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MOLECULAR EFFECT OF *LANTANA CAMARA* LEAVES AGAINST DENGUE VECTOR *AEDES AEGYPTI*

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Abstract

The aim of the study was to investigate the effect of *Lantana Camara* leaves on Dengue Vector *Aedes Aegypti.* Larvae on molecular DNA extraction. The plant *Lantana Camara Linn (Verbenaceae)* is described in Ayurveda and Siddha as a potent drug for a variety of ailments.The plant was collected, authenticated and extracted using hexane and methanol solvent The phytochemical, physico chemical and TLC studies were done and further larvicidal activity was performed.The results of the methanolic extract of *Lantana camara* showed the presence of terpenoids, flavanoids and glycoside. TLC studies showed the results of 9 spots with R_f value range from 0.06-0.96 in the mobile phase Hexane: Ethanol (3:7). *Lantana camera* extracts showed potent larvicidal activity. The LC_{50} value for 24 hr observation was very high indicating potent activity. Among the extracts, methanol showed comparatively better activity. The LC50 value of methanol was 5.5 lesser than hexane extract against A. *Aegypti*. The toxic effects resulting in the mortality manifested differently on exposure to the extracts at different times. However, more than 80% mortality was observed only at higher concentrations. The larvae was subjected for DNA isolation by standard procedure and run in Agarose Gel Electrophoresis and observed in UV light. The DNA fragment pattern was observed for methanolic extract of *Lantana camara* which showed potent effect on Larvae *AEDES AEGYPTI.* Thus the molecular studies on Dengue vector Larvae *AEDES AEGYPTI* was potent with methanolic extract of Lantana camara leaves.

Keywords: *Lantana camera*, Dengue vector *Aedes Aegypti*, Larvicidal, Gel Electrophoresis.

Introduction

Mosquitoes are one of the most medicinally significant vectors and they transmit parasites pathogens which continue to have devastating impact on human beings. Several numbers of species belong to genera *Anopheles, Culex, Aedes* and vectors for the pathogens of various diseases like malaria, filarial, Japanese encephalitis, dengue and yellow fever. Thus, one of the approaches for control of these is interruption of disease transmission by killing mosquitoes or preventing mosquito bites.

The plant *Lantana Camara Linn (Verbenaceae)* is described in Ayurveda and Siddha as a potent drug for a variety of ailments. In many parts of the world the plant has been used to treat a wide variety of disorders, in the folk medicine especially for tumors and cancer. A tea prepared from the leaves and flowers is taken against fever, influenza and stomach ache. With other preparations of the plant fever, cold, rheumatism, asthma and high blood pressure are treated.

In Central and South America the leaves were made into a poultice to treat sores, chicken pox and measles. In Ghana infusions of the whole plant are used against bronchitis. The powdered root in milk was given to children for stomach ache. In Asian countries leaves are used for cuts, rheumatism, ulcers, and as a vermifuge. Decoctions are applied externally against leprosy and scabies. In India the leaves of the plant are boiled for tea and the decoction is a remedy against cough. The decoction of the whole plant is given as treatment against tetanus, rheumatism, malaria and ataxia of abdominal viscera. It is used as a lotion for wounds, too. Pounded leaves are applied to cuts, ulcers and swellings.[12] Thus we planned to investigate the plant *Lantana camara Linn* (*Verbenaceae*) for larvicidal activity against Dengue mosquito.

Materials and Methods

Preparation of Extract:

The leaves of the plant *Lantana camara* were collected from the village of Kunnathur in Krishnagiri district, Tamilnadu, India during January 2017. The plant material was identified and authenticated by Professor Dr. J. Jayaraman, Ph.D. Director, Plant Anatomy Research Center, West Tambaram, Chennai. A voucher specimen was submitted at C.L.Baid Metha College of Pharmacy, Chennai-97. The Collected material was shade dried

and extracted through soxhlet apparatus by using hexane and methanol. The extract obtained was studied for preliminary phytochemical studies and physic chemical constant as per standard method. The extract was evaluated by Thin Layer Chromatography method.

Thin layer chromatography:

Procedure: The solvent system is taken in the T.L.C chamber and kept for saturation. The extracts of crude drug were spotted in the pre-coated TLC plate and placed inside the chamber. After development of the spots the plate is taken out, then dried in hot-air oven and observed in UV chamber to detect the number of spots developed. Tha stationary phase precoated TLC plate was used with Mobile phase Chloroform : Ethanol. The Detecting Reagent were UV visible light, and iodine vapour.The Rf value was taken by colour obtained Bluish green, Yellow, fluorescence, Orange colour spots.

Larvicidal activity

Test organisms

The larvae of the mosquito species *Aedes aegypti* were collected from the aquatic environments of Chennai District. Biscuits were served as larval food. The larvae were kept at 25 ± 2ºC and proper photoperiod was given for their growth. Late third and early fourth instars larvae were (4-5 mm in length) used for larval bioassay purpose. The morphological and anatomical characteristics of the collected larvae were observed and identified through microscopic analysis and by comparing to the standard keys.

Bioassay

Larvicidal bioassay was performed based on WHO protocol. *A. aegypti* mosquito larvae were exposed to a wide range of concentration of crude extracts i.e. 10, 25, 50, 100 and 200 µg/ml and control to find out activity. Batches of 25 healthy fourth instar larvae were transferred to the 250 ml water containing chambers and different concentrations of three non-polar and polar extracts of crude extracts were added to assess the desired target dosage. Three replicates were performed for each concentrations and equal number of controls were also setup with tap water. Larval mortality was observed after 24 h. Mortality percentage was calculated using Abbott's formula. LC_{50} and LC_{90} values were calculated using Probit analysis.

$$\text{Mortality}(\%) = \frac{X-Y}{X} \times 100$$

Where, X= survival in the untreated control. Y= survival in treated sample.

Dose-response bioassay

The crude extracts were subjected to dose-response bioassay for larvicidal activity against the larvae of *A. aegypti*. The number of dead larvae was counted after 24 h and the selected samples turned out to be equal in their toxic potential.

Statistical analysis

The larvicidal activity of plant extracts were expressed in terms of lethal concentrations (LC_{50} and LC_{90}) of the average of larval mortality data that were subjected to Probit analysis (Finney, 1971) for calculating LC_{50}, LC_{90} and other statistics at 95% fiducial limits of upper confidence limit and lower confidence limit and chi-square values were calculated using the software type, SPSS (2007). Results with $p<0.05$ were considered to be statistically significant.

Isolation of Genomic DNA

The 24 h dead larvae 1.5 ml was added in to an eppendorf tube and centrifuged at 10000 rpm for 10 minutes. Then pellet was collected and re-suspended in to 450µl of TE buffer (vortex mixer).

To that pellet 5µl of lysozyme and 50µl of 10% SDS (sodium dodecyl sulphate) was added and incubated at 37ºc for 1 hours with regular interval. After incubation, equal volume of phenol; chloroform (1:1) was added and mixed well by inverting the tubes and centrifuged at 10000 rpm for 10 minutes. The aqueous phase was transferred to fresh tube and phenol: chloroform extraction step was repeated. The supernatant was transferred to a fresh tube without disturbing the bottom layer. To the aqueous solution 50µl of 3M NacH3 was added and 300µl of isopropanol was added to precipitate the DNA. Then centrifuged at 1000 rpm for 10 minutes and the supernatant were discarded. Pellets were washed with 70% ethanol and centrifuged at 8000rpm for 1-2 minutes. Ethanol was discarded and evaporated the excess of ethanol without losing DNA. Then dissolved it with 50µl of TE buffer. Dissolved genomic DNA samples were stored at -20 ºC.

Agarose Gel Electrophoresis

0.25g of 0.8% agarose was dissolved 1x Tris Acetate Ethylene Diamine tetra acetic acid (TAE).

Agarose mixture was heated in the microwave oven for 90 sec with constant shaking. The mixture was swirled and was made sure that agarose has melted without any formation of lumps or particles. Agarose was allowed to cool for several minutes and 1 drop of ethidium bromide (4mg/ml) was added in to it. Agarose solution was poured into a sealed gel tray without any air bubbles. After the gel had completely hardened, the comb was carefully removed and the gel was immersed using 1x TAE running buffer. Sample DNA was mixed with loading dye (Bromophenol blue- xylene mixture) and loaded in the wells. The marker DNA was added for the reference. Electrical leads were connected to the electrophoresis chamber and the gel was allowed to run with a current of 50-100 V for 30 min. The gel was carefully removed and viewed with gel documentation.

Results

Table-1: Preliminary Phytochemical Studies.

Phytoconstituents in Methanolic extract	
Alkaloids	-ve
Saponins	-ve
Tannins	-ve
Glycosides	+ve
Flavanoids	+ve
Phenols	-ve
Steroids	-ve
Terpenoids	+ve
Quinones	-ve
Proteins	-ve

+ve indicates the presence of the constituent

-ve indicates the absence of the constituent

Flavanoid
Fig : 1

Terpenoid
Fig : 2

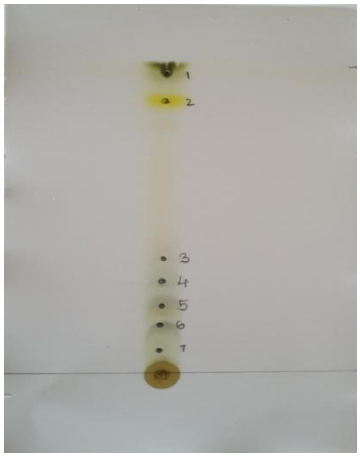
Glycoside
Fig : 3

Table-2: Physiochemical Analysis of *Lantana camara* leaves.

SL. NO.	PARAMETERS	RESULTS
1.	Ash values:	
	➢ Total ash	19%
	➢ Insoluble ash	15%
	➢ Sulphated ash	26.5%
	➢ Water soluble ash	12%
2.	Extractive values:	
	➢ Alcohol soluble extractive	28%
	➢ Water soluble extractive	8%
3.	Loss on drying	5.15%

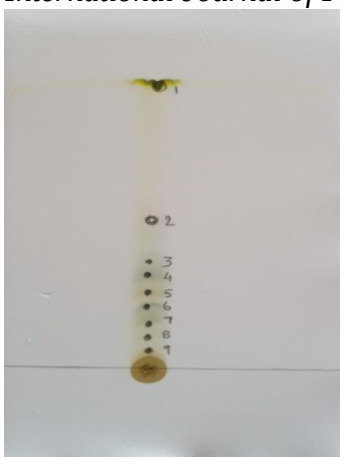
Table-3: Thin Layer Chromatography of *Lantana camara* leaves.

Stationery phase	Mobile phase	Detecting method	No. of spots	R_f values
Precoated silica gel plates	Hexane : Ethanol (3:7)	Visual / UV	9	0.976, 0.511, 0.372, 0.325, 0.255, 0.209, 0.162, 0.116, 0.069
Precoated silica gel plates	Chloroform : Ethanol (4:6)	Visual / UV	7	0.956, 0.869, 0.369, 0.282, 0.217, 0.152, 0.065



Chloroform: Ethanol

Fig : 4



Hexane: Ethanol

Fig : 5

Table-4: Larvicidal Activity of Extracts against *A. AEGYPTI.*

Test larvae	Extracts	Conc. (µg/ml)	LC_{50} 95%confidence (LCL-UCL)	LC_{90} 95%confidence (LCL-UCL)	χ^2 Value
A. aegypti	Methanol	Control 10 25 50 100 200	22.558 (17.132-28.182)	153.026 (110.907-244.310)	9.217
	Hexane	Control 10 25 50 100 200	28.010 (20.832-35.766)	266.862 (175.629-508.815)	2.535
	Standard (Bleeching powder)	Control 10 25 50 100 200	60.913 (47.676-79.569)	621.678 (362.126-1474.746)	0.653

Where, LC_{50} & LC_{90} - lethal concentration at 50 % and 90 % of samples, respectively; LCL- lower confidential limit; UCL- upper confidential limit; χ^2 value – chi square value at $p > 0.05$ significant level.

Table-5: Larvicidal Activity of *Lantana camara* Leaves Against Aedes AEGYPTI.

Aedes	Methanol	Hexane	Std
0	0	0	0
10	8	7	4
10	9	8	4
10	10	8	4
25	11	9	7
25	12	11	8
25	12	12	8
50	15	15	11
50	16	16	11
50	18	16	12
100	20	19	15
100	21	20	16
100	21	20	16
200	23	21	18
200	25	22	18
200	25	22	19

LARVICIDAL ACTIVITY OF *Lantana camara* LEAVES AGAINST AEDES AEGYPTI

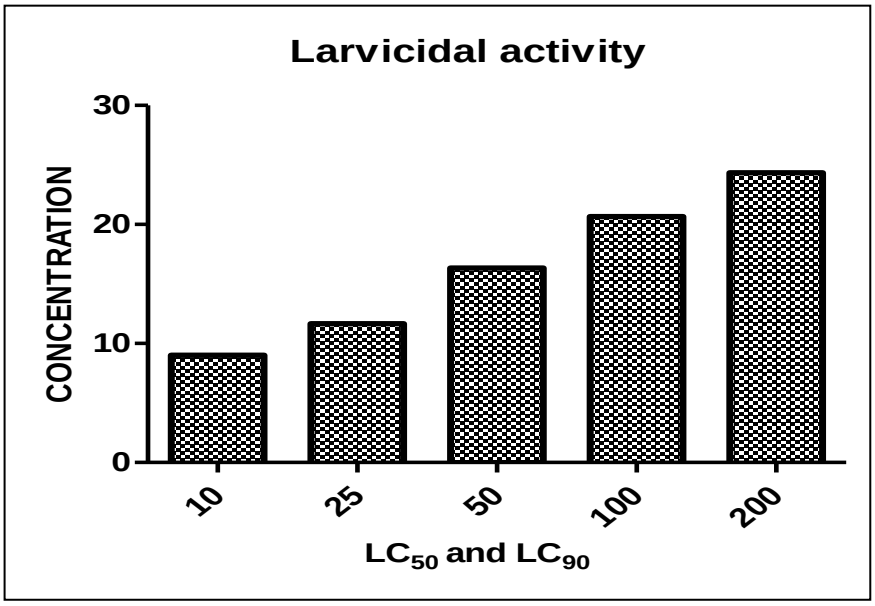
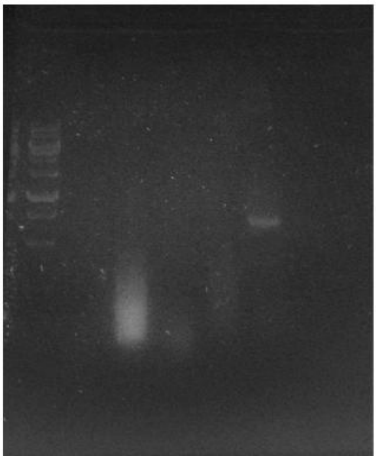


Table-7: Mean Death Count Of Larvae.

Methanol	Hexane
9	7.666667
11.66667	10.66667
16.33333	17
20.66667	19.66667
24.33333	21.66667

LARVICIDAL ACTIVITY OF *Lantana camara* LEAVES AGAINST AEDES AEGYPTI



Agarose Gel image.

The methanolic extract of *Lantana camara* showed the presence of terpenoids, flavanoids and glycoside. This reveals the activity may be due to presence of this phytoconstituents.

TLC studies showed the results of 9 spots with R_f value range from 0.06-0.96 in the mobile phase Hexane : Ethanol (3:7). This reveals many phytoconstituents was present in *Lantana camara.*

Plant extracts that causes high level of mortality at reduced concentration and those which can cause effective mortality within a short span of time can be considered to possess potential phytotoxicity. In the present study, *Lantana camera* extracts showed potent larvicidal activity. The LC_{50} value for 24 hr observation was very high indicating potent activity.

Discussion

Among the extracts, methanol showed comparatively better activity. The LC50 value of methanol was 5.5 lesser than hexane extract against A. *Aegypti*. The toxic effects resulting in the mortality manifested differently on exposure to the extracts at different times. However, more than 80% mortality was observed only at higher concentrations. Comparatively the methanol extracts showed early toxicity resulting in mortality in a short duration of time on exposure to the extracts against *A.aegypti*. The larvicidal activity of *Lantana camera* may be due to the phytoconstituents present in it. The larvae was subjected for DNA isolation by standard procedure and run in Agarose Gel Electrophoresis and observed in UV light. The DNA fragment pattern was observed for methanolic extract of *Lantana camara* which showed potent effect on Larvae *AEDES AEGYPTI.* Thus the molecular studies on Dengue vector Larvae *AEDES AEGYPTI* was potent with methanolic extract of Lantana camara leaves. This is the first report on this plant on DNA fragmentation.

Conclusion

The methanolic extract showed potent larvicidal activity and good DNA fragmention pattern on gel electrophoresis studies. Further this studies was explored for lead compound through chromatographic and spectroscopical studies. This will lead to get a new compound for Dengue larvae *AEDES AEGYPTI.*

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Conflict of interest

Authors would like to declare that they have no conflict of interest.

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