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Phytochemical Evaluation and Larvicidal Activity of *Lantana camara* Leaf Extracts

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Abstract: Across the world many infectious diseases are transmitted by mosquitoes which are otherwise called as 'flying syringes', 'tiny buzzing vampires', 'wing devil' and 'tiny assassins'. They serve as a vector to spread numerous infectious pathogens, like bacteria, protozoa, viruses and nematodes, which can result in devastating illnesses like dengue, zika, yellow fever, filariasis, and malaria. There are now no commercially accessible vaccines or preventive medications for mosquito borne disease. Controlling mosquito populations is the sole method of lowering the disease's prevalence. Numerous methods of mosquito control have been proposed since ancient times. Chemical pesticides are widely regarded as the best method of controlling mosquitoes. Insecticide resistance has developed in mosquitoes as a result of enhanced selection pressure brought on by the careless use of pesticides. Many investigations on natural plant products against mosquito vectors have been conducted over the past ten years as a potential substitute for manufactured chemical pesticides. One setback in vector management has been identified as mosquito species' tolerance to chemical pesticides. In order to manage insect vectors, the current study focuses on naturally occurring plant-based compounds that have insecticidal qualities. Lantana camara leaves water, methanol, chloroform, hydro alcoholic and petroleum ether extracts were tested against *Culex quinquefasciatus* larvae in their third instar. Tannins, alkaloids, flavonoids, proteins, and carbohydrates were among the phytocompounds found in the leaves according to a phytochemical screening. This plant's extracts showed strong larvicidal activity and may be worth looking into further for mosquito control.

Keywords: *Lantana camara*, Phytochemicals, Larvicidal activity, *Culex quinquefasciatus*

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Introduction

Worldwide, mosquitoes constitute the main threat to public health. More than a hundred species of the 3492 mosquito species known to exist in the world are capable of spreading a wide range of diseases to humans and other vertebrates (Rueda, 2008). Humans can suffer with filariasis, malaria,

dengue fever, yellow fever, Japanese encephalitis, and chikungunya from mosquitoes (Nour *et al*., 2008). Mosquito-borne illnesses are a major global cause of sickness, mortality, poverty, and societal debility, especially in tropical nations. Of these illnesses, malaria continues to be the most dangerous vector-borne disease, impacting between 300 and 500 million people worldwide and accounting for 1.4 and 2.6 million fatalities each year. Malaria-prone areas are home to about 40% of the world's population (Ghai *et al*., 2000) Dengue fever can present as either the hemorrhagic type, which frequently results in death, or the classic form, which leaves the patient severely disabled for a week or more (Neves-Filho *et al*., 2009). A member of the alpha virus genus, the chikungunya virus poses a serious threat to public health in Southeast Asian and African nations (Pastorino *et al*., 2005). Due to their ease of control and limited mobility in their nesting environments, mosquitoes in their larval stage make them a desirable target for control operations (Howard *et al*., 2007). In developing nations, controlling mosquito populations is a crucial part of efforts aimed at preventing disease. Interest in substitute substances for mosquito control has increased as a result of mosquitoes' growing resistance to pesticides (Lima *et al*., 2006). Since they are a natural source of many different substances, plants are known to contain larvicidal agents, which can function alone or in combination. The best alternative is to utilize natural products because they do not hurt non-target organisms or the environment as much. Numerous chemicals and extracts from various plant groups have been investigated for potential novel larvicides (Ghayal *et al*., 2010). The most important criteria in the search for a new insecticide these days is that it must originate from plants and not harm the ecology in any way. Scholars have demonstrated the efficacy of secondary chemicals produced from plants, including but not limited to saponin (Ester Innocent *et al*., 2008), steroids (Wiseman *et al*., 2005), isoflavonoids (Chowdhurry *et al*., 2008), essential oil (Joseph *et al*., 2004), alkaloids, and tannins (Cavalcanti *et al*., 2004), as larvicides against mosquitoes. An alternate supply of insect repellent chemicals is offered by plant components and their essential oils (Khana *et al*., 2007). *L. camara*, a significant medicinal plant used in traditional medicine, is a member of the Verbenaceae family. Phenolic compounds have been found in the essential oils of various parts of *L. camara*; the main phytochemical groups are flavonoids, carbohydrates, proteins, alkaloids, glycosides, iridoid glycosides, phenyl ethanoid, oligosaccharides, quinine, saponins, steroids, triterpenes, sesquiterpenoides, and tannin (Yang *et al*., 2004). The current study examined the larvicidal activity of plant extracts from *L. camara* against *Cx. quinquefasciatus* larvae in their third instar.

Materials and Methods

Selection of Plant:

The leaves of *Lantana camara* were collected from Marudhamalai hills, Coimbatore, Tamil Nadu, India in the month of April. The whole plant was shade dried under room temperature for about 15 days. The Plant was authenticated from Botanical survey of India,Tamil Nadu Agricultural University, Coimbatore, India.

Preparation of extracts:

Cold Maceration Technique: Shade dried pulverized *L. camara* leaves (100 g) were defatted with petroleum ether (40-60 °C). Then defatted marc was successively extracted with chloroform, petroleum ether, methanol, hydro alcoholic and water by cold maceration method with intermittent stirring for 72 h. Then, the prepared extracts (chloroform, ethyl acetate and methanol) were concentrated by superfit vacuum rotary evaporator and the percentage yield was calculated and subjected to phytochemical analysis.

Reflux Condensation Method: Defatted *L. camara* leaf marcs were immersed in respective solvents (successive manner) in a round-bottomed flask and connected to the reflux condenser and heated

until it reaches the boiling point. which was connected to a condenser. Then, solvent was heated until it reaches its boiling point and continued for 6 h. Final extracts were concentrated by superfit vacuum rotary evaporator and the percentage yield was calculated.

Phytochemical analysis of Lantana camara leaf extracts:

Several phytochemical assays were performed on the crude leaf extracts of LC (*Lantana camara*) in order to determine the presence of mucilage, sterols, terpenoids, carbohydrates, flavonoids, proteins, alkaloids, glycosides, saponins, tannins, volatile oils, and carbohydrates.

Test for Alkaloids: A smooth paste containing around 2 g of the powdered leaf material, 1 g of calcium hydroxide, and 5 ml of water were combined and allowed to stand for 5 min. After that, it was dried off on a porcelain dish submerged in water. This was combined with 200 ml of chloroform, thoroughly mixed, and refluxed on a water bath for 30 min. Then it was filtered and the chloroform was evaporated. This was mixed with 5 ml of diluted hydrochloric acid and 2 ml of each of the subsequent reagents.

(i) Mayer's Reagent - White cream precipitate

(ii) Dragendorff's Reagent - Orange brownish precipitate

(iii) Hager's Reagent - Yellowish precipitate

(iv) Wagner's Reagent - Brownish red precipitate

The above test reports indicated presence of alkaloids.

Test for flavonoids:

Shinoda test: The addition of magnesium turnings and diluted hydrochloric acid to 1 g of alcoholic LC leaf extracts resulted in the appearance of a red color, which is indicative of the presence of flavonoids.

Ammonia test: The presence of flavonoids is shown by the formation of a yellow spot on filter paper that was created after it was dipped in an alcoholic solution containing 1 g of drug extract and subjected to ammonia gas.

Vanillin HCl test: An alcoholic drug extract solution with vanillin hydrochloric acid added showed the presence of flavonoids by turning pink.

Test for terpenoids:

Salkowaski's test: 2 ml of chloroform and 3 ml of strong sulfuric acid were added to 5 ml of extract, and the emergence of a reddish brown color indicated the existence of terpenoids.

Trichloro acetic acid test: The presence of triterpenes was indicated by the colored precipitate in 5 ml of extract with the addition of saturated trichloro acetic acid.

Antimony trichloride test: Chloroform was added to the dry leaf extract and thoroughly mixed. Subsequently, a saturated $SbCl_3$ solution was added to chloroform that contained 20% acetic anhydride. When heated, the formation of a pink color signifies the presence of triterpenoids and steroids.

Test for steroids:

Libermann Burchard test: Added a few drops of acetic anhydride and conc. sulfuric acid from the test tube's side wall to the dry alcoholic LC leaf extracts, which was extracted using chloroform. The presence of a sterol group was indicated by the appearance of a violet ring with a pale green color on the upper layer.

Salkowaski's test: To the mixture of chloroform and extract of LC leaf, concentrated sulfuric acid was poured from the test tube's side wall. The appearance of yellow ring and becoming red at the intersection of the two liquids, indicated the presence of a sterol group.

Test for saponins:

3 ml of the aqueous solution of the extract was mixed with 10 ml of distilled water in a test tube. The aqueous mixture was shaken vigorously for about 5 min and then it was allowed to stand for 30 min. Formation of froth indicated the presence

of saponins.

Test for tannins:

1 ml of distilled water was added to each LC extract, which was then heated, boiled, and filtered. When ferric chloride reagent was added to the filtrate, a blue-green precipitate formed, which suggested the presence of tannins.

Tests for Cardiac Glycosides:

Keller-kilani test: Equal volumes of water and 0.5 ml of a potent lead acetate solution were added to the LC alcoholic extract, thoroughly mixed, and filtered and equal volume of chloroform was added to the filtrate. After evaporating the chloroform extract the residue was produced. Subsequently, it was dissolved in 3 ml of glacial acetic acid and a few drops of ferric chloride solution was added. The final mixture was poured into a test tube along with two ml of conc. H_2SO_4.

Legal test: 0.5 ml of lead acetate solution was added to a mixture containing equal volume of extract and water. After extracting the filtrate with an equivalent volume of chloroform, it was dried by evaporation. After dissolving the residue in 2 ml of pyridine and sodium nitropruside, the residue was made alkaline by adding sodium hydroxide solution. The appearance of pink color indicated the presence of glycosides.

3, 5-dinitro benzoic acid test: Added a few drops of sodium hydroxide and then 2% solution of 3, 5-dinitro benzoic acid to the LC alcoholic solution. The absence of pink color indicated the presence of cardiac glycosides.

Test for Volatile oil: A volatile oil estimation equipment (BP 1980) was used to hydrodistill a weighed quantity of fresh leaves (250 g). There was no volatile oil found, indicating that young leaves did not contain it.

Test for Proteins:

Millon's Test: Millon's reagent test was done by heating a little amount of acidulous alcoholic extract. Formation of white precipitate and turning into red indicated the presence of proteins.

Biuret Test: One ml of a 10% sodium hydroxide solution and a diluted $CuSO_4$ solution was added to a different volume of the acidic alcoholic extract. The violet color of the mixture indicated the presence of proteins.

Larvicidal activity of different extracts of Lantana camara:

C. quinquefasciatus larvae in their third instar were collected from the neonatal mosquito in the National Institute of Communicable Disease, Southern India branch field station. This located at Mettupalayam, Coimbatore District, Tamil Nadu, India. The larvicidal activity against *C. quinquefasciatus* was carried out according to the standard method given by WHO. In total 120 larvae were taken and divided into 6 batches for assessing the larvicidal activity. Stock solutions of all the extracts were prepared using DMSO and different dilutions were made to obtain required concentrations from the range of 10 to 1000 µg. The larvae were transferred in 249 ml distilled water along with 1 ml of the extract. 1 ml of DMSO was used as a control. After 24 h the dead larvae were counted and the whole procedure was repeated five times and the average was calculated. Any larvae that were immobile were considered dead and removed to prevent contamination of surviving larvae and were included in the mortality counts. The percentage of mortality was calculated by Abbott's formula (Abbott, 2005).

(% test mortality -% control mortality/ 100 - control mortality x 100)

The LC_{50} of each extracts were calculated using AAT Bioquest calculator.

Results and Discussion

In the present study different methods of extraction were carried out and the yields were compared. Extraction yield observed in hydro-alcoholic extract of LC leaf was higher (14.3% w/w) than in other extracts. Also the yield obtained by reflux condensation method was

Table 1: Extraction yield obtained from LC leaf by different extraction techniques

S. No.	Solvent used	Extraction yield (% w/w)	
		Cold Maceration extraction (% w/w)	Reflux condensation extraction (% w/w)
1.	Methanol	6	8
2.	Chloroform	4.7	5.8
3.	Aqueous	8.3	10.1
4.	Hydro-alcoholic	12.6	14.3
5.	Pet-ether	5.9	7.01

Table 2: Phytochemical Analysis of *Lantana camara* Leaf extract

S. No.	Phytochemicals	Aqueous	Chloroform	Methanol	HydroAlcoholic	Pet.ether
1	Carbohydrates	**++**	**++**	**+**	**++**	**--**
2	Tannins	**++**	**-**	**++**	**++**	**++**
3	Proteins	**-**	**-**	**-**	**-**	**-**
4	Alkaloids	**++**	**++**	**++**	**-**	**++**
5	Flavonoids	**++**	**++**	**++**	**++**	**--**
6	Glycosides	**++**	**+**	**++**	**+**	**+**
7	Saponins	**+**	**+**	**++**	**+**	**-**

Table 3: Larvicidal Activity of *Lantana camara* leaf extract

LC leaf extracts	LC_{50}	LC_{90}
Aqueous	121.34	256.62
Methanol	134.77	281.06
Chloroform	228.14	459.53
Hydro-alcoholic	98.23	197.94
Pet-ether	397.01	801.63

higher than that of cold maceration extraction (Table 1).

The phytochemical analysis of the prepared extracts of LC leaf revealed that terpenoids and glycosides present in all the extracts (Table 2).

LC_{50} and LC_{90} values of each extract are depicted in Table 3 and Figure 1.

The prepared extracts of LC leaves were subjected to larvicidal activity. Interestingly all the extracts showed moderate larvicidal activity against larvae of *Culex quinquefasciatus.* The hydro alcoholic extracts showed highest larvicidal activity with LC_{50} of 98.23 and LC_{90} of 197.94. The LC_{50} of aqueous and methanolic extracts were found to be 121.34 and 134.77 ppm, respectively and LC_{90} of aqueous and methanolic extracts were found to be 256.62 and 281.06 ppm, respectively. Chloroform and petroleum ether extracts showed lesser larvicidal activity as compared to other extracts (Table 3).

One of the main strategies for controlling vector-borne illnesses is the application of larvicidal chemicals to control mosquito larvae. At the community level, using plants as possible larvicides is regarded as a practical and preferred choice for controlling mosquito species. This study demonstrated the larvicidal activity of LC leaves. The hydro alcoholic extract followed by aqueous and methanolic extracts showed better larvicidal activity compared to other extracts. The highest larvicidal activity (LC_{50}) observed was 98.23 ppm.

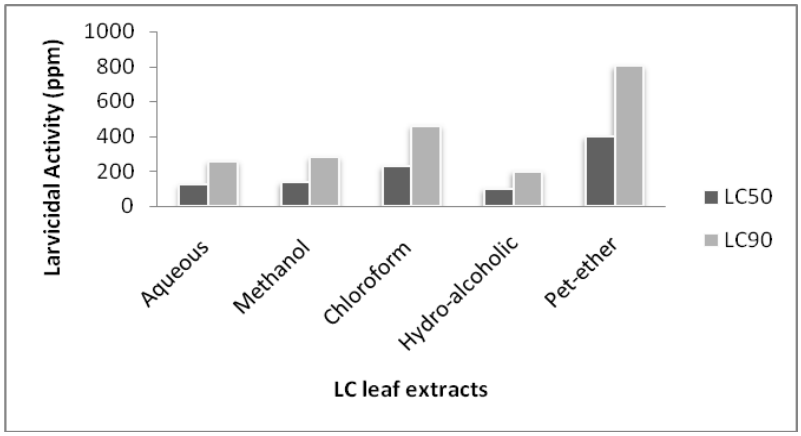

Fig 1: Larvicidal activity of activity of different extracts of *Lantana camara* leaf.

Petroleum ether extract showed least larvicidal activity with LC_{50} of 397.01 ppm. On the whole the larvicidal activity of LC leaves were explored through this study and it can be extrapolated with various larvae of vectors.

Conflict of Interest

The authors declare no conflicts of interest.

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