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Efficacy of botanical repellents against *Aedes aegypti*

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Abstract

A laboratory study was conducted to evaluate the efficacy of repellency of *Hyptis suaveolens* and *Ocimum gratissimum* against the adults of *Aedes aegypti*. The methanol extract of *H. suaveolens* at 0.03, 0.05, 0.075 and 0.1 mg/cm² offered 50.00, 66.67, 71.43 and 75.00 percent protection against the mosquito bite. The percent protection against the mosquito bite was 33.33, 50.00, 57.14, 60.00 and at 0.03, 0.05, 0.075 and 0.1mg/cm². Synergistic action was observed when the plant extracts were mixed in equal quantity and the percent protection was extended up to 83.33. Mosquito coil made with the extracts of *H. suaveolens* and *O. gratissimum* provided 75.31 and 70.37 percent protection from mosquito bite. Combined use of plant extracts increased the percent protection than with individual plant extracts.

Keywords: *Hyptis suaveolens*, *Ocimum gratissimum*, *Aedes aegypti*, Percent Protection, Synergistic Action.

1. Introduction

In terms of disease transmission and public health importance mosquitoes are considered as a very important group of insects. In tropical and subtropical countries, mosquito borne diseases are main problem [20]. Major cause for the chikungunya and dengue is *A. aegypti* that act as a vector for the disease and affect the 2.5 million people every year [12]. Most important reason for the increase of dengue fever are increased breeding places for the *Aedes* mosquitoes, less effective control of mosquito, more urbanization and enhanced growth of population [24]. The phytochemicals derived from plant resources can act as larvicides, insect growth regulator, repellents and ovipositional deterrent [15]. In southern India, leaves of *Vitex negundo* are burned to repel mosquitoes from houses [8]. Fumigants evaporated using heat or mosquito coils containing the plant may repel and kill mosquitoes. In general, chemical or natural compounds can produce a range of insect responses, contact, irritancy, spatial repellency, knock-down and toxicity [26].

Plants like *O. gratissimum*, *Clausena dentate*, *Eclipta prostrate*, *Tagetes erecta* have been reported to possess strong repellent activity against mosquitoes [17, 16, 9]. Repellents have been used as personal protection against mosquitoes for decades. Plant derived essential oils are considered non-toxic alternative insect repellents for humans [28, 13]. Mosquito coils made from dried plants and combustible materials such as sawdust are also a cheap and often an effective method of repelling mosquitoes. They are probably derived from the incense used in religious ceremonies by Hindus, Buddhists, and the followers of Confucius. In Java today, the same incense used in ceremonies to honor ancestors is also used on a daily basis to repel mosquitoes [21]. A laboratory study was conducted to evaluate the efficacy of repellency of *H. suaveolens* and *O. gratissimum* against the adults of *A. aegypti* to avoid the adverse effect of synthetic repellents on the safety of user and inexpensive indigenous molecule.

2. Materials and Methods

2.1 Stock Culture of *A. aegypti*

The eggs of *A. aegypti* were collected from National Institute of Communicable Diseases Centre, Coimbatore, Tamilnadu. The eggs were transferred to 24 x 18 x 4 cm size enamel coated tray containing 500 ml of water and maintained in the laboratory at 27±2° C and 85 % of relative humidity. Freshly hatched larvae were maintained in the tray and fed with the stock

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solution of dog biscuit and yeast at 3:1 ratio. Second, third and fourth instars larvae fed with the same powder till the larvae entered in to pupation. The pupae were collected from the culture tray and transferred to the glass beaker and kept in a mosquito cage (50 x 50x 50 cm) for adult emergence. The cage was made up of wooden frame and covered with fine mosquito net. The bottom of cage was fitted with strong card board. The cage door was fitted with muslin cloth to avoid escapes of adults. The adults were maintained at the laboratory condition 27±2° C and 75 to 85 % relative humidity, under 14 L:10 D photoperiod cycle. The adults were fed with 10% sugar solution for a period of three days before they were provided an animal for blood feeding. The adult females were fed with the blood of chick. Ovitrap were placed inside the mosquito cage to allow them to lay eggs.

2.2 Preparation of botanical extracts

The leaves of *O. gratissimum* and *H. suaveolens* were washed in tap water and shade dried. Dried leaves were grinded with the help of mixer grinder. 100 gm of finely grinded powder of respective plants extracted with 300 ml of methanol with the help of soxhlet apparatus [29]. The extracted liquid was subjected to rotary evaporation in order to remove the chemicals. The dried residues dissolved in ethanol to prepare the stock solution. From the stock solution different concentrations of test solutions (50 to 800 ppm) were prepared as recommended by WHO [30].

2.3 Bioassay for repellence

The repellent bio assay was conducted by adopting the standard procedure of WHO [30]. Evaluation of repellent property of methanolic extracts of *H. suaveolens* and *O. gratissimum* was carried out against the adults of *A. aegypti*. Twenty, 3 to 4 day old starved adult females were introduced into the chamber with one side nylon net opening. The arms of test person were cleaned with isopropanol. After drying under electric fan, only 25 cm³ of the dorsal side of the skin was exposed by wearing rubber hand gloves. The exposed area was treated with the plant extracts at 25, 50, 75 and 100 percent concentration. After drying of test solutions, both treated and untreated hands were introduced in to the mosquito cage. The untreated was treated with ethanol as control. The experiment duration was maintained for 30 minutes. Observations made on the mosquitoes landed and bitten on the exposed area were recorded. The percentage protection was calculated by the following formula

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

2.4 Bioassay for smoke toxicity

The mosquito coils were prepared using *H. suaveolens* and *O. gratissimum* leaves by following method of Saini *et. al.* [19] with minor modifications by using 4 gm of coconut shell and charcoal powder as burning material. These ingredients were thoroughly mixed with distilled water to form a semisolid paste. A mosquito coil (1.6 cm thickness and 4 cm height) was prepared manually and shade dried. The control treatment coils were prepared without the addition of plant extracts. The experiment was conducted in a glass chamber (140 X 120 X

60 cm). A window measuring 60 X 30 cm was placed at mid bottom of one side of the chamber. Three to four day old starved 100 adult female mosquitoes were introduced from the stock culture. A belly shaven pigeon was kept inside the cage in immobilized condition. The ignited coil was placed inside the chamber and allowed to emit smoke. A control chamber with mosquitoes was also maintained. Experiments repeated five times. Observations made on the mortality and landed number of mosquitoes were recorded.

3. Results and Discussions

Repellency activity of *H. suaveolens* is presented in Table - 1. The methanol extract of *H. suaveolens* at 0.03, 0.05, 0.075 and 0.1 mg/cm² were tested for its repellency against *A. aegypti*. At the concentration of 0.03 mg/cm² *H. suaveolens* offered 50 percent protection for 6 hours against the mosquito bite. 66.67 and 71.43 percent protection was made by 0.05 and 0.075 mg/cm² dose.

Table 1: Repellent activity of *Hyptis suaveolens* against *Aedes aegypti*

Treatment (mg/cm ²)	% Protection after		
	4 Hours	5 Hours	6 Hours
0.03	33.33	40.00	50.00
0.05	40.00	50.00	66.67
0.075	50.00	60.00	71.43
0.1	60.00	66.67	75.00

The percent repellency was extended up to 6 hours with 75 percent mosquito bite protection when the arms were treated at 0.1 m/cm². 33.33 percent repellency was registered for *O. gratissimum* when treated at 0.03 mg/cm² concentration against mosquito bite (Table-2).

Table 2: Repellent activity of *Ocimum gratissimum* against *Aedes aegypti*

Treatment (mg/cm ²)	% Protection after		
	4 Hours	5 Hours	6 Hours
0.03	0.00	20.00	33.33
0.05	25.00	33.33	50.00
0.075	42.86	50.00	57.14
0.1	50.00	57.14	60.00

The protection was extended upto 6 hours. The percent protection against the mosquito bite was 50.00, 57.14 and 60.00 at 0.05, 0.075 and 0.1mg/cm². These observations comparable with the earlier research findings. Plants like *O. gratissimum*, *Clausena dentate*, *Eclipta prostrate*, *Tagetes erecta* have been reported to possess strong repellent activity against mosquitoes [16, 9]. The repellent activities were found to be in the order *Cytopogen nardus* and *Eucalyptus globules* (100%) > *O. sanctum* (97.94%) > *S. aromaticum* (95.81%) > *C. sinensis* (93.75%) > *C. longa* (89.56%) > *V. negundo* (85.44%) > *A. indica* (81.25%) [18]. Repellent activity of *Adansonia digitata* extracted with a different solvent was studied against *Anopheles stephensi* and methanol extract was reported the most effective and provided 100% protections for 210 minutes [11]. Recently increasing efforts are being made to find and develop repellents derived from plant extracts [28, 7]. In the present study synergistic repellent action was observed when mix the methanolic extracts of *H. suaveolens* and *O. gratissimum* (Table-3). The percent protection ranged

between 57.14 to 83.33 when treated with the extracts ranged from 0.03 to 0.1 mg/cm² concentration. The percent protection was significantly higher than the extracts of *H. suaveolens* and *O. gratissimum* individually used against the mosquitoes.

Table 3: Synergistic repellent activity of *Hyptis suaveolens* and *Ocimum gratissimum* against *Aedes aegypti*

Treatment (mg/cm ²)	% Protection after		
	4 Hours	5 Hours	6 Hours
0.03+0.03	40.00	42.86	57.14
0.05+0.05	50.00	60.00	71.43
0.075+0.075	57.14	62.50	66.67
0.1+0.1	66.67	71.43	83.33

Various plant extracts, such as neem (*Azadirachta indica* A.Juss.), basil oil (*O. basilicum* L., *O. basilicum* L., *Fa. Citratum* Bach, *O. gratissimum* L., *O. americanum* L., *O. tenuiflorum* L.) citronella grass (*Cymbopogon nardus* Rendle), galingale (*Alpinia galangal* L.) clove (*Syzygium aromaticum* L.) and thyme (*Thymus vulgaris* L.) have been recorded as mosquito repellents^[25,23,6,27,5,3].

The synergistic effect of mixing of *A. indica* and *P. pinnata* oils is well exhibited against adult females of *A. aegypti*^[14]. 4 mg concentration of ethanol extracts of stilt root and bark of *R. mucronata* showed maximum protection of 100 and 97.5% up to 10 hours^[1]. 6 to 8 hour complete protection from *C. tritaeniorhynchus* was achieved, when treated with the petroleum ether extracts of *Vitex negundo* at 1.5 and 2.0 mg/cm² concentration^[10]. The oil of *Dalbergia sissoo* showed strong repellent action when 1 ml oil was applied on exposed parts of human volunteers. They were protected from mosquito bites for 8 to 11 hours. The protection (91.6±2%) obtained with sissoo oil was comparable to that with commercial mylol oil (93.8±1.2%) consisting of di-butyl and dimethyl phthalates^[2].

3.1 Smoke toxicity

Smoke toxicity effect of *H. suaveolens* and *O. gratissimum* are presented in Table - 4. Mosquito coil incorporated with *H. suaveolens* provided 75.31 percent protection than the control treatment coil. The percent protection was 70.37 when treated with *O. gratissimum* coil. When both the plant extracts with the mosquito coil, the percent protection was increased upto 77.78%. The commercial good knight mosquito coil offered superior protection than the plant extracts added coil (85.19%).

Table 4: Smoke toxicity of *Hyptis suaveolens* and *Ocimum gratissimum* against *Aedes aegypti*

Treatment (mg/cm ²)	% Protection
<i>H. suaveolens</i>	75.31
<i>O. gratissimum</i>	70.37
<i>H. suaveolens</i> + <i>O. gratissimum</i>	77.78
Goodknight coil	85.19

Alkaloid compounds may be used as repellents by burning plant material, either on a fire or in a mosquito coil to create an insecticidal smoke that repels the insects through direct toxicity. Alkaloids are found in large quantities in many members of the berberidaceae, fabaceae, solanaceae, and ranunculaceae families, all of which are used extensively as

traditional insect repellents^[4]. The remarkable repellent properties of *M. piperita* essential oil were established against adult's of *A. aegypti*. The application of oil resulted in 100% protection till 150 min. After next 30 min, only 1-2 bites were recorded as compared with 8-9 bites on the control arm^[22].

4. Conclusion

The present study inferred that the extracts of *Hyptis suaveolens* and *Ocimum gratissimum* protected the treated from mosquito bite. The higher concentration of methanolic extracts offered 75 percent protection up to 6 hours. The percent protection was increased when the extracts were mixed in equal volume.

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