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

M Govindarajan*, R Sivakumar

Division of Vector Biology and Phytochemistry, Department of Zoology, Annamalai University, Annamalai Nagar 608 002, Tamil Nadu, India

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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

*Corresponding author: M. Govindarajan, Principal Investigator, DST Fast Track Young Scientist Project & UGC Major Research Project, Division of Vector Biology and Phytochemistry, Department of Zoology, Annamalai University, Annamalai Nagar – 608 002 ,Tamil Nadu, India.
E-mail: drgovind1979@gmail.com
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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