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CASE REPORT

IN VITRO SENSITIVITY ASSAY OF *LANTANA CAMARA* AGAINST *AEDES AGYPTI* WITH SUPPLEMENTARY FACTS FROM GC MS AND *IN SILICO* ANALYSIS

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ABSTRACT

In search of novel plant derived bioactive compounds, advanced methods like GC-MS and computational techniques play a crucial role to characterize and assess their effectiveness. Present study analyzed the larvicidal activity of *Lantana camara* against *Aedes aegypti* larvae to find out whether this plant can be used as a source to formulate a novel naturally derived larvicide. Larvicidal screening of petroleum ether extracts of *Lantana camara* showed a dose dependent growth inhibitory effect on *Aedes aegypti* larvae. Chemical profiling of petroleum ether extract with GC-MS showed the presence of Beta caryophyllene as a major compound and the *in silico* docking analysis recognized the compound as a competitive inhibitor of acetylcholine preventing its binding with acetylcholine esterase and thus nervous functioning.The results suggest that *Lantana camara* leaf extracts could serve as a source of compounds to formulate ecofriendly larvicides to prevent the spreading of vector-borne diseases.

Keywords: *Lantana camara, Aedes aegypti*, Beta caryophyllene, docking, Acetyl choline esterase

INTRODUCTION:

Mosquitoes are medically significant vectors responsible for the biological transmission of several deadliest diseases, mainly in tropical countries where the favorable environmental conditions are responsible for the proliferation of *Aedes aegypti*. The WHO currently estimates there may be 50 million cases of dengue fever infection worldwide every year [WHO, 2011]. Despite an array of control measures taken to suppress this mosquito population, this scourge has not left us but flourish unabatedly to take heavy toll of life every year, and left the present day overpopulated world on the edge of resurgence and outbreaks of mosquito borne diseases. As well *Aedes aegypti* being a fresh water breeding mosquito it is very difficult to control it during rainy season. The adverse effects of chemical insecticides based intervention measures for the control of mosquito vectors have received wide public apprehension because of several problems like insecticide resistance, resurgence of pest species, environmental pollution resulting in bio amplification of food chain contamination and harmful effects on beneficial non target animals [2].Therefore, prospection for new insecticidal molecules based on rich plant biodiversity is essential to combat the erosion of the efficiency of these antique approaches.

Co-evolution has equipped plants with a plethora of chemical defenses against insect predators and several plants have been reported significant for their mosquitocidal activity [3]. But only a few botanicals have moved from the laboratory to field use, as they are poorly characterized, in most cases active principals are not determined and most of the works are restricted to preliminary screening. This has necessitated the need for search specific, easily biodegradable, eco-friendly, accessible and affordable preparation from botanicals for vector control. Search for eco-safe, low cost and a highly potential insecticide for the control of mosquitoes needs the preliminary screening of plants to evaluate their insecticidal activities.

The present study evaluated the larvicidal potential of *Lantana camera,* a species of flowering plant within the verbena family, Verbenaceae. This plant is widely distributed in tropical and subtropical regions of the world including India and leaves of this plant are easily available but are not involved many manufacturing processes. In the view of that a larvicide preparation for field use from this plant shall not be causing any toxic effect to environment, biodiversity or human health.

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MATERIALS & METHODS:

Collection of plant material

Fresh leaves of *Lantana camera* was collected from local regions of Kalyan, Thane, and authenticated at Blatter's herbarium; St. Xavier's College, Mumbai and the specimens voucher were deposited in the St. Xavier's College. The leaves were washed and shade dried and pulverized to a coarse powder in a mechanical grinder and passed through a sieve.

Extraction

10gm of powder was suspended each in 100ml of petroleum ether. The powdered materials were stored in airtight, dark, glass container to prevent photochemical reactions.

Raring of *Aedes aegypti* larvae

The eggs of *A. aegypti* were procured from Haffkine Institute at Mumbai, India and authenticated at Department of Entomology, NIV, Pune. The egg rafts of *A. aegypti* were kept in the tray containing tap water at laboratory condition. After incubation, the eggs were observed to hatch out into first instar larvae. Larvae were fed with a diet of dog biscuit. The 3rd instar larvae were used in the study.

Bioassays and larval mortality

The plant extracts were dissolved in DMSO to prepare the stock solution. Larvicidal activity was determined according to WHO protocol [4]. The 3rd intstar larvae of *Aedes aegypti* was treated with plant extract of 100ppm, 200ppm, 300ppm, 400 ppm 500ppm concentrations. A corresponding control was maintained. The larval mortality of third instar of *A. aegypti* was observed. The number of larvae surviving at the end of 24 and 48 hours were recorded and the percent mortality was calculated.

The percentage of mortality was calculated by (No. of larva dead /No. of larvae)*100.

Phytochemical analysis

The plant extract that showed least LC50 value for *A.aegypti* larvae were screened for the phytochemicals present according to Prashant Tiwari et al [5].

GC/MS analysis

To identify the phytochemicals present in the petroleum ether extract of *Lantana camara* GC/MS analysis was performed on an Agilent gas chromatograph directly coupled to the mass spectrometer system (JoelAccuTOF GCV). Samples were injected using the split mode (split ratio 1:30), by applying the following temperature program: 50°C for 5 min, 50º to 100ºc at 8°C /min, 100º to 270ºc at 3°C/min. Helium was used as carrier gas with flow rate of 1ml/min. MS scan range was 50 to 500 a.m.u. The identification of volatile phytochemicals was achieved by comparing the mass spectra with the data system library (NIST) and other published spectra (Mass Spectrometry Data Centre., 1974), supported by retention index data, which were compared with available literature retention indices

In silico analysis

Biocomputational investigation was carried out to analyze whether the candidate phytochemical compound, caryophellene identified in GC MS analysis have the potential for inhibiting the growth of mosquito larvae. The paralytic movement of the larvae after treatment explored the neurotoxic behavior of bioactive compound in *Lantana* extract. The 3D crystal structure of the insect acetylcholine esterase (AchE) protein was retrieved from the protein data bank (www.rcsb.org) [6]. Docking preparation was done by using UCSF CHIMERA (https://www.cgl.ucsf.edu/chimera) [7]. The phytochemical and acetylcholine structure was downloaded from ChemSpider database (http:// www. chemspider.com).

Energy minimization

Energy minimization is considered as one of the important step towards docking as it optimizes the molecules towards stable state. Energy of a molecule is inversely proportional to its stability. Hence to mimic the in-vivo environmental stability of AChE molecule, energy minimization was carried out using Chimera software.

Docking analysis

Molecular Docking is the process in which two molecules fit together in 3D space. It is a key tool in structural biology and computer-aided drug design [8].The molecular docking analysis was performed using CLC drug discovery workbench [9][10]. PDB structure of target protein and MOL2 files of ligand, both acetyl choline and caryophyllene were submitted to the software to compare their binding mode with target protein.

RESULTS AND DISCUSSION:

Larvicidal activity

Petroleum ether extract was highly toxic to the 3rd instar larvae *Aedes aegypti even in 100ppm* concentration. The mosquito larvae exposed to plant extract showed significant behavioral changes. The most obvious sign of behavioral changes observed in *Aedes aegypti* was restlessness, loss of equilibrium, paralytic movement which finally led to death. No death was observed in the control with DMSO.

Phytochemical analysis

Mortality of *Aedes aegypti* larvae in Petroleum ether extract of *Lantana camara* shows the presence of some specific larvicidal secondary metabolites in it. Phytochemical screening of the extract showed the presence of terpenes, anthroquinones and fixed oils (Table1).

Table 1: phytochemical screening of *Lantana camara* petroleum ether extract

Phytochemicals	Presence
Phenol	-
Alkaloid	-
Terepenes	+
Flavones	-
Saponin	-
Tannins	-
Anthraquinones	+
Fixed oils	+

Presence '+', Absence '-'

GC MS analysis

GC profile showed the presence of a range of compounds with maximum peak area (1161736.94 intens.*sec) for a compound with retention time 17.1min. Comparison of mass spectral fragmentation pattern (Fig 1) of the same compound with those reported in the library showed similarity with Beta caryophyllene (C15-H24) of molecular weight 204.355.

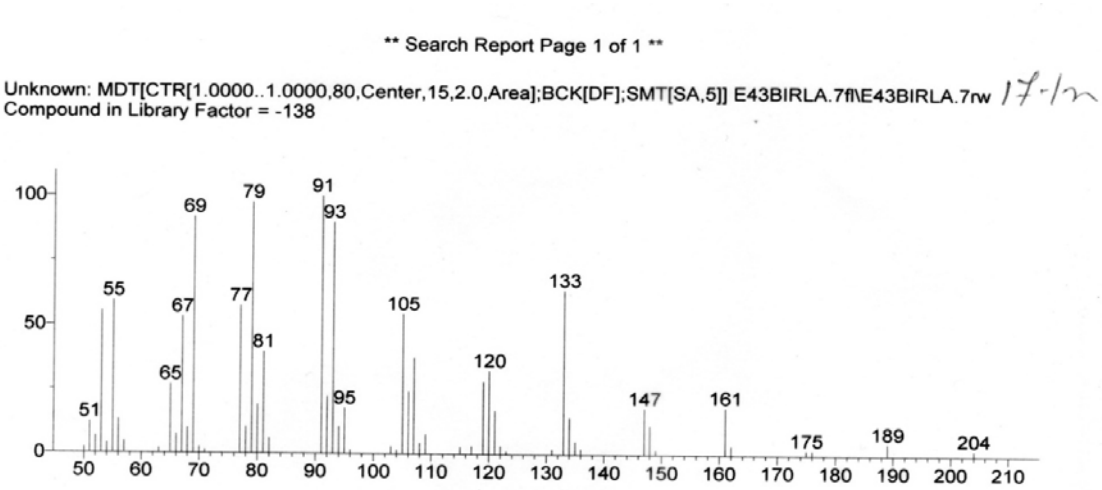

Figure 1: MS spectra of Beta Caryophyllene

Molecular docking analysis

The 3-D crystal structure of the insect acetylcholine esterase (AchE) protein was retrieved from the protein data bank (PDB) (Fig 2A). Energy minimization was carried out using Chimera software. Energy minimized target protein and ligands, both acetyl choline and caryophyllene formed complexes facilitating the comparison of their binding modes. The various complexes were visualized and the best run and poses of both acetylcholine (Fig 2B) and caryophyllene (Fig 2C) were studied. Docking Score estimated for AChE with acetyl choline was -32.14 and that for caryophyllane was -48.48. They bound to similar active sites which are shown in Figure 3. Since caryophyllane has best (least) estimated free energy of binding than the original acetyl choline, it can act as competitive inhibitor of acetylcholine preventing its binding and recycling and thus nervous functioning (Figure 4).

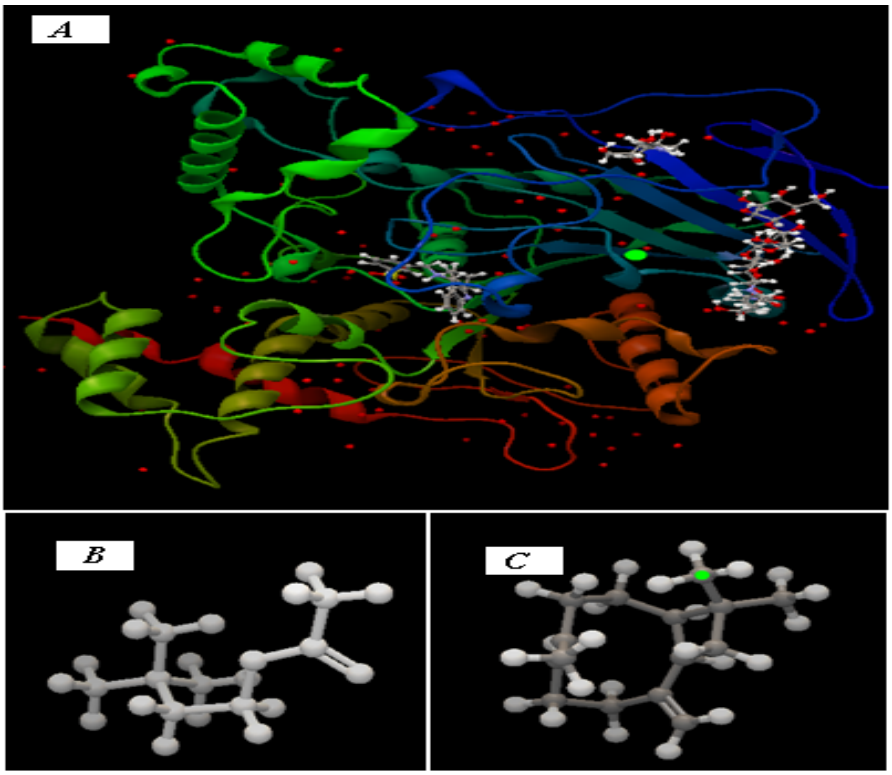

Figure 2: A: Acetylcholine esterase B: Acetylcholine C: Caryophyllene

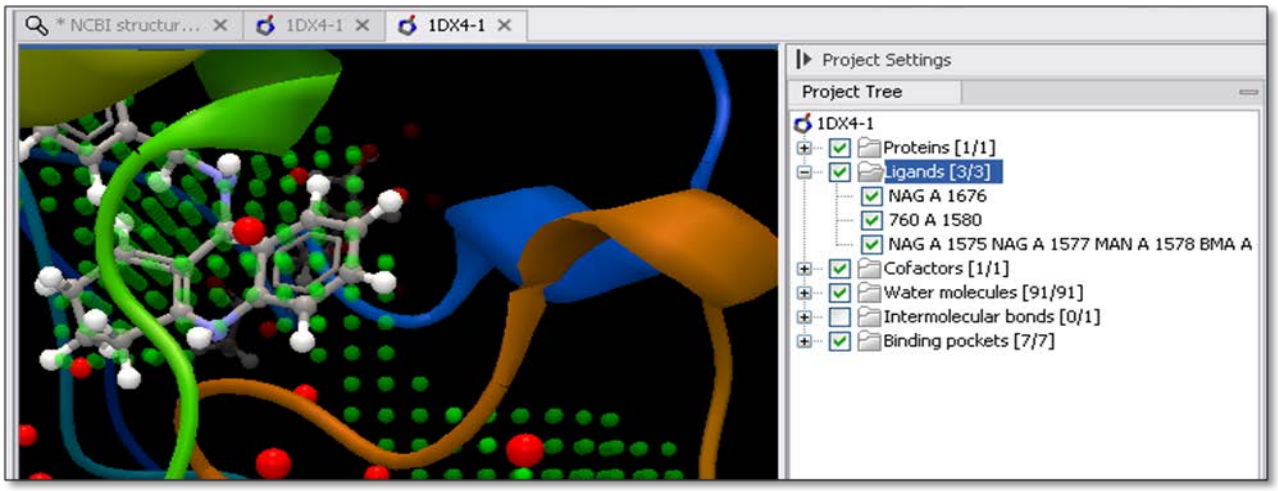

Figure 3 Acetyl choline esterase receptor specifications

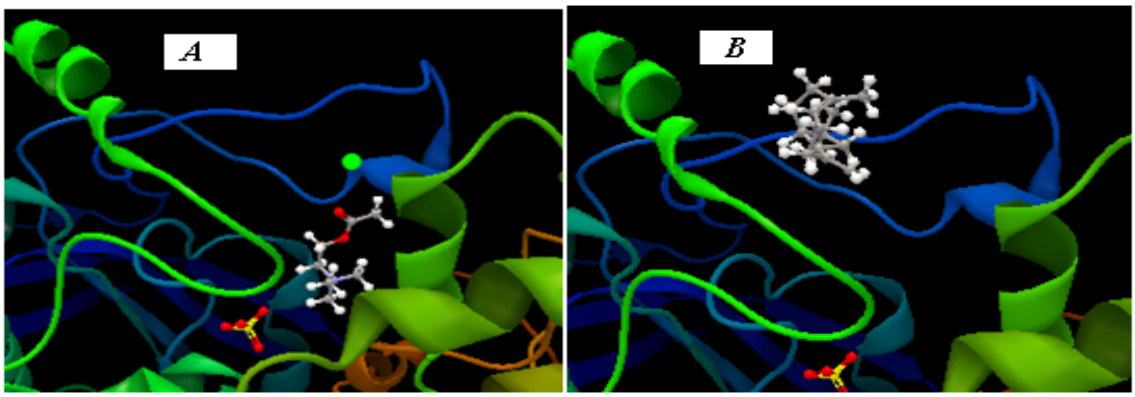

Figure 4: Acetyl choline esterase receptor with ligands A: Acetyl choline B: caryophyllene

CONCLUSION:

The findings of the present study demonstrated the larvicidal activity of petroleum ether extract of *Lantana camara* against *Aedes aegypti*. The docking analysis of Beta-Caryophellene showed its potential to disrupt the nervous functioning and thus it can be the bioactive compound in the extract with growth inhibition potential. Caryophellene being a natural compound, stands as a better option for designing an efficient larvicide than the existing synthetic larvicides and can be a candidate for the formulation of ecofriendly larvicide to prevent the vector-borne diseases.

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REFERENCES:

1. World health Organization. *Dengue and Dengue haemorrhaghic fever*. Factsheet 2011, 117.
2. Kumar., Sarita., Wahab., Naim., Mishra., Monika., Warikoo., Radhika., *Evaluation of 15 Local Plant Species as Larvicidal Agents Against an Indian Strain of Dengue Fever Mosquito, Aedes aegypti L. (Diptera: Culicidae)*, Frontiers Research Foundation, 2012.
3. S. Arivoli.,Samuel Tennyson., *Studies on the Mosquitocidal Activity of Murraya koenigii (Rutaceae) Leaf Extracts Against and Aedes aegypti, Anopheles stephensi, Culex quinquefasciatus*, ASIAN J. EXP. BIOL. SCI, VOL 2(4),2011, 721-730.
4. World Heath Organization. *Instructions for determining the susceptibility or resistance of mosquito larvae to insecticides.* WHO/VBC, 1981, 81, 807-962.
5. Prashant Tiwari., Brimless Kumar., Mandeep Kaur., Gurpreet Kaur., Harleen Kaur., *Phytochemical screening and Extraction: A Review.* Internationale pharmaceutica sciencia. 2011, 1:1.
6. H.M. Berman., J. Westbrook, Z. Feng, G. Gilliland, T.N. Bhat, H. Weissig, I.N. Shindyalov, P.E. Bourne, *The Protein Data Bank*., Nucleic Acids Research, 2000, 28, 235-242.
7. Pettersen E F., Goddard T D., Huang C C., Couch G S., Greenblatt D M., Meng E C., Ferrin T E. *UCSF Chimera -- a visualization system for exploratory research and analysis.* J Comput Chem. 2004 5(13), Oct, 1605-12.
8. John Dogulas Palleti et al., *Virtual Screening and Molecular Docking Analysis of Zap-70 Kinase Inhibitors*, International Journal of Chemical and Analytical Science, 2011, 2(9), 1208-1211.
9. *CLC Inc A*, Denmark. S J Daharwal., *Computer aided drug design and bioinformatics: A current tool for designing Pharmacology*.

