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Mosquito repellent action of *Blumea lacera* (Asteraceae) against *Anopheles stephensi* and *Culex quinquefasciatus*

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ABSTRACT

Petroleum ether extract of *Blumea lacera* was screened under laboratory conditions for repellent activity against mosquito vector *Anopheles stephensi* Liston and *Culex quinquefasciatus* Say (Diptera: Culicidae). The repellent activity of *Blumea lacera* extract was tested against mosquitoes in comparison with the DEET, which was used as a positive control. Results obtained from the laboratory experiment showed that the extract was effective against mosquito vectors even at a low dose. A direct relationship was observed with concentrations of *Blumea lacera* extract and the repellent activity. Percent repellency obtained at 6% concentration of the extract against *An. stephensi* and *Cx. quinquefasciatus* were 97and 98% at 0 hour and 78.8 and 76.2% after 6 hrs. DEET-2% however showed 100% repellency against *An. stephensi* and against *Cx. quinquefasciatus* up to 4 hours and 1 hour, respectively. These results show that *Blumea lacera* extract has the potential as an effective mosquito repellent.

Keywords: *Blumea lacera*, Leaf extract, Mosquitoes Repellent

1. Introduction

The control of mosquitoes remains a challenge even after continuous use of synthetic insecticides in public health. The main technical reason is development of insecticide resistance in mosquito vectors and thus these mosquitoes continue to transmit vector borne diseases in endemic areas. *Anopheles stephensi* Liston is a major vector of urban malaria in India, while *Culex quinquefasciatus* Say transmits filariasis in coastal areas. As an alternative to the use of insecticides, mosquito repellents are convenient, inexpensive, and afford advantages in protection against a wide range of vector [1]. Repellents can be used by individuals for personal protection and thus help in prevention of the disease transmission [2]. They are also the primary means of mosquito-borne disease prevention available in areas, where vector control is not practical [3, 4]. The majority of commercial repellent products contain, a synthetic insect repellent N, N-Diethyl-3-methylbenzamide (*N*,*N*-Diethyl-*meta*-toluamide) commonly known as DEET [5, 6]. While some commercially formulated natural repellent products include promising botanicals, such as *citronella,* Lemon eucalyptus, neem, and peppermint oils [7-15]. However, the repellency effect of these natural products is still lower in both efficacy and duration than that of most commonly used chemical DEET. Nevertheless, the possible health risks associated with use chemical repellent should be taken in to consideration.

Keeping in mind that mosquitoes have developed resistance to insecticides and that plants are resources of active components for prevention of mosquito bite, the present study was carried out to investigate the repellent activity of the Indian medicinal plant, *Blumea lacera* against the mosquito vector species of malaria and filariasis, which may provide a new source of repellent to protect humans from the mosquito bites. *Blumea lacera* species is a medicinal plant commonly used for antipyretic, bronchitis, disease of blood, fever, disease of mouth. In homeopathic system, it is given in enuresis, neuralgia, and headache. Roy *et al.* [16] reported the use of *Blumea lacera* leaves extracts as botanical insecticides against lesser grain borer and Rice Weevil.

2. Materials and Methods

2.1. Extraction of *Blumea lacera*

Aerial parts were collected and washed with water, dried in shade and completely powdered. The powder material of this plant was subjected to extraction. One hundred grams powder of leaves was immersed in 500ml petroleum ether. After 24-hours it was stirred for one hour by magnetic stirrer and filtered through a filter paper. The extract was made solvent free and the final residue was dried in a desiccator and later reconstituted to prepare various dilutions.

2.2. Mosquito Strains

An. stephensi and *Cx. quinquefasciatus* used for the study were reared in a laboratory maintained at a constant temperature (27±2ºC) and relative humidity (70%) with a photoperiod of 12 hours (Light: Dark). Adult mosquitoes were provided with 10% sucrose. Prior to evaluation, one hundred 5-7 days old sugar fed female mosquitoes of the respective species, to be used for study, were kept in mosquito cages and starved for overnight. A minimum of three replicates were prepared for each mosquito species. Needed standardization was made for experiments as to the determination of the suitable age of the mosquito for experiments and method of recording the data [17].

2.3. Preparation of the Repellent and Control Replicates

Repellent surface were prepared in a six cm diameter plastic bowl. Sufficient quantity of cotton was taken to be stacked in to 250ml plastic bowl. Ten percent sugar solution was prepared in the water and 230 ml of the sugar solution was poured into the plastic bowl and the cotton was soaked. The cotton at the top was stretched out side in to circular form and covered with single layer of nylon net. Remaining 20 ml was used to prepare repellent formulation. To 20 ml of the sugar solution, required quantity of the extract concentrate was mixed to arrive at the desired concentrations, *viz*, 2, 4, and 6% and was poured evenly on the sugar soaked cotton in the above plastic bowl. Similarly DEET 2% in 10% sugar soaked cotton was prepared for use as positive control and only 10% sugar soaked cotton was used as negative control, respectively. Various test concentrations *viz*. 2, 4, and 6% were prepared in distilled water using freshly made stock solution. Tween-80 was used as an emulsifier at 0.05% concentration in the final test solution.

2.4. Repellency test

The repellency test was carried out in a room maintained at 27 ^{0}C and 70% relative humidity. The experimental cages with the mosquitoes were placed in this room. In these cages, the plastic bowl along with cotton soaked in three different concentrations of extract of *Blumea lacera viz* 2, 4, and 6% sugar solution, DEET 2% (positive control) in 10% sugar solution and 10% sugar solution (negative control) were placed in four different corners and one in the centre of the cage. Five-minute landing counts were made at each hour for six hours (0, 1, 2, 3, 4, 5, and 6 hours). The bowls were removed from the cage after the five minute observation at each interval of time. The bowl was covered to avoid evaporation of the insecticide formulation and was placed in the refrigerator. For subsequent exposure the position of the bowls were inter changed to different corners [17].

2.5. Data analysis

Observations for mosquito cage studies were made with at least three replicates for the given species of the mosquito. Landing rates of the mosquitoes on the treated bowls with different concentrations of the formulation of petroleum ether extract of *Blumea lacera* 2, 4, and 6%, DEET 2% and sugar (10%) was recorded. The data were recorded as mean of the observations for each of the formulation. Results were expressed as average landing and percent repellency per exposure interval compared to control using the following formula [11].

$$\text{Percent repellency} = \frac{\text{Control(C) - Treatment (T)}}{\text{Control (C)}} \times 100$$

Where, C= the mean number of landing on negative control (10% sugar solution); T= mean number of landing on the repellents (DEET and extract of *Blumea lacera*).

Table 1: Percent repellency of petroleum ether extract of leaf of *Blumea lacera* against important mosquito vectors.

Species	Doses (%)	Repellency in hours						
		0 hour	1 hour	2 hours	3 hours	4 hours	5 hours	6 hours
An. stephensi	2-Treat	84.6	85.3	84.6	75.0	75.2	72.2	72.2
	4-Treat	91.6	85.7	87.2	84.5	80.2	82.2	76.2
	6-Treat	97.0	95.7	89.7	86.5	82.8	82.8	78.8
	2- DEET	95.3	97.8	97.4	92.3	100	93.0	90.1
Cx. quinquefasciatus	2-Treat	85.3	90.5	84.4	75.8	66.7	66.7	66.7
	4-Treat	90.3	95.2	87.5	86.2	76.2	76.2	76.2
	6-Treat	98.0	97.6	90.6	86.2	84.2	80.2	76.2
	2- DEET	100.0	100.0	87.5	87.5	94.6	92.4	90.2

3. Results

The leaf extract of *Blumea lacera* showed strong repellent activity against adult mosquitoes. The results showed a highly significant difference between the mean number of mosquitoes landing on the treated and control surfaces (Table 1). The percent repellency at different observation periods (0 hour, 1 hour, 2 hours, 3 hours 4 hours, 5 hours and 6 hours) ranged from 66.7 to 98% against different concentrations. The repellency rates at 0 h varied between 84.6–98% at different concentrations of *Blumea lacera* extract. The extract showed 85.3-98% repellency at 2-6% in 0 h, and 66.7-76.2% after 6 h against *Cx. quinquefasciatus* and 84.6-97% in 0 h and 72.2-78.8% after 6h against *An. stephensi*. DEET- 2% showed 100% repellency against *An. stephensi* and *Cx. quinquefasciatus* up to 4 and 1 h, respectively. It is evident from the data that the overall repellency rates against two mosquito species. varied between from 84.6–98% at 0 h and 66.7-78.86.2% after 6 h at 2 to 6% concentrations of the extract.

4. Discussion

Leaf extract of *Blumea lacera* showed up to 98% repellency against *An. stephensi* and *Cx. quinquefasciatus* in laboratory bioassays. These results show that *Blumea lacera* leaf extract has the potential as an effective mosquito repellent. The efficacy of *Blumea lacera* leaf extract is comparable to that of currently used commercial repellent product DEET. Though DEET a synthetic chemical compounds provided better and longer protection, the possible health risks associated with the use of these chemicals should be taken in to consideration [6]. In recent years, trend for the use of natural repellent products is gaining importance and several botanicals have been tested for the repellent activity against mosquitoes [12-15]. Sharma *et al*. [18] reported the repellent effect of neem oil and showed 37.5% protection against *Cx. quinquefasciatus* with neem oil, whereas in the present study, leaf extract of *Blumea lacera* showed upto 98% repellency against the same species, but the efficacy declined after one hour. Most of the plant based repellents are shown to repel mosquitoes, but their effect lasts from few minutes to some hours. Their active ingredients tend to be highly volatile, so although they are effective repellents for a short period after application, they rapidly evaporate leaving the user unprotected [15]. PMD (para-menthane 3, 8 diol), a potent natural repellent extracted from the leaves of lemon eucalyptus *Corymbia citriodora* (Myrtaceae) trees is an exception, which is less volatile than monoterpeines found in most plant oils and provides very high protection from a broad range of insect vectors over several hours and it is the only plant-based repellent that has been advocated for use in disease endemic areas by the CDC (Centres for Disease Control) as it considered to pose no risk to human health [15]. More research is needed to develop new repellents from substance of natural original that can offer effective mosquito management to reduce the indiscriminate use of harmful chemical insecticide. Though the results of the present study showed the potential of *Blumea lacera* extracts mosquito repellent against *An. stephensi* and *Cx. quinquefasciatus* in laboratory bioassays, these results pertain to the effectiveness in cage experiments using only sugar solution as attractant and thus, confirmation by testing this repellent on human subjects to evaluate the repellency effect is needed and further studies should be undertaken against as many different vectors as possible under both laboratory and field conditions.

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