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FULL LENGTH ARTICLE

Repellent properties of *Delonix elata* (L.) Gamble (Family: Fabaceae) against malaria vector *Anopheles stephensi* (Liston) (Diptera: Culicidae)

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Abstract Mosquito control is facing a threat because of the emergence of resistance to synthetic insecticides. Insecticides of botanical origin may serve as suitable alternative biocontrol techniques in the future. The purpose of the present study was to assess the effects of leaf and seed hexane, ethyl acetate, benzene, chloroform and methanol extract of *Delonix elata* on repellent activity against the malaria vector mosquito *Anopheles stephensi* (Diptera: Culicidae). Evaluation was carried out in a net cage (45 × 30 × 45 cm) containing 100 blood starved female mosquitoes of *An. stephensi*. Repellent activity was carried out in laboratory conditions. Plant crude extracts of *D. elata* were applied at 1.0, 2.5, and 5.0 mg/cm^2 separately in the exposed forearm of volunteers. Ethanol was used as the sole control. Applied leaf and seed crude extracts were observed to protect against mosquito bites. There were no allergic reactions experienced by the volunteer subjects. The repellent activity of the extract was dependent on the strength of the extract. Among the tested solvents, both the leaf and seed methanol extracts showed maximum efficacy. The highest concentration of 5.0 mg/cm^2 provided over 210 and 180 min protection for the leaf and seed extracts, respectively. Crude extracts of *D. elata* (leaf and seed) exhibit the potential for controlling *An. stephensi*.

1. Introduction

Malaria remains one of the most prevalent diseases in the tropical world. With 200–450 million infections annually worldwide, it causes up to 2.7 million deaths (WHO, 2010). In India, malaria is transmitted by six vector species, in which *Anopheles stephensi* is responsible in urban areas. It is endemic in all parts of India, and periodic epidemics of malaria occur every 5–7 years. Malaria continues to be a major public health problem in the tropical world. Of the total world population of about 5.4 billion people, 2200 million are exposed to malarial

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infections in some 90 countries or areas. The most recent estimates indicate that there may be 300–500 million clinical cases each year, with countries in tropical Africa accounting more than 90% of these. Malaria is also the cause of an estimated 1–4 to 2–6 million deaths worldwide every year, with more than 90% in Africa alone (Breman, 2001).

DEET, a synthetic mosquito repellent, is recognized as one of the few products effective against mosquitoes and biting flies. The efficacy of DEET in providing long-lasting protection against a wide variety of mosquito species has been documented in several studies (Fradin and Day, 2002). Although DEET is an effective repellent against mosquitoes, there are concerns associated with its use. Human toxicity has been reported with DEET, with symptoms varying from mild to severe (Briassoulis et al., 2001). It is irritating to mucous membranes, and concentrated formulations dissolve plastic. DEET may be unsafe for children possibly causing encephalopathy (Abdel-Rahman et al., 2001). Research regarding insect repellents derived from plant extracts is needed to find alternatives that are safer but still effective. Some plant species contain insecticidal or repellent substances. Some plant extracts, such as neem (*Azadirachta indica*), sweet basil (*Ocimum basilicum*), and lemon eucalyptus (*Corymbia citriodora*) have been studied as possible mosquito repellents and have demonstrated good efficacy against some mosquito species (Kirton, 2005).

Repellent activity of hexane, ethyl acetate, benzene, chloroform and methanol extract of *Cardiospermum halicacabum* was evaluated against *Cx. quinquefasciatus*, *Ae. aegypti* and *An. stephensi* (Govindarajan and Sivakumar, 2012). Seyoum et al. (2002) studied the repellency of live potted plants against *A. gambiae* from human baits in semi-field experimental huts. *Ocimum americanum*, *Lantana camara*, and *Lippia uckambensis* repelled at an average of 39.7%, 32.4%, and 33.3% of the mosquitoes, respectively. The combination of *O. americanum* with either *L. camara* or *L. uckambensis* repelled 31.6% and 45.2% of the mosquitoes, respectively. 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 *Cx. tritaeniorhynchus* and *An. subpictus* (Govindarajan, 2011). The larvicidal, ovicidal, and repellent activities of crude benzene and ethyl acetate extracts of leaves of *E. coronaria* and *C. pulcherrima* were assayed for their toxicity against three important vector mosquitoes, viz., *An. stephensi*, *Ae. aegypti*, and *Cx. quinquefasciatus* (Govindarajan et al., 2011a).

Delonix elata (Syn. *Poinciana elata*) commonly known as white gold mohur (Fabaceae) is used by folklore for joint pains and in flatulence. In Indochina, the bark is considered as febrifuge and antiperiodic. The leaf and bark in the form of paste are used by local people to reduce inflammation and pain. It has been used in traditional Indian medicine for the treatment of rheumatism and stomach disorders, and its leaves are used in the treatment of bronchitis and pneumonia in infants. Leaf extracts of *D. elata* are reported for strong anti-inflammatory activity (Sethuraman and Sulochana, 1986). As far as our literature survey could ascertain, no information was available on the repellent activity of the experimental plant species given here against malarial vector *An. stephensi*. Therefore, the aim of this study was to investigate the mosquito repellency of the different solvent extracts of *D. elata*. This is the first report on the mosquito repellent activity of the solvent extracts of leaf and seed of the selected plant.

2. Materials and methods

2.1. Collection and identification of plants

Fully developed leaves and seeds of *D. elata* (L.) Gamble. (Family: Fabaceae) were collected from different regions of Thanjavur District (between 9°50′ and 11°25′ of the north latitude and 78°45′ and 70°25′ of the east longitude), Tamilnadu, India. The plants are taxonomically identified by a taxonomist, Department of Botany and voucher specimens have been deposited in the plant Phytochemistry Unit, Department of Zoology, Annamalai University.

2.2. Preparation of plant extracts

The fully developed fresh leaves and seeds of the plant *D. elata* were washed with tap water and shade dried at room temperature. The dried leaves and seeds were powdered with the help of an electrical blender. The powdered leaf and seed material (1.0 kg) was then subjected to extraction in various solvents viz, hexane, benzene, chloroform, ethyl acetate and methanol (5.0 L) using soxhlet extraction apparatus for 8 h individually. The extract was filtered through a Buchner funnel with a Whatman number 1 filter paper. The filtrate was evaporated to dryness under reduced pressure using a rotary vacuum evaporator. The residue was then made into a 1% stock solution with ethanol. For the repellent activity a varied range of stock solutions (1.0, 2.5 and 5.0 mg/cm^2) was prepared by dissolving the residues in ethanol.

2.3. Laboratory colonization of mosquitoes

For the different bioassays an enormous amount of different stages of the mosquito colony is needed. The eggs of *An. stephensi* were obtained from the vector control laboratory, Department of Zoology, Annamalai University, Tamilnadu, India. The laboratory colony was maintained at 70–85% RH, 28 ± 2 °C temperature and a 12:12 light and dark photoperiod cycle. The larvae were fed on a powdered mixture of dog biscuits and yeast powder in 3:1 ratio. The adults were provided with 5% glucose solution and honey was given to male and females with one week old chicks for blood meal.

2.4. Repellent activity

The repellency of the plant crude extracts against *An. stephensi* was evaluated by using the percentage of protection in relation to the dose method (WHO, 2009). Three day old blood starved female *An. stephensi* mosquitoes (100) were kept on a net cage (45 × 30 × 45 cm). Two cages with hungry mosquitoes for test and control were kept aside. The volunteers have no contact with lotions, perfumes, oils or perfumed soaps on the day of the assay. The skin of the volunteers arms were washed and cleaned with ethanol. Ethanol served as control. After air drying the arms of the volunteers, only 25 cm^2 of skin on the dorsal side of each arm was exposed. The remaining area was covered by rubber gloves. The different concentrations of crude extracts were applied. The experiment was tested during the night from 19.00 to 05.00 h. The control and treated arm