



## Efficacy of Leaves of *Lantana Camara* as Mosquito Repellent

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### ABSTRACT

There are so many diseases which are spread by mosquitoes and for their control aspects there are numbers of products which are available. To overcome the harmful effects of commercially available mosquito repellent formulations, herbal formulations can help in developing eco friendly and cheaper alternatives. With this view in mind *Lantana camera* leaf powder was utilized to make incense sticks and used to evaluate mosquito repellence by smoke toxicity test and compared with commercial product. The test revealed that the control could show that the control (commercial product) 100% mortality in just 2 minute and took 30 minutes for complete burning. It created some side effects like headea, watery eye and coughing in some member. The experimental product took 40 minutes for complete burning with 100% mortality with any side effect. The cost of experimental product just 25% of commercial product. The presence of different phytochemicals in different solvent extract corroborates the insecticidal activity. Antibacterial activity was also found against three test organism *Escherichia coli*, *Pseudomonas sp.* and *B.megaterium*. This result may be use for conformation and development drugs.

### INTRODUCTION

Mosquitoes are the major public health problem throughout the world. Among the 3492 species of mosquitoes recorded worldwide, more than a hundred species are capable of transmitting various diseases in human and other vertebrates [1]. Mosquitoes transmit malaria, dengue fever, yellow fever, filariasis, and chikungunya to humans [2]. Mosquitoes are tiny blood-sucking insects that depend on standing water to reproduce. Female mosquitoes must feed on blood to lay eggs. They feed by piercing their mouthparts

into the skin of an animal and sucks blood rapidly. More often, they carry viruses that can be transmitted to a person while they are feeding [3].

Environmental conditions like temperature and moisture are where the length of mosquito life cycle between species depends. Nonetheless, the life cycle of all mosquitoes consists of the egg, larval, pupal and adult stages. Male mosquitoes feed on the nectar of the plants only, while females extract the blood of the hosts in order to develop and nourish eggs. Nearly all mosquitoes lay their eggs directly into water and others lay their eggs just near but not in the water. Within 24 to 48 hours, the eggs will hatch into larvae. The larvae will soon grow approximately 5mm in length. They breathe through siphon or air tubes. The larger ones can be seen floating just above the surface of waters that are infested. Within a span of seven to 10 days, the larvae will enter the stage of a pupa. Pupae are also visible upon the surface of the breeding site. When a mosquito is fully developed, it will emerge from its pupal case and will become a mosquito. The new adult, at this time, will stand upon the water to dry its wings and prepare for its flight. The female mosquitoes will then seek an animal to suck on and feed. They are capable of flying for miles and lay over 100 eggs at a time. Larvae and pupae ordinarily cannot survive without water. If a water source evaporates while they are still in these stages, they will die [4].

Several approaches were extensively used to control the threat posed by these mosquitoes. Over the years, synthetic insecticides were introduced. But, although these are effective, the insects tend to develop resistance to such products [5]. Aside from being

costly, the use of these repellents also generates problems such as environmental pollution and has toxic side effect on humans [6]. This creates a need for other alternative methods which are efficient, economical and environmentally safe. Botanical derivatives can materialize as a possible larvicides for the *Aedes aegypti* or the common household mosquitoes [7].

Plants, being a natural source of various compounds are known to contain larvicidal agents, which may act in combination or independently [8]. Phytochemicals act as general toxicants both against the adult as well as larval stages of mosquitoes, while others interfere with the growth and development, reproduction, produce olfactory stimuli action as a repellent. Natural products are best option because they are less harmful to environment and non-target organisms. Several extracts and compounds from different plants families have been evaluated for new and promising larvicides [9].

*Lantana camara* Linn (*Verbenaceae*) is also known as wide sage or lantana weed. It is a hefty extensive evergreen shrub which can grow up to 3 m in height and has a strong scent. It is a perpetual shrub found growing up to 2000 m altitude in tropical, subtropical and climate parts of the world. Different parts of this plant have been used conventionally for numerous illnesses through the world. The leaves of this plant were used as an antibacterial and antihypertensive agent, roots for the treatment of malaria, rheumatism, and skin rashes [10]. Extract from the leaves of *Lantana camara* possess larvicidal activity while extract from flowers of the plant showed repellent activity against adult mosquitoes [11].

### Scientific classification

- Kingdom : Plantae
- Order : Lamiales
- Family : Verbenaceae
- Genus : *Lantana*
- Species : *camara*

### OBJECTIVES

- To produce an effective and natural mosquito coil/incense stick using *L. camara* leaves.
- To find out if the product is an alternative to already available mosquito coil/stick in the market.

- To demonstrate the significant difference between experimental product and the commercial product in terms of effectiveness.

## 2. Materials and methods:

### 2.1 Collection of plant material

#### Collection of leaves

*Lantana camara* leaves were collected during month of October (2017), from rural area near Uka Tarsadia University, Bardoli. The Leaves were washed in running tap water, air dried in shade and then homogenized to make fine powder. This powder was packed in air tight polythene bags until further use.

**Fig: 1 Collection of leaves**



### 2.2: Phytochemical Analysis:

#### 2.2.1 Qualitative Analysis:

##### 2.2.1.1: Alkaloids

Crude extract was mixed with 2ml of 1% HCl and heated gently. A Mayer's And Wagner's reagent was then added to the mixture. Turbidity of the resulting precipitate was taken as evidence for the presence of alkaloids [12].

##### 2.2.1.2: Glycosides

Crude extract was mixed with each of 2ml of chloroform and 2ml of acetic acid. The mixture was cooled in ice. Carefully concentrated H<sub>2</sub>SO<sub>4</sub> was

added. A colour change from violet to blue to green indicated the presence of steroidal nucleus, glycine portion of glycoside [12].

### 2.2.1.3: Flavonoid

When dilute sodium hydroxide was added to 0.2 ml of extract it created intense yellow colour, On addition of HCl it turned colourless which suggests the presence of flavonoids [13].

### 2.2.1.4: Carbohaydrate

1 ml of distilled water and 5-8 drop of fehling's solution was added to 0.5 ml of plant extract and allowed to 60°C for 15 min. Formation brick red precipitate indicated the presence of carbohaydrate.

### 2.2.1.5: Tannins

Crude extract was mixed with 2ml of 2% solution of FeCl<sub>3</sub>. A blue-green or black coloration indicated the presence of tannins [13].

## 2.3 Preparation of incense sticks

The incense sticks were prepared using the powder, binding agent and burning agent at Mahavir Agarbatti Works at Vyara.

## 2.4 Mosquito repellence test

### 2.4.1 Mosquito Larvae Collection

The mosquito larvae and pupae were collected from Uka Tarsadia University campus. The mosquito larvae were maintained at room temperature. The larvae were fed with Bagasse.

### 2.4.2 Microscopic examination larva

Larva sample was taken with the help of a dropper and placed on a slide. It was observed under 10x in compound microscope.

### 2.4.3 Smoke toxicity test

Smoke toxicity test was conducted in a chamber measuring 34.5x24x29.5 cm. Then adult mosquitoes were released in to the chamber and they were exposed to the smoke of burning incense stick for 45 min. The mortality data were recorded after every 15min. The smoke toxicity was compared with the commercially available citronella incense stick as tested above [14].

### 2.4.4 Evaluation of burning on users

For investigating mosquito repellent activity the prepared incense sticks were checked for its burning time with respect to flammability. Further its causal effect such as irritation, coughing, tears were

observed and recorded. Mosquito repellence test was done by simply selecting the mosquito prone areas in the evening and night period. The public remarks were noted down after the test [14].

## 2.5 Larvicidal activity

### 2.5.1 Preparation of plant extracts:

The prepared powder was put in each of water, petroleum ether, chloroform, and ethyl acetate solvents (plant material to solvent ratio was 1:10, w/v) and extracted for 24h at room temperature with shaking at 150 rpm. The extracts were filtered and dried at 40°C. The dried extracts were resuspended in 1 ml of acetone.

### 2.5.2 Biological assay:

Prepare different concentration of extract in distilled water. Every experimental exposure was done in petri-plate. Twenty-five (25) larvae were collected with a pasture pipette, placed on a filter paper for removal of excess of water and placed in 25 ml tap water containing various concentration of crude extracts. Control test was done in 25 ml distilled water with 25 larvae. The petri-plates were covered with muslin cloth to avoid entry of any foreign material. Sufficient control was also kept for each extracts. The observed mortality was recorded at 24 h of period exposure to test solution. From this crude mortality, if any, was recorded and percentage crude mortality was obtained [15].

$$\text{Percentage of mortality} = 1 - \frac{\text{population in treated plot after treatment}}{\text{population in control plot after treatment}} \times 100$$

## Results

### 3.1 Phytochemical Analysis:

The phytochemicals and elemental analysis of leaf of *Lantana camara* showed the presence of useful classes of chemical compounds such as Alkaloids, Glycosides, Carbohydrate, Flavonoid and Tannins compounds. Table 1 shows the phytochemical results of *Lantana camara*.

**Table: 1 Phytochemical Analysis of leaf extract**

No	Test	Methanol	Aqueous
1	Alkaloids	-ve	+ve
2	Glycosides	+ve	+ve
3	Carbohydrate	+ve	-+ve
4	Flavonoids	+ve	+ve
5	Tannins	+ve	+ve

**Fig : 2 Result of Qualitative Analysis**



**Methanol extract**



**Aqueous extract**

**3.3 Preparation of incense sticks**

Incense sticks with leaf powder were prepared and used to test the efficacy against mosquito by smoke toxicity test.

**Fig: 3 Incense Stick**



**Incense Stick (80% charcoal )**



**Incense Stick (75% charcoal )**

**Table: 2 Cost of stick**

	<b>Incense Stick (80% charcoal )</b>	<b>Incense Stick (75% charcoal)</b>
Weight of crude power	300gm	50gm
Total number of incense stick	3200	270
Total cost of incense stick	Rs 750	Rs 27
stick cost	20 paisa	10 paisa

**Table: 3 Ingredient in sticks**

<b>Ingredient</b>	<b>Composition (%)</b>	<b>Composition (%)</b>
Leaf material	20%	25%
Charcoal and binding agent	80%	75%
Total amount	100%	100%

**3.4 Mosquito repellence test**

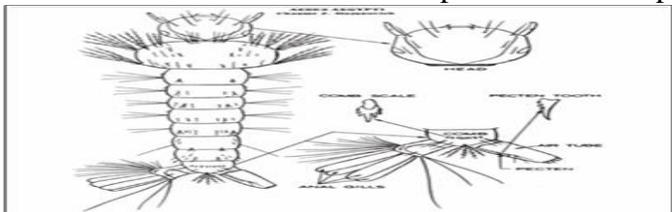
**3.4.1 Mosquito Larvae Collection**

The mosquito larvae and pupae were collected from Uka Tarsadia University campus.

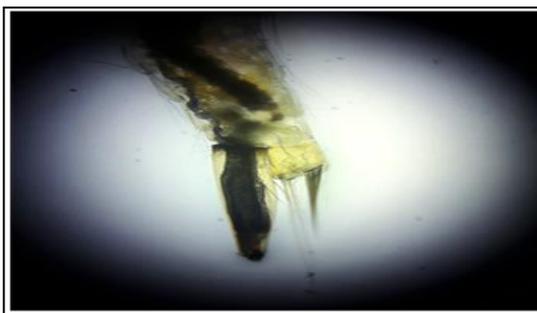
**Fig : 4 Mosquito Larvae Collection**



**Fig: 5 Microscopic examination larva** By the help of dropper took larva sample and than place it on slide and observe under 10x in compound microscope.



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**3.4.2 Smoke toxicity test**

**Table: 4** Showed smoke toxicity of three types of repellent products used in smoke toxicity test such as commercial coil (Control I), incense stick with 75% concentration of charcoal and binding agent and incense stick with 80% concentration of charcoal and binding agent. In this test 75% charcoal plus 25% powder containing incense sticks is more effective when compared with 80% charcoal plus 25% powder containing incense stick.

**Table: 4 Result of smoke toxicity test  
Incense sticks (80% charcoal and 20% leaf sample)**

Sr. no	No. of mosquitoes	No. of dead mosquitoes	No. of live mosquitoes	Time (min)	Temperature (°C)	Burning time (min)
Control	25	25	0	2	31	31
Test 1	25	30	5	40	31	40
Test 2	30	28	2	40	36	42
Test 3	35	33	2	44	38	39

**Table: 5 Result of smoke toxicity test  
Incense sticks (75% charcoal and 25% leaf sample)**

Sr. no	No. of mosquitoes	No. of dead mosquitoes	No. of live mosquitoes	Time (min)	Temperature (°C)	Burning time (min)
Control	15	10	5	2	31	31
Test 1	15	10	5	30	37	46
Test 2	10	8	2	28	36	45
Test 3	10	8	2	20	37	42

**Fig: 6 Smoke toxicity**



**3.4.2 Evaluation of burning on users**

Test was done by simply selecting the mosquito prone areas in the evening and night period. The public remarks were noted down after allowing them. For investigating mosquito repellent activity the prepared incense sticks were checked for causal effect such as irritation, coughing, and tears were observed and recorded. Ash produced by the stick was weighed and recorded.

**Table: 6 Investigating mosquito repellent activity in open**

Sr. No.	Areas	Observation given by people	Remarks
1	Hostel	Mosquitoes escaped , Less irritation, no coughing, tears.	Mosquito repelled
2	Canteen	Mosquitoes escaped ,No irritation, no coughing, no tears	Mosquito repelled
3	Rural area near pond	Mosquitoes moved outside the room, No coughing, no tears	Mosquito repelled
4	Hospital	Mosquitoes moved outside the room, No coughing	Mosquito repelled
5	Hotel	Mosquitoes escaped ,No irritation, no coughing, no tears	Mosquito repelled

**3.5 larvicidal activity**

**Table: 7 Result of biological assay**

Extract	Concentration ( µg/ml )	No. of larvae		Time duration	Percentage of mortality
		Exposed	Dead		
Distilled water	50 µg/ml	20	10	24 hours	40%
	100 µg/ml	20	12	24 hours	48%
Petroleum ether	50 µg/ml	20	0	24 hours	0%
	100 µg/ml	20	8	24 hours	32%
Chloroform	50 µg/ml	20	14	24 hours	56%
	100 µg/ml	20	18	24 hours	72%
Ethyl acetate	50 µg/ml	20	2	24 hours	8%
	100 µg/ml	20	1	24 hours	4%

**Fig : 8 Result of larvecidal activity**

#### 4. Discussion

Mosquitoes have been posing a deadly threat to man for a long time. Through some repellents in the market help in the control of mosquitoes to some extent at the same time they induce certain side effects due to the presence of synthetic chemicals. Plants have been helpful in providing remedies in such cases. Such plant products are safe, environment friendly, cheaper, and without any side effect.

Lantana camara is believed to have insecticidal properties and this plant grows evergreen in tropical climates. This plant was selected to test the mosquito repellent activity. Leaf powder was selected to make incense sticks which were burnt under different conditions to observe the repellent activity of mosquitoes using smoke toxicity as a parameter.

Incense sticks were prepared at a factory in Vyara with

20% & 25% leaf powder plus 80% and 75% charcoal and binding agent. Commercially available incense sticks (Sleep Well brand) were used.

The cost of the product was calculated for both. The burning time of the experimental sticks was about 45 minutes & that of the commercial product was about 30 minutes. 100% mortality was observed in just 2 minutes when the commercial product was burnt in the experimental chamber. But its smell & smoke created side effects like uneasiness, itching in the eyes, and coughing in some members. This may be due to the presence of some synthetic chemicals. The experimental product took about 40 minutes for complete burning and 100% mortality. The smoke did not cause any side effect like coughing, uneasiness, or itching in the eyes as in commercial products. The cost of production is found to be just 25% of the commercial product.

Phytochemical evaluation of leaf extract in distilled water and methanol revealed that alkaloids, glycoside, carbohydrate, flavonoids, and tannins were present in the distilled water extract. In the methanol extract, alkaloids were found to be absent while glycoside, carbohydrate, flavonoids, and tannins were present.

Antimicrobial activity against three bacterial species (*Escherichia coli*, *Pseudomonas sp.* and *B. megaterium*) using leaf extract in acetone, ethanol, and distilled water in 20  $\mu$ l & 40  $\mu$ l. The antimicrobial activity was found in all the extracts with 40  $\mu$ l concentration. The activity was absent against *B. megaterium* only at 20  $\mu$ l concentration in the ethanolic extract but present against all three organisms in all three extracts at both concentrations.

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