SPECIMEN FORMAT FOR THESIS OF MONTH

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Title of the thesis	:	*In silico* studies to screen ovicidal and repellent activity of selected plant extracts against the filarial vector, *Culex quinquefasciatus* (Diptera : Culicidae)
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Abstract:

Mosquitoes are considered as "Public Enemy Number One" because they cause various problems to the human society by their biting and blood feeding mode of habit. *Culex quinquefasciatus* is an important vector of filariasis which occurs in human habitations in the tropical and sub-tropical regions. The present *in silico* study was undertaken to screen the ovicidal and repellent activity of leaves and flowers of *Calotropis gigantea, Thevetia peruviana, Tagetes erecta, Lantana camara and Bauhinia acuminata* against *C.quinquefasciatus*. Ovicidal activity was assessed by the method of Su and Mulla, 1998. It was determined at concentrations ranging from 100 - 300 ppm and hatch rates were assessed 48 h post treatment. The repellent study was assessed by the method of WHO (1996). The crude extract was applied at 1.0, 2.5 and 5.0 mg/cm^2, separately in the exposed area of the fore arm. The test was carried out for a time period of 30, 60, 90, 120, 150 and 180 minutes. Preliminary phytochemical screening of was carried out using the standard procedures of Raman, 2006. Fourteen ligand molecules were retrieved from PubChem database of which only ten were processed. The ligands were docked against mosquito odorant binding protein (PDB id 2L2C) and the corresponding glide scores were recorded. The best docked complexes were carried forward for molecular dynamic simulation. The results suggest that, among the leaf extracts, minimum hatching rate was noted in chloroform extract of *T. peruviana* followed by ethanol extract of *C. gigantea.* Among the flower extracts, minimum hatching rate was recorded in chloroform extract of *T. erecta* followed by ethanol extract of *C. gigantea.* In repellent activity, highest repellency was observed in chloroform extract of *T. erecta* flower followed by ethanol extract of *C. gigantea* flower. Maximum repellency was observed in ethanol extract of *C. gigantea* leaf followed by chloroform extract of *T. peruviana* leaf. Ethanol extract of *C. gigantea* leaf and flower, chloroform extract of *T. peruviana* leaf and chloroform extract of *T. erecta* flower exhibited high ovicidal and repellent activity and were further carried for qualitative analysis and GC MS analysis. The phytochemical analysis showed the presence of alkaloids, tannins, phenols, flavonoids, sterols, terpenoids, saponins, antraquinones, proteins and quinones in the leaf and flower extracts. The GC-MS analysis confirmed

the presence of hydrocarbon, methyl, hydroxyl, nitrogen, carbonyl and carboxylic acid functional groups in the extracts analyzed. *In silico* studies were carried out to analyse the biological activity of the ligands structures retrieved from PubChem using PASS. Among the ten ligands, peruvianoside I, beta amyrin, cis-ocimene and linalool recorded good glide scores. Molecular dynamic simulation experiments were performed to check the stability of the best docked complexes and was found to be stable. From these results, it can be concluded that the integration of computational and experimental approaches proposed in this study exemplifies a genuine computer-aided discovery of mosquito ovicides and repellents that may be a better option against the mosquitoes than the existing harmful synthetic ones.

i) MAJOR OBJECTIVES :

- To conduct the bioassay studies and to evaluate the efficacy of selected plant extracts for the ovicidal activity against *C.quinquefasciatus.*
- To evaluate the repellent activity of selected plant extracts against *C.quinquefasciatus.*
- To identify the nature of the phytochemicals by spectral studies.
- To find the efficacy of the identified phytochemicals on an *in silico* platform.

ii) HYPOTHESIS:

To test the ovicidal and repellent activity of selected plants, namely *Calotropis gigantea, Thevetia peruviana, Tagetes erecta, Lantana camara and Bauhinia acuminata* against *C.quinquefasciatus*.

iii) METHODOLOGY :

a. Studies on the potency of selected botanicals

Laboratory culture of eggs

Hay infusion method was adopted for culturing mosquito eggs. After one or two days eggs were laid by female mosquitoes in clusters forming an egg raft. The egg rafts were collected, maintained in the laboratory and was allowed to hatch for the ovicidal bioassay studies.

Collections of test materials

Leaves and flowers of the selected five plants namely *Calotropis gigantea* (L.) R. Br.*, Thevetia peruviana* (Pers,) Merr*, Tagetes erecta* L.*, Lantana camara* L. var. aculeate (L.) Moldenke and

Bauhinia acuminata L. were collected (BSI/SRC/5/23/2013-14/Tech.883) from the natural habitat of Coimbatore locale.

Preparation of leaf and flower extracts

Fresh leaves and flowers were collected and left to shade dry at room temperature and were ground using an electric pulverizer to get the fine powder.The leaf and flower powders were subjected to extraction using a Soxhlet apparatus (Harbourne, 1973; Vogel, 1978). Petroleum ether extraction was followed by chloroform and ethanol. The extracts thus obtained were concentrated by distillation and were used for further bioassays.

b. Ovicidal activity of plant extracts

Each leaf and flower extracts were diluted in the appropriate solvents to achieve various concentrations ranging from 100 – 300 ppm. Eggs of the mosquito species of *C.quinquefasciatus* were exposed to each concentration of leaf and flower extracts for hatching assessment after counting the eggs under microscope.

The hatch rates were assessed 48 h after treatment by the following formula.

$$\% \text{ of egg mortality} = \frac{\text{Mortality in the treatment} - \text{mortality in the control}}{100 - \text{mortality in the control}} \times 100$$

Statistical analysis

The data on bioassay studies were also subjected to one way analysis of variance (ANOVA) as described by Panse and Sukahtme (1985).

c. Repellent activity of selected plant extracts

Repellent activity of the leaves and flowers of *C.gigantea, T.peruviana, T.erecta, L. camara* and *B.acuminata* were tested. Larvae obtained from laboratory colony were fed with dog biscuits and yeast powder in the ratio of 3:1. Three day old blood starved *C.quinquefasciatus* mosquitoes were used for repellent bioassay studies.

Evaluation of selected plant extracts

The repellent study was followed by the method of WHO (1996). Dorsal side of the right arm was treated with extracts, at 1.0, 2.5 and 5.0 mg/cm^2, left arm was kept as control and the remaining area was covered by rubber gloves. The test was conducted at each concentration by inserting the treated and control arms into the same cage for one full minute for every five minutes. The mosquitoes that landed on the hand were recorded and then shaken off before imbibing any blood. The percentage of repellency was calculated by the following formula.

% Repellency= [(Ta – Tb) / Ta] ×100

Where Ta is the number of mosquitoes in the control group and Tb is the number of mosquitoes in the treated group.

d. Phytochemical and GC MS analysis

Phytochemical screening of ethanol extract of *C. gigantea* leaf and flower, chloroform extract of *T. peruviana* leaf and chloroform extract of *T. erecta* flower were carried out using the standard procedures of Raman, 2006. Mass experiments were performed on GC (T8000 Top CE) combined with Mass Spectrometer (Md 800 FIS ONS).

e. Molecular docking studies

Maestro is Schrödinger's powerful, unified, multi-platform Graphical User Interface (GUI). Three dimensional structure of mosquito odorant binding protein (PDB id: 2L2C) is obtained from PDB databank. Glide was used for receptor grid generation. Ligand molecules were retrieved from PubChem database. All 14 compounds were prepared using LigPrep module from Schrodinger. In the LigPrep process 10 compounds were processed, unwanted structures were eliminated and optimized. Ligand structures of the processed 10 compounds were retrieved from PubChem database in standard 3D SDF format. Totally ten compounds activity were predicted using PASS. All 10 ligands were docked against mosquito odorant binding protein. When the ligand binds with protein, the conformation of the protein structure will change so the function of the protein will alter automatically. The entire docked complex was visualized by using XP visualizer.

f. Molecular dynamic simulation of docked complex

Dynamics is performed using following parameter such as keeping the constant temperature at 300 K and in the integration step at 1.0 ps. MD simulations for complex structure was run. Finally, Root Mean Square Deviation (RMSD) was calculated for checking the stability of target protein with their native motion. The entire coordinate file was saved every 0 ps upto 100 ps and the results were analyzed by Scatter Plot.

iv) FINDINGS:

- ✓ In ovicidal bioassay, among the leaf extracts, maximum ovicidal activity was noted in chloroform extract of *T. peruviana* followed by ethanol extract of *C. gigantea.*
- ✓ Among the flower extracts, zero percentage egg hatchability was recorded in chloroform extract of *T. erecta* and ethanol extract of *C. gigantea.*

- ✓ In repellent activity, among the flower extracts, highest repellent activity was observed in chloroform extract of *T. erecta* flower followed by ethanol extract of *C. gigantea* flower.
- ✓ Among the leaf extracts maximum repellency was observed in ethanol extract of *C. gigantea* leaf and chloroform extract of *T. peruviana* leaf.
- ✓ The phytochemical analysis showed the presence of alkaloids, tannins, phenols, flavonoids, sterols, terpenoids, saponins, antraquinones, proteins and quinones in the leaf and flower extracts.
- ✓ The GC-MS analysis confirmed the presence of hydrocarbon, methyl, hydroxyl, nitrogen, carbonyl and carboxylic acid functional groups in the extracts analyzed.
- ✓ Compound Peruvianoside I exhibited good glide score (-7.55 Kcal/mol**)** and formed 2 H-bonds, followed by compound Beta amyrin which showed a glide score (-6.73 Kcal/mol**)** and formed 1 H-bond with target odorant binding protein. It was followed by compound Cis-ocimene having glide score (-3.7 Kcal/mol) which formed 1 H- bond followed by the compound Linalool, which displayed glide score (-3.0 Kcal/mol) and formed 1 H- bond with target odorant binding protein.
- ✓ While running MD simulation for Peruvianoside I - 2L2C complex, Beta amyrin - 2L2C complex and Cis-ocimene - 2L2C complex the Root Mean Square Deviation plot shows the stability of the complex structures at 80 ps, 90 ps and 90 ps respectively.

Examiners

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