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Mosquito repellent activity of *Calotropis gigantea* (Apocynaceae) flower extracts against the filarial vector *Culex quinquefasciatus*

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ABSTRACT

Plan: *To assess the repellent efficacy of the flower extracts of Calotropis gigantea against Culex quinquefasciatus mosquito and to screen the bioactive compounds present in the flower extract.*
Methodology: *The flower extracts of Calotropis gigantea were extracted with petroleum ether, chloroform and ethanol and the efficacy of the extracts as repellent were assessed on three day blood starved female Culex quinquefasciatus mosquito. The repellent study was following the method of WHO (1996).*
Outcome: *The results suggested that flower ethanol extract of Calotropis gigantea showed a higher repellency on the adult of female Culex quinquefasciatus mosquito than the other two extracts. The repellent activity was found to be dose dependent and the percentage of protection was found to be directly proportional to the concentration of extract. The results of phytochemical screening showed the presence of bioactive compounds such as alkaloids, tannins, phenol, flavonoids, sterols, anthraquinone, proteins and quinones in the flower extract. It may be concluded from the result that ethanol extract of Calotropis gigantea flower was effective in mosquito vector control and has an excellent potential in controlling the mosquito.*
Keywords: *Culex quinquefasciatus, flower extracts, repellent activity, Calotropis gigantea*

1. INTRODUCTION

Mosquitoes were recognized as a health and nuisance problem only in the last century. They are carriers of number of vector born diseases, such as chikungunya, dengue fever, malaria, filariasis, yellow fever, etc[1]. The *Aedes aegypti*, *Anopheles stephensi* and *Culex quinquefasciatus* are the major urban vectors of dengue, malaria and lymphatic filariasis respectively. Thus, one of the approaches for control of these mosquito-borne diseases is the interruption of disease transmission by killing or preventing mosquitoes from biting human beings[2]. Much of the literature about mosquitoes provided by government agencies recommends regular use of a mosquito repellent, which is the chemical DEET. As there are many health and environmental problems associated with the usage of DEET, many people are looking for repellents based on other chemicals[3]. Repellency is known to play an important role in preventing the vector borne diseases by reducing man-vector contact.

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Synthetic chemicals and insecticides used for control of vectors are causing irreversible damage to the eco-system, as some of them are non-degradable in nature. Some repellents of synthetic origin may cause skin irritation and affect the dermis[4]. It has been reported that these chemical repellents are not safe for public use[5, 6].

Because of unpleasant smell, oily feeling to some users[7, 8] and potential toxicity[9, 10, 11] some prefers to use natural insect repellent products. Repellents of plant origin do not pose hazards of toxicity to human and domestic animals and are easily biodegradable. The natural products are safe for human when compared to that of synthetic compounds[12, 13].

Most plants contain compounds that they use in preventing attack from phytophagous (plant eating) insects. The primary functions of these compounds are defence against phytophagous insects, in which many of them are also effective against mosquitoes and other biting Dipterans, especially those volatile components released as a consequence of herbivory[14]. Insects detect odours, when that volatile odour binds to odorant receptor (OR) proteins displayed on ciliated dendrites of specialized odour receptor neurons (ORNs) that are exposed to the external environment, most often on the antennae and maxillary palps of the insect and some ORNs, such as OR83b that is very important in olfaction and blocked by the gold-standard synthetic repellent DEET (N, N-diethyl-3-methylbenzamide)[15], are highly conserved across insect species[16, 17].

Although vector control programs have been established for a long time, the main method for control of vectors is the use of chemical insecticides. The conventional chemical pesticides have resulted in the development of resistance, undesirable effects on non-target organism and fostered environmental and human health concerns. An alternative approach for mosquito control is the use of natural products of plant origin. Phytochemicals have proven that they are potential mosquito control agent and also alternative to synthetic insecticides[1]. In an effort to develop low cost plant-based household protection methods that can be used by communities with minimal external input, several plant species were recently evaluated in terms of their repellent properties[18, 19].

Herbal products with proven potential as repellents can play an important role in the interruption of the transmission of mosquito-borne diseases at the individual as well as at the community level[2]. Diligent investigations into such grassroots protection methods by the scientific community is leading to the development of new biorational, effective and affordable products as well as increasing knowledge and confidence in traditional protection methods and reducing vector-borne disease[20]. Therefore, it is the hour to launch extensive search to explore eco-friendly biological materials for control of insect pests.

2. MATERIALS AND METHODS

2. 1. Origin and laboratory maintenance of the mosquito colonies

Mosquitoes used in study were *Culex quinquefasciatus*. Individuals were reared for several generations in the Department of Zoology, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore by Hay infusion method under laboratory conditions.

Adult *Culex quinquefasciatus* mosquitoes were obtained from laboratory colony maintained at 28±2° C, 70% - 85% relative humidity with a photo period of 14:10 light and dark photo period cycle. Larvae were fed with dog biscuits and yeast powder in the ratio 3:1. Adults were provided with 10% sucrose solution and the three days blood starved female mosquitoes were used for repellent bioassay.

2. 2. Collection of test materials and preparation of flower powder

Fully developed fresh flowers of the plant *Calotropis gigantea* were collected from natural habitat of Coimbatore locale. Fresh flowers were collected, washed in water and left to shade dry at room temperature for 2 to 3 weeks and finely powdered separately using an electric pulverizer. These powders were subjected to extraction[21, 22]. Petroleum ether extraction was followed by chloroform and ethanol extraction in their increasing order of polarity. The flower extracts thus obtained were concentrated by distillation and dried by evaporation in a water bath. The residue thus obtained was used for further bioassays.

2. 3. Phytochemical Screening

The preliminary qualitative phytochemical analysis has been attempted in *Calotropis gigantea* flower extracts to find out the presence or absence of certain bioactive compounds. The preliminary screening was carried out by using standard procedures described by Sofowara[23], Trease and Evans[24] and Harbourne[21].

2. 4. Repellent Bioassay

The repellent study was following the method of WHO[25]. Three day old blood starved female *Cx. quinquefasciatus* (100) were kept in a net cage (45cm × 30 cm × 45 cm). The arms of the volunteer, only 25 cm^2 dorsal side of the skin on each arms was exposed and remaining area covered by rubber gloves. The extract was applied at 1.0, 2.5 and 5.0 mg/cm^2, separately in the exposed area of the forearm. The volunteer conducted their test by inserting the control and treated arms simultaneously into the same mosquito cage for one full minute for every five minutes. Mosquitoes that landed on the hand were recorded and then shaken off before imbibing any blood making out a 5 minute protection.

3. RESULTS AND DISCUSSION

In the present study, the repellent efficacy of petroleum ether, chloroform and ethanol extracts of the flower of *C.gigantea* were analysed against *C.quinquefasciatus* mosquito.

The data were recorded and were statistically analysed. The results of the skin repellent activities of *C.gigantea* flower extracts against the three days blood starved female *C.quinquefasciatus* mosquitoes were summarized in the Table.

The results revealed that the ethanol extract of *Calotropis gigantea* flower was found to be more repellent against C.*quinquefasciatus*.

A higher concentration of 5.0 mg/cm^2 provided 100% protection up to 150 minutes against C.*quinquefasciatus* (Fig: 1). The repellent activity was very high at the initial stage of exposure. The results of repellent activity of *C.gigantea* flower extracts were comparable with earlier reports. In accordance to the results of the present study similar observations were reported by Govindarajan *et al*[26] in which the methanol leaf extract of *Ervatamia coronaria* showed remarkable repellent properties at the higher concentration of 5.0 mg/cm^2 which provided 100% protection up to 150 minutes against C.*quinquefasciatus* mosquitoes.

Table 1: Repellent activity of *Calotropis gigantea* flower extracts against *Culex quinquefasciatus*

S.No	*Solvent used*	*Concentration mg/cm^2*	*% of repellency*					
			30 min	*60 min*	*90 min*	*120 min*	*150 min*	*180 min*
1	Control	-	0±0	0±0	0±0	0±0	0±0	0±0
2	Petroleum ether	1.0	94.33±0.47	94±0	94.33±0.47	93±0.0	93±0.81	93±0.0
		2.5	99.66±0.47	96.33±0.47	95±0.81	94.33±0.47	94±0.81	94±1.63
		5.0	100±0	97.33±0.47	96±0.0	95±0.81	95±0.0	95.33±0.47
3	Chloroform	1.0	94.33±0.47	94.33±0.47	94±0.81	93.66±0.47	93±0.0	92.66±0.47
		2.5	95.33±0.47	95±0.81	94.33±0.47	93.66±0.47	94±0.0	93.33±0.47
		5.0	95.66±0.47	96±0.81	96±0.81	94.33±0.47	95±0.81	94.33±0.47
4	Ethanol	1.0	100±0.0	100±0.0	100±0.0	99±0.81	97.66±0.47	96.66±0.47
		2.5	100±0.0	100±0.0	100±0.0	99.33±0.47	97.66±0.47	97.66±0.47
		5.0	100±0.0	100±0.0	100±0.0	100±0.0	99±0.81	98.33±0.47

Each value (χ ± SD) represents average of three values

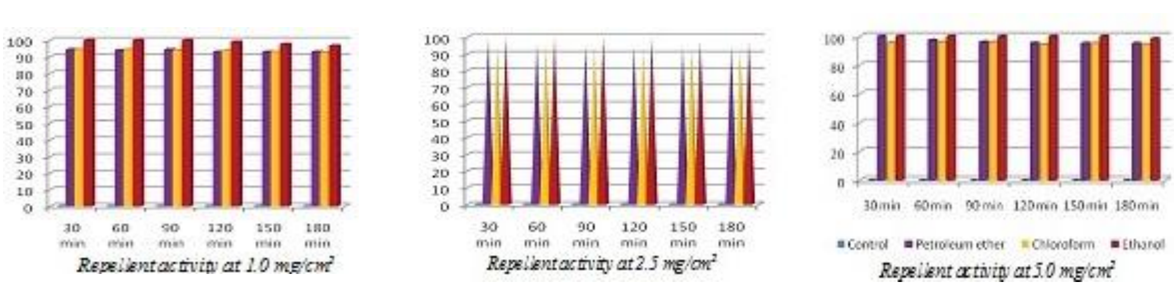

Fig 1: Graph showing repellent activity of Calotropis gigantea flower extracts against Culex quinquefasciatus

The repellent efficacy of ethanol flower extract was followed by petroleum ether flower extract which provided 100% protection up to 60 minutes at a higher concentration of 5.0 mg/cm^{2}. Increase in the exposure period showed reduction in the repellent activity. Govindarajan[27] reported similar findings from his study in which the crude extract of *Sida acuta* showed significant repellent activity against *C.quinquefasciatus*, at a higher concentration of 5.0 mg/ cm^{2} providing 100% protection up to 120 minutes.

In the present study the chloroform flower extract provided a protection of 95.66% at the higher concentration of 5.0 mg/cm^{2}. Dua *et al*[28] reported similar results in which *Lantana camara* flower extracts provided 94.5% protection against *Aedes albopictus* and *Aedes aegypti* mosquitoes. Swathi *et al*[29] reported that the ethanolic extract of *Pongamia pinnata* leaves provided complete protection of 99.96, 141.35 and 144.73 minutes against *A.aegypti, An.stephensi* and *C.quinquefasciatus.* The control treatment did not provide any protection even during the first trial.

The preliminary phytochemical screening was carried out in the highly active ethanol extract of *Calotropis gigantea* flower. The results showed the presence of phytochemical compounds of alkaloids, tannins, phenol, flavonoids, sterols, anthraquinone, proteins and quinones in the flower extract. Whereas terpenoids and saponins were found to be absent. Similar observations were reported by Sampathkumar and Ramakrishnan[30] in which phytochemical analysis of *Naringi crenulata* stem revealed the presence of phytochemical compounds such as carbohydrates, proteins, lipids, phenols, flavonoids, saponins, alkaloids, quinones, anthraquinones and terpenoids.

The increase in the concentrations of flower extract increased the mean protection time against the bites of C.*quinquefasciatus*. The skin-irritant potential test for all the concentrations indicated that the flower extract did not cause irritation to human skin. The results clearly showed that repellent activity depends upon the concentration of the extract and density of mosquito and is dose dependent. From the above results it can be stated that the plant based repellents can be used for the control of vector borne diseases which would replace the currently used synthetic repellents that cause many side effects.

In accordance with the results of the present study in which the repellent activity was dose dependent similar trend were reported in the work by Rajkumar and Jebanesan[31] in which both oviposition deterrent and skin repellent activity of *Solanum trilobatum* against the malaria vector *Anopheles stephensi* were dose dependent. Other studies done on essential oil from leaves of *Ocimum basilicum* by Prajapati *et al*[32] showed effective repellency of 82.4±0.7, 75.0±1.2 and 115.3±1.9 mg/mat against *Ae. aegypti, An. stephensi* and *C.quinquefasciatus.*

Earlier studies with petroleum ether extract of *Zanthoxylum limonella* fruits by Choochote *et al*[33] provided protection time of 296 minutes and 223.5 minutes against *Aedes albopictus* in mustard oil base and coconut oil base respectively. Mandal[34] evaluated the repellent activity of *Eucalyptus* and *Azadirachta indica* seed oil against filarial vector *C.quinquefasciatus* and reported that the test oil showed excellent repellent action against *C.quinquefasciatus*.

The *Azadirachta indica* seed oil provided 90.26% and 88.83% protection, and the Eucalyptus oil provided a protection of 93.37% and 92.04%, at concentrations 50% and 100% (v/v), respectively, with the protection time up to 240 minutes. There was no bite within 120 minutes and 180 minutes, respectively, due to the action of Eucalyptus and *Azadirachta indica* seed oil.

4. CONCLUSION

Results of our study indicate that the flower extracts of *C. gigantea* have higher repellent efficacy against the vector *C.quinquefasciatus*. Further characterization and isolation of bioactive molecules from the extracts of *C. gigantea* flower will provide further clarity about the nature of these bioactive compounds which could become an alternative to the conventional insecticides used for repelling annoying mosquito species. However toxicity tests of the flower extract did not caused any irritation to human skin which ascertained the safety in its usage. To summarize, *C.gigantea* flower showed good mosquito repellent activity in all the performed tests.

Hence, *C.gigantea* flower, alone or in combinations with those obtained from other mosquito repellent plant species, could be potentially used for the preparation of mosquito repellent products. Such formulations could help in reducing the harmful effects of synthetic mosquito repellents on human health. It may be concluded from the result that medicinal plants can be used alone or combined for effective protection against mosquito bites and also can be used for control of mosquito breeding under integrated disease vector control programme in various situations. They also offer safer alternative to synthetic chemicals and can be easily obtained by individuals and communities at a very low cost.

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