

Prevention of Dengue fever through plant based mosquito repellent *Clausena dentata* (Willd.) M. Roem (Family: Rutaceae) essential oil against *Aedes aegypti* L. (Diptera: Culicidae) mosquito

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Abstract. – Background: Plant based repellent against mosquito borne diseases are used recently because synthetic repellents cause side effects like breathing problem, eye irritation, head ache, cough, etc. The use of natural products for dengue control would protect the environment, reduce dependence on expensive synthetic repellents and also generate local employment.

Material and Methods: Essential oil was isolated by steam distillation which was used against the bites of *Aedes aegypti* and duration of protection period was assessed. Skin-irritant potential test was also conducted on 25 healthy volunteers by using four-point scale.

Results: The increase in the concentrations of essential oil increased the mean protection time against the bites of *Aedes aegypti*. The lowest mean protection time was 180.0 min for 2.5% and highest time of 255.0 min for 10%. The mean score of zero for skin-irritant potential test for all the concentrations indicated that the essential oil did not cause irritation to human skin.

Conclusions: Results indicated that the use of plant based repellent for the control of dengue fever would replace the currently used synthetic repellents which causes many side effects.

Key Words:

Dengue, Essential oil, Repellent, Skin-irritant potential.

spicuous nuisance pests as well. They are potential vectors of malaria, filariasis, yellow fever, brain fever, dengue fever, etc¹. Among them, the dengue fever is the most common mosquito-borne viral disease of humans world wide with an estimated 50 million infections occurring in tropical and subtropical regions each year². In the absence of vaccine, dengue prevention and control mainly through prevention of human vector contact by repellents. The use of repellents already has been accepted as part of an overall integrated mosquito-borne disease control program³. Currently a variety of repellents are marketed in India, which mainly contain synthetic pyrethroids and N,N-diethyl-m-tolnamide (DEET) as a active ingredient. The protection provided by these repellents generally lasts for 2 to 4h and also cause side effects like breathing problem, eye irritation, headache, cough, asthma, etc⁴.

Therefore, it is time to search for effective alternatives to synthetic repellents. In many parts of the world, plant derived natural products have traditionally been used as repellents against insects bites⁵, in particular essential oils, have been investigated and described as potentially natural sources of insect repellents⁶. Essential oils are natural volatile substances obtained from non-woody parts of the plant, particularly leaves, through steam or hydrodistillation. In this study, we report the skin irritant potential test and skin repellent activity of the leaf essential oil of *Clausena dentata* against day bite mosquito *Aedes aegypti*. *Clausena dentata* is a small tree plant that is widely spread in South India. It is used by peoples of Tamil Nadu for its medicinal and nutritive values.

Introduction

Mosquitoes are still the world's number one vector of human and animal diseases and are con-

Materials and Methods

Collection of Plant Material

Fresh leaves of *Clausena dentata* were collected from three years old tree in natural forest of Sirumalai hills, Tamil Nadu, India.

Isolation of Essential Oil

The essential oil was isolated by steam-distillation using a clavenger apparatus for six hour; moisture in the oil was removed over anhydrous sodium sulphate and stored at 5°C for further experimentation.

Mosquito

The vector of dengue fever *Aedes aegypti* was used in the tests. The species was obtained from a laboratory colony maintained at 12L: 12D photoperiod, 70±10% RH and 26±2°C in an insectary in the Zoology Department, A.V.C College, India.

Repellent Activity

Duration of protection period against bite of *Aedes aegypti* was evaluated in essential oil of *Clausena dentata* by using the method of Fradin and Day⁷. In this test, 25 disease-free, laboratory-reared, unfed female mosquitoes that were between 7 and 10 days old were placed into separate cages (45×38×38 cm). An undressed skin area of 30 cm² located on the forearm of a human volunteer was treated either with 100 µl of ethanol (solvent control) or with oil diluted in ethanol (2.5, 5, 7.5 and 10% V/V). The rest was covered by a paper sleeve. The volunteer inserts his or her treated or control arm for 2min every 30 min into a mosquito cage. Test was carried out either two bites occurred in a single 2-min exposure period or one bite occurs in each of two consecutive exposure periods, at this point test was terminated. The entire tests were carried out in daytime. The duration period between the application of a repellent and the first two bites or two bites in successive observation was recorded as the protection period. Each test solution and control were repeated six times in separate cages, and in each replicate, different volunteers were used to nullify any effect of skin differences on repellency.

Skin-Irritant Potential Test

Skin-irritant potential of *Clausena dentata* oil (10, 25, 50 and 100%) was evaluated in 25 male and female healthy volunteers aged between 18

and 50 years, who showed no signs of dermatological diseases. A piece of filter paper embedded (100 µl/cm²) either in different concentrations of essential oil or in a ethanol (70% V/V) was applied on the skin of antecubital arm and tightly covered by a surgical tape. Following a 4-h period of exposure, the occlusive patches were removed, and the sites of application were gently washed with water. The skin was examined and scored at 24, 48 and 72h after patch removal. Skin reactions were scored, as suggested by Basketter et al⁸, by using a four point scale: (0) absence of reaction; (+) weakly positive reaction, i.e. mild redness or dryness across most of the treatment site; (++) moderately positive reaction, i.e. a more intense redness generally spreading beyond the treatment site; and (+++) strongly positive reaction, i.e. strong redness generally accompanied by edema. A volunteer with a “+” or greater reaction at any of the assessments was considered to have shown a positive skin-irritant reaction.

Ethical Requirements

All volunteers who took part either in the skin-irritant potential test or in the mosquito repellency test signed an informed consent form after having received a full explanation of the test objectives procedures and foreseeable risks to subjects. The Ethics Committee of the Annamalai University approved the repellent and skin-irritant tests protocols.

Statistical Analysis

The significance of difference between the protections times of different concentrations was determined by Tukey's test.

Results

The main objective of our work is to prevent man-mosquito contact by using plant based natural repellents instead of commercially available electronic liquidator, mosquito coil and skin lotion which contain synthetic compounds. The yield of essential oil obtained by steam distillation of leaves of *Clausena dentata* was 10 ml/kg. Observation on appearance of essential oil showed no colour and a pleasant odour. The mean protection period of *Clausena dentata* essential oil at different concentrations against bite of *Aedes aegypti* are summarized in Table I.

Table I. Protection period of *Clausena dentata* essential oil against female *Aedes aegypti*.

Concentration % (v/v)	Protection Time (Minutes)
2.5	180 ± 6.7 ^a
5.0	200 ± 8.9 ^b
7.5	215 ± 10.5 ^c
10.0	255 ± 11.7 ^d
Ethanol (70% v/v)	0.0 ± 0 ^e

Each value (mean ± SEM) represents mean of six replicates values. Different letters in a column indicate significant differences in protection period at $p < 0.05$ level (Tukey's Test).

Ethanol was used as diluent of essential oil and acts as a control. In laboratory cage tests, maximum protection time of 255.0 min was observed at higher concentration of 10% where as the lower concentration of 2.5% gave 180.0 min protection against mosquito bite. The in between concentrations of 5 and 7.5% showed 200.0 and 215.0 min protection, respectively. However, the ethanol-applied arm did not provide at least 30 min protection against the bite of *Aedes aegypti* mosquito. The mean protection period of different concentrations and control significant at 0.05 levels. The above results clearly indicate that the protection period directly proportional to concentration of essential oil and also ethanol in essential oil not influence the protection period of essential oil.

The skin-irritant potential test results given in Table II. The 25 volunteers who took part in the skin-irritant potential test did not show positive irritant (slight, moderate and severe) reactions to essential oil of *Clausena dentata* at concentra-

Table II. Skin irritant potential test of *Clausena dentata* essential oil.

Concentration % (v/v)	No of individuals	Scoring for skin irritation			
		0	+	++	+++
10	25	25	0	0	0
25	25	25	0	0	0
50	25	25	0	0	0
100	25	25	0	0	0
Ethanol (70%v/v)	25	25	0	0	0

0 absence of irritation, + slight irritation, ++ moderate irritation, +++ severe irritation.

tions of 10, 25, 50 and 100%. The foregoing results thus suggest that topical application of essential oil was not irritant to human skin and do not cause dermatitis. The results also suggest that the organic solvent ethanol used in dilution of essential oil in protection period test did not show positive irritant reaction. The results of both protection period and skin-irritant tests recommend essential oil could be used as natural repellent that prevent man-dengue vector contact.

Discussion

Today, insect vector management has to face ecological as well as economic costs. Synthetic repellents such as DEET and pyrethroid containing formulations are the most commonly sold in supermarkets. The side effects of these two ingredients in repellent reported by many researchers^{9,10}. In fact, plant based repellent not only minimizes the use of synthetic chemicals but also protects environment, health of users and save huge amount of money spent on chemical compounds¹¹. In this way, the results obtained suggest that the leaf essential oil of *Clausena dentata* are promising as repellent against *Aedes aegypti*. Further more, the crude essential oils are more effective compared to the individual compounds, due to natural synergism that discourages the development of resistance in the vectors¹². The people do not need an expert's advice on using plant products as people of India are already using them in various forms like fumigants, live potted plants, etc against bite of mosquitoes. Now a day's plant essential oils used in flavouring, pharmaceuticals, confectionary and are considered non-toxic to humans¹³. A large number of different plant species representing different geographic areas around the world, which encourage natural product based mosquito abatement practices. Such practices would generate local employment, reduce dependence on expensive imported products and stimulate local efforts to enhance public health¹⁴. The most promising botanical groups are Meliaceae, Rutaceae, Asteraceae, Annonaceae, Labitae and Malvaceae. Some of them are characteristics as aromatic plants¹⁵. Consequently, these plant extracts including essential oil have a great potential for vector management.

In the present study, the experimental plant *Clausena dentata* belongs to the Rutaceae family.

Based on the present results, the protection period of *Clausena dentata* is well comparable with our previous studies^{16,17}. Side effects of ethanol used as topical application are not reported in our study. It is mostly used in commercially available body spray and do not cause any irritation to the skin. Hence we select ethanol for dilution of essential oil. The plant *Clausena dentata* is distributed in most part of the country, the repellent properties of this plant species can be well utilized while planning alternate vector control strategies based on integrated vector control measures through community-based approaches. Further studies on preparation of formulations based on creams, polymer mixtures or microcapsules thereby prolong the repellent effect of essential oil is going on in our laboratory.

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