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Full Length Case Report

REPELLENT ACTIVITY OF *CALOTROPIS GIGANTEA* LEAF EXTRACT AGAINST MOSQUITOE *AEDES A EGYPTI*

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ABSTRACT

The results of the repellent activity of *C. gigantea* at three different extracts (methanol, ethanol and acetone) and three different concentration of 1.0, 2.5 and 5.0 mg/cm2 were applied on skin of fore arm in man and exposed against 3 to 4 days old unfed adult female Ae. aegypti mosquito. In this observation the *C. gigantea* extract gave protection against mosquito bites without showing any allergic to the test person and also the repellent activity dependent on the strength of the lowest concentration extract of 1.0 mg/cm2 against *Ae.aegypti* for the exposure period of 2.0 hours and 30 minutes.

Key words: Aedes aegypti (L.), Calotropis Gigantea, Repellent Activity.

INTRODUCTION

Aedes aegypti (L.) 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 continue to increase, especially with more severe forms of the disease, dengue haemorrhagic fever and dengue shock syndrome, or with unusual manifestations such as central nervous system involvement (Hendarto and Hadinegoro 1992, Pancharoen *et al*., 2002). There are a variety of control measures that can be employed to reduce the disease risk to humans and animals from these insects. It may be directed against the immature or adult stages of mosquitoes. However, it is undoubtedly the best method of protecting the community against the diseases (Sharma *et al*., 1989). (Kumar, 1984). The demand for more food and adequate maintenance of public and animal health will not permit significant elimination of broadspectrum synthetic pesticides a problem that has led to an increased interest in the discovery of new chemicals. The organic, synthetic insecticides are more hazardous to handle, leave toxic residues in food products, not easily biodegradable, besides their influence on the environment is deleterious. Unlike synthetic that kill both pests and predators outright the natural insecticides are relatively inactive against the later. Conventional synthetic insecticides require special safety procedures and equipment during production and application despite precautions, exposure to humans, the environment (Franzen, 1993) and food (FAO, 1992). The synthetic insecticides are expensive and have in many cases only produced moderate results along with major ecological damage (Franzen, 1993). The botanical insecticides are generally pest-specific and are relatively harmless to non-target organisms including man. They are also biodegradable and harmless to the environment (Jacobson, 1975). Furthermore, unlike conventional insecticides which are based on a single active ingredient, plant derived insecticides comprise an array of chemical compounds which act concertedly on both behavioral and physiological processes. Thus the chances of pests developing resistance to such substances are less likely (Saxena, 1987). One plant species may possess substances with a wide range of activities, for example extracts from the the neem tree *Azadirachtaindica* are antifeedant, antioviposition, repellent and growth-regulating

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(Rembold,1994;.Schmutterrer, 1995). In contrast, the low toxicity of botanical insecticides makes processing and application of the product inexpensive. In many cases, the materials are locally available and affordable (Childs *et al*., 2001). The supply of natural insecticides could be made continuous at a cheaper rate by regular cultivation. The objectives of the present study were Collection and identification of Calotropis gigantea (L.)Extraction of the leaves *Calotropis gigantea* (L.) with different solvents viz., methanol ethanol and acetone by soxhlet apparatus. Testing the repellent activity of crude extract of *Calotropis gigantea* (L.) against *Ae.aegypti*.

MATERIALS AND METHODS

***Collection of plant materials*:** The plant Calotropis gigantea (L.) T. Aiton, (Asclepiadaceae) was collected from Annamalai University Campus, Annamalainagar, Chidambaram (11:24 N lat. and 79°: 5E long; 5.79 above MSL) Tamilnadu, India. The vernacular name of the plant is Eruku. The plant was taxonomically identified at the Department of Botany, Annamalai University and voucher specimen was deposited at the Department of Zoology, Annamalai University.

Preparation of plant extract: The leaves of Calotropis gigantea were carefully examined and old, insect-damaged, fungus infected leaves, twigs and flowers were removed. Healthy leaves were washed with tap water and shade dried at room temperature (28 + 2°C) for 5-8 days or until they broke easily by hand. Once completely dry, leaf material (1.0 kg) was ground to a fine powder using electrical blender. Three literéthanol, methanol, acetone and petroleum ether separately was used for the extraction of 1.0 kg in the Soxhlet apparatus followed by the standard procedure (Vogel, 1978). The plant material was loaded in the inner tube of the Soxhlet apparatus and then fitted into a round bottomed flask containing ethanol. The solvent was boiled gently (40°C) over a heating mantle using the adjustable rheostat. The extraction was continued until complete extraction was effected (8 hrs.) and the solvent was removed at the reduced pressure with the help of rotary vaccum evaporator to yield a viscous dark green residue (12.5 g) of each solvent of ethanol, methanol and acetone leaf extracts.

Laboratory colonization of Aedes aegypti: The eggs of *Ae. aegypti* procured from Vector Control Research Centre (VCRC) at Puducherry, India. The mosquito colony maintained at 70-85% RH, 28 + 2°C temperature and 14:10 light and dark photoperiod cycle The larvae were fed on powdered mixture of dog biscuits and yeast tablets in 3:1 ratio. The blood meal was given to the female adult mosquitoes and 5.0% glucose solution and honey were given to the male adult mosquitoes.

Testing for repellent activity: The repellent activity was determined by the percentage protection time in relation to dose method (WHO, 1996). Three to four days old blood-starved 100 adult female of Ae. aegypti mosquito were kept in a net cages (45 x 30 x 45 cm2). The arms of the test person were cleaned with isopropanol. After air drying the arm of the test person, only 25 cm2 dorsal side of the skin on each arm was exposed and the remaining area being covered by rubber gloves. The plant extract was dissolved in isopropanol and this alcohol served as control. The leaves of Calotropis gigantea plant extract of 1.0, 2.5, 5.0 mg/cm2 concentration was applied. The control and treated arms were Introduced simultaneously into the cage, The first bit was noted to 5 minutes for every 30 minuites, from 20:00 h for Cxqninquefasciatus and 08:00 to 18:00 h for Ar. Aegypti. The experiment was conducted for five times. It was observed that there was no skin irritation from the plant extract (Plate 1). The percentage protection was calculated by using following formula.

$$\% \text{ Protection} = \frac{\text{No.of bites by control} - \text{No.of bites received by treated}}{\text{No.of bites received by control}} x\ 100$$

RESULTS

Repellent activity of extract of *C. gigantea* against dengue vector *Ae. aegypti* in laboratory condition: The results of the repellent activity of *C. gigantea* at three different extracts (methanol, ethanol and acetone) and three different concentration of 1.0, 2.5 and 5.0 mg/cm2 were applied on skin of fore arm in man and exposed against 3 to 4 days old unfed adult female *Ae. aegypti* mosquito. In this observation the *C. gigantea* extract gave protection against mosquito bites without showing any allergic to the test person and also the repellent activity dependent on the strength of the lowest concentration extract of 1.0 mg/cm2 against *Ae.aegypti* for the exposure period of 2.0 hours and 30 minutes.

Acetonic extract: The repellent activity acetonic extract of *C. gigantea* at different concentration 1.0, 2.5 and 5.0 mg/cm2 on the fore arm of the volunteer against *Ae.aegypti* is presented in Table 5.12.Fig 1. In this observation that the highest protection of 100 percent was recorded up to 2.0 hours and 30 minutes of exposure period at the concentration of 5.0 mg/cm2 against *Ae.aegypti*, whereas the lowest repellent activity was exerted by the lowest concentration extract of 1.0 mg/cm2 against *Ae.aegypti* for the exposure period of 1.0 hours and 30 minutes.

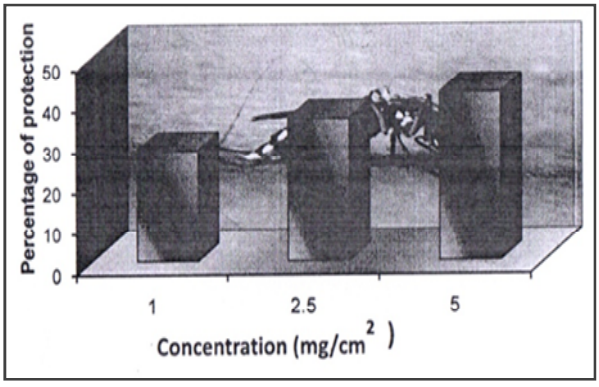

Figure 1. Repellent activity of acetonic extrct. *C.* gigantea against dengue vactor Ae. *Aegypti*

Table 1. Repellent activity of acetonic leaf extract of C. gigantea against dengue vactor Ae. Aegypti

Concentration of Plant extract (mg/cm^2)	No. of mosquito bites		Total % of protection for 12 hours	Mean number of 100% protection (hours)
	Control	Treated		
1.0	68.64 ± 2.64	50.00 ± 3.05	27.15	1.30
2.5	66.52 ± 4.62	43.30 ± 2.06	34.90	2.0
5.0	72.00 ± 2.28	42.38 ± 4.8	41.13	2.30

Values are mean of six replication standard ±error

DISCUSSION

There is a renewed interest in the use of natural products to control destructive insects and vectors of diseases due to the prevalent occurrence of vector resistance to synthetic insecticides and the problem of toxic nonbiodegradable residues contaminating the environment and adversely affecting nontarget organisms. More than 2000 plant species are already known to have insecticide properties (Sukumar *et al*., 1991). Humans have used plant parts, products, and metabolites in pest control since early historical times. Plants are the chemical factories of nature, producing many chemicals, some of which have medicinal and pesticidal properties. By using plant parts in early historical times and plant extracts and concentrated components in more recent times, man has been able to control certain pests with these remedies quite successfully. Repellency is known to play an important role in preventing the vector borne disease by reducing man-vector contact. Insect repellent properly applied to the skin and clothing of individuals are on inexpensive and practical means of reducing the biting activity of hematophagous arthropods and for the prevention of arthropod borne disease transmission. At present, in India mosquito repellents for domestic use are mainly available in the form of coils, vapourizing mats and liquid sprays containing chlorinated hydrocarbons and organophosphates with potential health hazards. Also by their continuous use, mosquito has developed resistance against these chemicals. Majority of commercial repellents are prepared by using chemicals like allethrin, N-N-diethyl-m-toluamide (DEET), dimethyl phthalate (DMP) and N,N-diethyl mendelic acid amide (DEM). It has been reported that these chemical repellents are not safe for public use (Zadikoff, 1979 and Ronald, 1985). Natural products are safe for human when compared to synthetic compounds (Sharma *et al*., 1993 and Sharma and Ansari, 1994). Ansari *et al.* (1999) reported that the essential oil extract by steam distillaion of *Mentha piperita* and *Dalbargiasisoo* provided 84.5 to 100% Protection against *Cx.quinquefasciatus* and *An.culicifacies*. Tyagi et al. (1994) reported the high degree of repellency (>90% protection for 2h and >50% up to 4 hour) was observed in the essential oil extract of Tagetesminuta against *An.stephensi*, *Culex quinquefasciatus* and *Ae.aegypti*. Protection for 8 hours was achieved by the application of formulation containing 1% oil fraction of A.sativum in bees wax (89:10) at a dose of fatigans used (Bhuyan*et al.*, 1974). Das and Ansari (2003) carried out a field study to evaluate the mosquito repellent action of *CymbopoganmartiniiStapf* var sofia oil 10ml provided 98.7% protection in indoor and 96.52% in outdoor conditions during 12h period of observation from the bite of An. Sundaicus. Hadis, (2003) reported that the repellent activity of essential oils in field study of lemon eucalyptus (*Eucalyptus maculata*), rue (Rutachalepensis), Oleoresin of Pyrethrum (*Chrysanthemum cinerariaefolium*) and neem (*Azadiractaindica*) at 50% concentration provided highest repelleny was recorded. The protection was 91.6% for rue, 87.0% for neem, 96.0% for pyrethrum and 97.9% for DEET.

In this present study, An insect repellent of plant origin ought to be well defined and harmless to human and other non-target organisms. Therefore, use of these botanical insecticides could reduce the cost and environmental effects. The results of the preliminary screening of laboratory evaluation of repellent activity of five plant essential oils confirmed their broad spectrum mosquito repellent properties. Further studies on identification of active compounds toxicity and active fraction of these plant essential oils for development of eco-friendly chemicals and indigenous plant base oil for protection against bites of haematophagous insects needed for commercial exploitation.

CONCLUSION

This study reveals that the extract of *C.gigantea* has remarkable repellent properties. The flora of India has rich aromatic plant diversity with potential for development of natural insecticides for control of mosquito and other pests. These results could encourage the search for new active natural compounds offering an alternative to synthetic repellents and insecticides from other medicinal plants.

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