



Mosquito repellent activity of chandana and sarshapa essential oil

Gazala Hussain^{1*}, Vinaykumar R Kadibagil², A Jebanesan³

¹ Associate Professor, Department of Rasashastra and Bhaishajya Kalpana, Sri Dharmasthala Manjunatheshwara College of Ayurveda and Hospital, Hassan, Karnataka, India

² Professor, Department of Rasashastra and Bhaishajya Kalpana, Sri Dharmasthala Manjunatheshwara College of Ayurveda and Hospital, Hassan, Karnataka, India

³ Professor, Vector Biology Laboratory, Department of Zoology, Annamalai University, Tamil Nadu, India

Abstract

Mosquitoes are vectors of various diseases. To keep them away the convenient option are repellents. The repellents minimize the contact with mosquitoes. The popular and commonly used mosquito repellent is DEET (N, N-diethyl-m-methylbenzamide). Though not much toxicity studies are reported of the usage of DEET, botanicals are always a safer option. Various crude drugs and methods are employed for repellent actions. In treatises of Ayurveda too, one can come across references of plants or compounds of various herbs used as repellants. One such combination is of *chandana* (*Santalum album*) and *sarshapa* (*Brassica campestris*). The essential oils of these two drugs have proved to have mosquito repellent action against *Aedes aegypti*.

The study was conducted by Cone bioassay and Cage Bioassay methods. The repellency activity of *Santalum album* and *Brassica campestris* oil were tested at 0.5%, 1.0 %, 2.5 % and 5.0 % against 3-4 days old, blood starved female mosquito of *Ae. aegypti*. The synergistic activity for the essential oil, 7 hours knock down effect was noticed at the concentration of 5 %. Thus plant extracts could be a safer alternative as mosquito repellants.

Keywords: mosquito repellent, herbs, *chandana*, *sarshapa*, essential oil

Introduction

Mosquitoes are considered the most dangerous animal in the world because of their ability to transmit numerous diseases, organisms which pose a serious threat to human and animal health [1]. The World Health Organization blames global warming for the expanding range of mosquitoes that are responsible for malaria, yellow fever and dengue fever, causing millions at risk. It is estimated by WHO that nearly 15,000 deaths per year at all ages occur only in the Indian Peninsula [2]. Arboviruses and parasites are transmitted to humans from arthropods, and mosquitoes are the most common arthropods associated with dengue, malaria, and yellow fever. Enormous efforts have been made to develop effective repellents against arthropods, and thus far synthetic repellents have been widely used. However, the use of synthetic repellents has raised several concerns in terms of environmental and human health risks and safety [3]. Personal repellents, applied to the skin or clothing, can provide protection from mosquitoes and other biting arthropods. The gold standard insect repellent for the past 55 years is DEET (N, N-diethyl-3-methylbenzamide). DEET provides good repellency against many mosquito species but has been criticized for its odor and skin feel and the fact that it is a plasticizer; in addition, some incidences of toxicity have been reported. Although the number of confirmed reports of DEET toxicity is extremely small compared with the number of people who use the compound, a portion of the population chooses to avoid products containing DEET, thereby exposing them to risk for mosquito-vector disease [4]. Plant-based repellents have been used for generations in traditional practice as a personal protection measure against

host-seeking mosquitoes. Knowledge on traditional repellent plants obtained through ethnobotanical studies is a valuable resource for the development of new natural products. Essential oils are natural volatile substances found in a variety of plants. Commercially, essential oils are used in four primary ways: as pharmaceuticals, as flavor enhancers in many food products, as odorants in fragrances, and as insecticides. Ayurveda treatise also has various references of combinations of herbs that are said to be effective against insects. *Santalum album* and *Brassica campestris* is one such combination which are said to be effective against microbes. The effect of using plant herbs has their own advantage in giving antimicrobial effect.

Materials and Methods

Preparation of Essential oil (*Santalum album* and *Brassica campestris*)

Santalum album barks and *Brassica campestris* (250 g) were crushed with distilled water. Then, the plant material was placed in a 5L bottom flask and submitted to hydro distillation during 3 h using a Clevenger –type apparatus. In the end, the essential oil was collected and stored at 4° C for further utilization.

Cage Bioassay

The protection time was tested by the method described by (Schreck and McGovern 1989; WHO 1996). The repellency of the *Santalum album* oil against dengue vectors was evaluated using the human bait technique. For investigation, 100 laboratories reared, blood starved, adult female *Aedes aegypti* that were between 5-7 days old were placed into

separate laboratory cages (45cm X 45 cm X 45 cm). The hand was washed with tap water dried with towel and then the essential oil was applied on the external surface of the first of human hand over an area of about 150 cm² at the rate of 1 mg/cm². The treated surface was exposed to 100 non blood fed female mosquitoes (5-7 days old) in the cage. Tests were conducted during 06:00 hr to 06:00 hr in the light room at 27±2° C and 70±5% RH. Each evaluator the number of bites was counted over 3 minutes every 30 minute biting counts and the experiments were performed in triplicate (n=6). DEET (N, N- diethyl-3-methylbenzamide) was used as standard. Tests were repeated at interval of 30 min and the experiment were completed when 5 mosquitoes had bitten. The repellent activity of DEET (N, N- diethyl-3-methylbenzamide) used as standard. The number of insects landing or biting was recorded for two volunteers (WHO, 1996).

The percentage protections were calculated by using the following formula

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

Cone bioassay

Cone tests were conducted following WHO guidelines. The bioassay was performed according the WHOPES guidelines. The set-up was prepared by cutting 25 cm× 25 cm piece of netting. Four plastic cones were attached to each piece of net and held together with two binder clips. The assembly was held at one of the test angles; 0°. Using an aspirator, five to eight non blood-fed females aged two to five days were introduced into each cone and the holes were plugged by pieces of cotton.

The mosquitoes were exposed for 3 min and subsequently transferred into labeled 150 ml plastic holding cups and provided with 10 % sugar solution. Knockdown and mortality were recorded every 1 hour and 24 hr after exposure, respectively. Mosquitoes were scored as alive if they were able to fly, irrespective of the number of legs still intact and dead, or knocked down, if immobile or incapable of flying or standing in a coordinated manner.

An untreated net was used as control and was tested each day the bioassay was performed. The bioassays were performed at temperature and relative humidity ranges of 25.9-29° C and 58-73%, respectively.

Bioassay for knocking- down and killing effects (Mat and Liquidizer)

The bioassay for knocking- down and killing effects was conducted by modifying the method of Jantan *et al.*, (2005). It was performed in a glass chamber measuring 120 x 120 x 60 cm at front and sides and wooden lamination with white colored interior at top bottom and backside. Sliding glass doors (60 x 30) were provided at both sides of the chamber. An electric plug point was provided at the interior back of the chamber. Mosquitoes (100) from the mosquito colony were introduced into this chamber through the sliding window at one side. The liquid mosquito insecticide oil/extract formulation (LMIEF) was allowed to vaporize for 2 min by using the vaporizer equipment provided with commercial liquid mosquito insecticide formulation. Knock down mosquitoes (i.e., those that no longer maintained

normal posture and were unable to fly or were on their backs) were recorded at 1 minutes intervals up to 12 hours or until total knock down was achieved. Knock – down mosquitoes were placed in a clean container containing cotton wool soaked with 5 % sucrose solution and the mortality of the mosquitoes was observed after 24 hr. Six replicate of the experiments were carried out for the each liquid mosquito formulation. One control was performed by exposing the mosquitoes to the vapour of the solvent (deodorized kerosene). A commercial reference standard liquid mosquito insecticide formulation (allethrin 3.6 % w/w) was also tested for comparison. Knock-down times KD50 and KD 90, as the minutes needed to Knock-down 50% and 90% mosquitoes, respectively) were determined by the probit analysis (Finney, 1971).

Data Analysis

$$\text{Mortality \%} = \frac{\text{Test mortality \%} - \text{Control mortality \%}}{100 - \text{Control mortality}}$$

The values of effective dose KD50 were obtained through Probit Analysis and by use of SPSS 16. Software and the values were drawn from six replicates. Effectiveness of the test oils was determined by comparing the 95% confidence intervals of the KD50 values.

Results

Cone Bioassay for Synergistic activity of Knock down effect of *Santalum album* and *Brassica campestris* essential oil

In the present study, the knockdown effect occurred at 0.5, 1.0, 2.5 and 5.0 % for *Santalum album* and *Brassica campestris* of essential oil synergistic activity for 12 hrs of exposure showed effective knockdown activity against *Ae. aegypti* mosquito. The statistical data are presented in Table 1. The highest concentration of 5.0 % provided cent percent (100%) protection up to 7 hours. At 0.5 % and 1.0 % concentration the knockdown was observed for 2 hours and at 2.5 % concentration. The knock down effect was observed for 3 hours.

The lower concentration of 0.5% provided cent percent protection upto 2 hours the total percentage protection observed up to seven hours exposure time increasing in exposure hours there is a reduction of knock down effect was noticed as 96.00, 88.02, 74.02, 64.00, 60.06, 55.00, 42.08, 30.06, 24.02, and 17.06 for 3, 4, 5, 6, 7, 8, 9, 10, 11, and 12 hours exposure period respectively.

The concentration of 1.0 % provided cent percent protection up to 2 hours. The total percentage protection observed up to seven hours exposure time increasing in exposure hours there is a reduction of knock down effect was noticed as 84.08, 70.00, 65.00, 59.06, 46.08, 40.00, 32.06, 27.33, 20.00 and 12.08 for 3, 4, 5, 6, 7, 8, 9, 10, 11, and 12 hours of exposure period respectively.

The concentration of 2.5 % provided cent percent protection up to 4 hours the total percentage protection observed upto seven hours exposure time increasing in exposure hours there is a reduction of knock down effect was noticed as 86.00, 72.06, 60.00, 56.02, 42.00, 36.08, 29.04, 18.06 and 9.01 for 4, 5, 6, 7, 8, 9, 10, 11, and 12 hours exposure period respectively. The higher concentration of 5.0 % provided cent percent protection up to 7 hours the total percentage protection observed up to seven hours exposure time

increasing in exposure hours there is a reduction of knock down effect was noticed as 40.00, 36.04, 29.03, 20.00 and

11.02 for 8, 9, 10, 11, and 12 hours exposure period respectively.

Table 1: Synergistic knockdown effect for the 12 hr exposure of essential oil *Santalum album* and *Brassica campestris* against *Aedes aegypti*

Name of the sample	Mortality (%)												
	% Con.	1hr	2hr	3hr	4hr	5hr	6hr	7hr	8hr	9hr	10hr	11hr	12hr
Essential oil <i>Santalum album</i> + <i>Brassica campestris</i>	0.5	100 ± 0.0	100 ± 0.0	96.00 ± 1.22	88.02 ± 1.3	74.02 ± 2.44	64.00 ± 2.35	60.06 ± 2.44	55.00 ± 0.34	42.08 ± 0.68	30.06 ± 2.42	24.02 ± 2.07	17.06 ± 2.23
	1.0	100 ± 0.0	100 ± 0	84.8 ± 2.73	70.00 ± 0.59	65.00 ± 1.26	59.06 ± 2.32	46.08 ± 0.69	40.00 ± 2.0	32.06 ± 2.07	27.33 ± 1.22	20.00 ± 0.00	12.08 ± 0.32
	2.5	100 ± 0.0	100 ± 0	100 ± 0.0	86.00 ± 2.73	72.06 ± 2.30	60.00 ± 1.26	56.02 ± 0.34	42.00 ± 1.4	36.08 ± 0.68	29.04 ± 1.83	18.06 ± 1.51	9.01 ± 0.21
	5.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	40.00 ± 2.72	36.04 ± 0.59	29.03 ± 2.44	20.00 ± 2.34	11.02 ± 0.59

Values are mean of six replicates ± SE

*Significant at p<0.001 level.

Cage Bioassay

Results of the synergistic repellent activity of essential oil *Santalum album* and *Brassica campestris* against *Ae. aegypti* mosquito. In terms of corrected mortality as defined therein, 100% mortality within 24 hours was observed against *Ae. aegypti*. Maximum of 100% protection time (7 hours) was obtained at the concentration of 5.0 mg/cm² the statistical data are presented in Table 3.

The statistically significant (1.0 mg/cm², X² = 0.709; 2.0 mg/cm², X² = 0.576; 3.0 mg/cm², X² = 0.792; 4.0 mg/cm², X² = 0.974 and 5.0 mg/cm², X² = 0.169, p < 0.001) between treated and control groups.

The investigations resulted in 6-7 hr of complete protection time with 5.0 mg/cm² of oil and extract applied on the arms

of humans volunteers. The essential oil and plant extract of Sandalwood oil and Mustard oil shows significant repellency against *Ae. aegypti* adults. It shows that repellency depends on the strength of essential oil and extracts concentration. A higher concentration of 5.0 mg/cm² provided 100% protection up to 7 hr. The essential oil of *Tagetes minuta*, providing a repellency of 90% protection for 2 hr was observed by Tyagi *et al.* (1994) and also reported that the repellent activity of DEET (N, N-diethyl-3-methylbenzamide) 23.8% ingredient provided protection for an average of 360 min. Although DEET has been claimed to be safe for use against biting insects for over 40 year it may still pose a risk to human health (Osimitz and Grothaus, 1995).

Table 2: Synergistic repellent activity of essential oil *Santalum album* and *Brassica campestris* against *Aedes aegypti*

Concentration of essential oil (mg/cm ²)	Mean number of bites ± SE		Mean number of 100% protection (hours)	Total % of protection for 12 hours	X ² (df =2)
	Control	Treated			
1.0	86.50 ± 2.39	42.83 ± 2.22	1.00	30.02	0.709
2.0	76.00 ± 2.05	35.62 ± 2.29	2.00	58.4.2	0.576
3.0	69.40 ± 2.21	24.00 ± 2.20	4.00	69.19	0.792
4.0	67.00 ± 2.88	10.00 ± 1.06	6.00	88.09	0.974
5.0	64.66 ± 1.26	0.0 ± 0.0	7.00	100.0	0.169

Values are mean of six replicates ± SE

*Significant at p<0.001 level.

Therefore, it is necessary to look for and find a better repellent, which could provide a safer and long-lasting protection against arthropod bites. The essential oil of *Santalum album* and *Brassica campestris* has exerted promising and remarkable repellent activity against *Ae. aegypti* adults. The present studies revealed the percentage protection in relation to time (hr). The results shows that

essential oils provide repellent activity against *Ae. aegypti* female mosquito and it's giving protection against the vector transmitted diseases. During the experimentation, essential oil did not show any adverse effects such as skin irritation, rashes, discomfort and other allergic reactions to the volunteer.

Table 3: Knocking down (KD) and killing effect of vaporizing mat with *Santalum album* and *Brassica campestris* plant essential oils against *Ae. aegypti*

Formulation	KD50 (minutes)	KD90 (minutes)	Mortality (%)
Control	26.00	52.00	7.00 (15.34)
1 % w/w	4.02	9.16	39.00 (44.43)
2 % w/w	3.17	8.67	72.00 (69.35)
3 % w/w	1.97	3.87	80.00 (69.77)

(Mortality (%) – values in parentheses are arcsine transformed)

The results showed that, the lower value of KD50 and KD90 of 1.97 and 3.87 minutes were observed with *Aedes aegypti* at 3 % w/w concentration. Highest mortality of 80.00 percent was observed at 3 % w/w whereas control recorded

only 7.00 % mortality. It could be inferred that the knockdown effect (KD50) and (KD90) and killing effects of vaporizing mats were lower than that of LMIEF liquid of *Santalum album* and *Brassica campestris*. This study

reveals that the essential oil of *Santalum album* and *Brassica campestris* cone assay have repellent properties. The flora of India has rich aromatic plant diversity with potential for development of natural product insecticides for control of *Ae. aegypti* mosquito. These results could encourage the search for new active natural compounds offering an alternative to synthetic repellents.

Conclusion

In the cone bioassay, the synergistic repellency of *Santalum album* and *Brassica campestris* essential oil was tested against *Ae. aegypti*. The synergistic repellency activity of *Santalum album* and *Brassica campestris* essential oil were tested at 0.5%, 1.0 %, 2.5 % and 5.0 % and the density of 100 mosquitoes against 3-4 days old, blood starved female mosquito of *Ae. aegypti*. Among the four concentration tested the higher concentration 5.0% gave better Knock down effect. The essential oil gave protection upto seven hours in dose method and effective Knock down effect up to seven hours. The synergistic repellency of *Santalum album* and *Brassica campestris* essential oil was tested against *Ae. aegypti* was evaluated by using the percentage of protection in relation to dose method. The repellency activity of these were tested at five different concentration (1.0, 2.0, 3.0, 4.0 and 5.0 mg/cm²) and at the density of 100 mosquitoes against 3-4 days old, blood starved female *Ae. aegypti*. The skin repellency activity of *Santalum album* and *Brassica campestris* essential oil gave maximum protection for 7.00 hours at the concentration of 5.0 mg/cm². The repellent activity was very high at the initial stage (h) of exposure. Increase in the exposure period showed reduction in repellent activity. In the present study, electric vaporizing mats were prepared from the two selected plants by impregnating the mats with *Santalum album* and *Brassica campestris* oils at three different concentrations viz; 1 %, 2 %, and 3 % w/w and their effect was tested against mosquito vector in term of knocking down and killing percentage. The *Santalum album* and *Brassica campestris* essential oil exhibited highest knocking down (KD50 1.97; KD50 3.87 minutes) and killing mortality 80 % effect was observed at 3 % concentration on *Aedes aegypti* mosquitoes. The maximum knock down effect of LMIEF was observed within a period of 2.51 minutes for the KD50 and 4.30 minutes for *Aedes aegypti* at 5% w/w concentration whereas the control recorded 25 minutes for KD50 and 52.00 minutes for KD90 against *Aedes aegypti*.

It was also observed that LMIEF showed a significant mortality of mosquitoes in all the three concentration tested. The maximum mortality of 86% was recorded 5 % concentration of liquid insecticide against *Aedes aegypti*, whereas control could record only 9.31 %.

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