Ervatamia coronaria* (*E. coronaria*) and *Caesalpinia pulcherrima* (*C. pulcherrima*) against *Cx. quinquefasciatus*, *Ae. aegypti* and *An. stephensi*. The larvicidal and repellent properties of essential oils from various parts of four plant species *Cymbopogan citrates, Cinnamomum zeylanicum, Rosmarinus officinalis* and *Zingiber officinale* against *Culex tritaeniorhynchus* and *Anopheles subpictus*[26]. The larvicidal, ovicidal, and repellent activities of crude benzene and ethyl acetate extracts of leaf of *E. coronaria* and *C. pulcherrima* were assayed for their toxicity against three important vector mosquitoes, *viz.*, *An. stephensi*, *Ae. aegypti*, and *Cx. quinquefasciatus*[27]. In Argentina, *Tagetes minuta* EO composed mainly of limonene (66%) and (E) ocimenone (19%) deterred *Ae. aegypti* from biting for 90 min at a 25% concentration. Limonene was also a main component of the EO of *Aloysia citriodora*, *Minthostachys mollis* and *Baccharis spartioides* from Argentina, all showing repellency against *Ae. aegypti*[28]. 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 repellent potential of the extracts from the medicinal plants against three medically important species of malaria, filariasis and chikungunya vector, *An. stephensi*, *Cx. quinquefasciatus* and *Ae. aegypti*.

2. Materials and methods

2.1. Plant collection

Fully developed fresh leaves of *Cardiospermum halicacabum* (*C. halicacabum*) 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. Preparation of the extract

The leaves were washed with tap water, shade dried at room temperature, and powdered by electrical blender. The powder (1.0 kg) was extracted with 90% methanol (3 L) at soxhlet apparatus for 8 h. The extract was filtered through a Buchner funnel with Whatman number 1 filter paper. The filtrate was evaporated to dryness under reduced pressure using rotary evaporator to yield a dark greenish, gummy extract. Standard stock solutions were prepared at 1% by dissolving the residues in ethanol, which was used for the bioassays.

2.3. Test organisms

Cx. quinquefasciatus, *Ae. aegypti* and *An. stephensi* were reared in the vector control laboratory, Department of Zoology, Annamalai University. The larvae were fed on dog biscuits and yeast powder in the 3:1 ratio. Adults were provided with 10% sucrose solution and 1-week-old chick for blood meal. Mosquitoes were held at (28±2)℃, 70%-85% relative humidity, with a photo period of 14-h light and 10-h dark.

2.4. Repellent activity

The repellent study was followed by the method of WHO[29]. Three-day-old blood-starved female, *Cx. quinquefasciatus*, *Ae. aegypti* and *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 fore arm. Only ethanol was served as the control. *Ae. aegypti* was tested during the day time from 07.00 to 17.00 h, while *Cx. quinquefasciatus* and *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 cages, 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.

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

Where T_a is the number of mosquitoes in the control group and T_b is the number of mosquitoes in the treated group.

3. Results

In the present observation, the skin repellent activity of hexane, ethyl acetate, benzene, chloroform and methanol extract of *C. halicacabum* against blood starved adult female of *Cx. quinquefasciatus*, *Ae. aegypti* and *An. stephensi* were given in Tables 1, 2, and 3. The present result showed that

Table 1
Repellency of different solvent extracts of *C. halicacabum* against *Cx. quinquefasciatus*.

Solvents	Concentration (mg/cm^2)	% of Repellency ±SD							
		30 min	60 min	90 min	120 min	150 min	180 min	210 min	240 min
Hexane	1.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	79.9±1.5	70.6±1.5	61.3±1.5	55.4±2.1
	2.5	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	76.6±1.2	65.9±1.9	58.6±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	79.6±1.7	68.4±1.9
Ethyl acetate	1.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	84.9±2.1	76.6±2.0	64.0±2.0	57.7±1.2
	2.5	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	82.4±1.4	69.6±1.6	62.0±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	81.2±1.3	71.9±1.5
Benzene	1.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	88.3±1.6	79.9±1.8	71.7±2.0	63.4±1.7
	2.5	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	86.0±1.3	76.9±1.8	68.6±1.6
	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	85.6±1.4	79.4±1.9
Chloroform	1.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	93.9±1.3	86.6±1.7	77.4±2.1	65.2±1.3
	2.5	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	90.3±1.9	82.0±1.6	70.3±1.8
	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	91.3±1.9	82.7±1.6
Methanol	1.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	96.3±1.8	89.8±1.5	82.2±2.2	77.6±2.1
	2.5	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	95.9±1.3	88.6±1.7	80.5±1.8
	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	94.0±1.4	86.2±1.4

Table 2
Repellency of different solvent extracts of *C. halicacabum* against *Ae. aegypti*.

Solvents	Concentration (mg/cm^2)	% of Repellency ±SD							
		30 min	60 min	90 min	120 min	150 min	180 min	210 min	240 min
Hexane	1.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	77.8±1.3	69.9±1.8	59.6±1.2	52.7±1.7
	2.5	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	73.3±1.6	62.6±1.8	56.0±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	77.0±1.6	65.3±1.5
Ethyl acetate	1.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	81.8±1.8	73.3±1.3	61.6±1.6	55.0±2.0
	2.5	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	80.2±1.7	67.3±1.8	60.9±1.6
	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	80.9±1.4	69.6±1.4
Benzene	1.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	87.0±1.4	77.6±1.4	68.6±2.1	61.3±1.8
	2.5	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	84.6±1.9	73.3±1.6	66.0±1.2
	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	83.0±1.7	78.9±1.3
Chloroform	1.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	91.6±1.7	83.3±1.5	75.9±1.5	63.7±1.6
	2.5	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	89.2±1.8	79.6±2.0	69.0±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	90.0±1.4	79.8±2.1
Methanol	1.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	94.9±1.2	87.6±1.7	80.6±1.9	75.3±1.8
	2.5	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	93.3±1.3	86.9±2.2	79.0±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	92.2±1.6	84.9±1.6

Table 3
Repellency of different solvent extracts of *C. halicacabum* against *An. stephensi*.

Solvents	Concentration (mg/cm^2)	% of Repellency ±SD							
		30 min	60 min	90 min	120 min	150 min	180 min	210 min	240 min
Hexane	1.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	76.6±1.2	68.3±1.6	56.5±1.6	49.6±1.8
	2.5	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	72.6±1.8	60.9±2.1	53.2±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	75.6±1.5	61.3±1.3
Ethyl acetate	1.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	79.6±1.6	70.9±1.3	57.3±1.8	51.7±1.8
	2.5	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	78.0±1.9	65.9±1.7	57.3±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	77.6±1.3	65.7±1.8
Benzene	1.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	85.6±1.8	73.9±1.5	63.7±2.0	57.9±1.4
	2.5	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	81.5±1.9	70.9±1.6	62.8±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	80.6±1.8	75.3±1.9
Chloroform	1.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	88.9±1.5	81.6±2.1	73.2±1.2	61.7±2.1
	2.5	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	86.6±1.5	76.8±2.1	67.3±1.6
	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	88.6±1.6	77.2±1.8
Methanol	1.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	92.7±1.3	84.9±1.7	77.3±1.4	72.6±1.5
	2.5	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	91.2±1.6	83.6±1.9	75.9±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	90.0±1.4	81.3±1.6

the percentage protection in relation to dose and time (minutes). The highest concentrations of 5.0 mg/cm^2 provided over 180 min protection and the lowest concentrations of 1.0 mg/cm^2 provided over 120 min protection in all extracts of *C. halicacabum* against *Cx. quinquefasciatus*, *Ae. aegypti* and *An. stephensi*. In this observation, the plant crude extracts gave protection against mosquito bites without any allergic reaction to the test person, and also, the repellent activity was dependent on the strength of the plant extracts. The tested plant crude extracts had exerted promising repellent against all the three mosquitoes.

4. Discussion

In our results showed that, crude extract of *C. halicacabum* have significant repellent activity against *Cx. quinquefasciatus*, *An. stephensi* and *Ae. aegypti* mosquitoes. The results are comparable with an earlier report by Elango *et al*[30] that the maximum repellent activity was observed at 500 ppm in methanol extracts of *Aegle marmelos*, *Acacia lineata*, and ethyl acetate extract of *Chamaecytisus hirsutus*, and the mean complete protection time ranged from 90 to 120 min against *Anopheles subpictus*. The hexane extract of *Andrographis paniculata* was more effective in exhibiting the repellent action against the mosquito as compared with *Acacia lineate* extract and the complete protections was observed for 150 min in hexane extract of *Andrographis paniculata* at 500 ppm against *Culex tritaeniorhynchus* bites[31]. Amer and Mehlhorn[32] studied 41 plant extracts and 11 oil mixtures were evaluated against the yellow fever mosquito, *Ae. aegypti*, the malaria vector, *An. stephensi*, and the filariasis and encephalitis vector, *Cx. quinquefasciatus* using the skin of human volunteers to find out the protection time and repellency and reported that 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 all three species. Mullai *et al*[33] 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. Similar results were observed by Phukan and Kalita[34] *Litsea salicifolia* recorded 70 and 50 per cent repellency for 3 and 4 h repectively against *Ae. aegypti* but failed to show much activity against *Cx. quinquefasciatus*. The hexane extract at 2 000 ppm exhibited 70 percent repellent activity for 3 h and 50 percent activity for 4 h against *Ae. aegypti* and 46 percent activity for 3 h against *Cx. quinquefasciatus*. Park *et al*[35] found that monoterpenes from the *Lamiaceae* could be used to repel mosquitoes of the genus *Culex*, Similarly, octacosane derived from *Moschosma polystachyum* (Lamiaceae) was effective in repelling *Culex*.

Karunamoorthi *et al*[36-42] 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*[43] have reported that the essential oils were extracted from 18 plant species, belonging to 11 families, and the oils were then prepared as 10% solution in absolute ethanol with additives evaluated the repellent effects and the result showed that the nightbiting mosquitoes (*Anopheles dirus* and *Cx. quinquefasciatus*) and *Aedes albopictus* were more sensitive to all the essential oils (repellency 4.5-8 h) than was *Ae. aegypti* (repellency 0.3-2.8 h), whereas deet and IR3535 provided excellent repellency against *Ae. aegypti*, *Aedes albopictus*, *Anopheles dirus*, and *Cx. quinquefasciatus* (repellency 6.7-8 h). Tawatsin *et al*[23] have reported repellent activity against *Ae. aegypti*, *Anopheles dirus*, and *Cx. quinquefasciatus* which is due to 5% vanillin which has

been added to the essential oil of *Curcuma longa*.

The methanol extract of *E. coronaria* found to be more repellent than *C. pulcherrima* extract. A higher concentration of 5.0 mg/cm^2 provided 100% protection up to 150, 180 and 210 min against *Cx. quinquefasciatus*, *Ae. aegypti* and *An. stephensi*, respectively[25]. larvicidal activity was observed in the essential oil from *Zingiber officinale* against *Culex tritaeniorhynchus* and *Anopheles subpictus* with the LC_{50} and LC_{90} values as 98.83, 57.98 ppm and 186.55, 104.23 ppm, respectively. The highest repellency was observed in *Zingiber officinale*, a higher concentration of 5.0 mg/cm^2 provided 100% protection up to 150 and 180 min against *Culex tritaeniorhynchus* and *Anopheles subpictus*, respectively[26]. Govindarajan *et al*[27] reported mosquito larvicidal, ovicidal, and repellent properties of botanical extracts against *An. stephensi*, *Ae. aegypti*, and *Cx. quinquefasciatus*. The benzene and ethyl acetate extracts of leaves of *E. coronaria* and *C. pulcherrima* show significant repellency against *An. stephensi*, *Ae. aegypti*, and *Cx. quinquefasciatus*. Traboulsi *et al*[44] showed that extracts of *Foeniculum vulgare* leaves were toxic against *Culex pipiens* larvae, and terpineol and 1,8-cineole were the most effective components in repellency tests. Omolo *et al*[45] evaluated six plant species growing in the Kenyan coast for repellency on the forearms of human volunteers against *Anopheles gambiae*. The results showed that some of these constituents from the different oils, such as R-pinene, limonene, γ-terpinene, and R-terpinene, showed high individual repellencies. In conclusion, an attempt has been made to evaluate the role of medicinal plant extracts for their repellent bioassay against *An. stephensi*, *Cx. quinquefasciatus*, *Ae. aegypti*. The results reported in this study open the possibility for further investigations of the efficacy of repellent properties of natural product extracts.

Conflict of interest statement

We declare that we have no conflict of interest.

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