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IN SILICO STUDIES FOR THE IDENTIFICATION OF NOVEL REPELLENT COMPOUNDS IN TAGETES ERECTA AGAINST THE ODORANT BINDING PROTEIN OF CULEX QUINQUEFASCIATUS

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ABSTRACT

Mosquito borne diseases are the major sources for death in developing countries. Control of mosquitoes is important in the present day with increase in number of mosquito borne illnesses. DEET is the active ingredient in insect repellent products and has been found to inhibit the activity of central nervous system, enzyme acetylcholinesterase, in both insects and mammals and is therefore harmful. The use of botanical derivatives in mosquito control as an alternative to synthetic insecticides is more eco-friendly. Therefore, the present study was carried out to determine the potential ability of the four selected repellent compounds, viz., Cis-ocimene, Lutein, Beta carophyllene and Piperitone of *Tagetes erecta* against the Odorant Binding Protein (PDB id 2L2C) of *Culex quinquefasciatus*. The results revealed that, Cis-ocimene showed good docking scores against the Odorant Binding Protein 2L2C and molecular dynamics confirmed the stability of the protein-ligand bonding. Further research is needed to develop new repellents from substance of natural origin that can offer effective mosquito management to reduce the indiscriminate use of harmful chemical insecticide.

KEY WORDS: *Tagetes erecta*, *Culex quinquefasciatus*, Odorant binding protein, Molecular docking, Molecular dynamics.

INTRODUCTION

Mosquito is the most indisputable medicinal significant arthropod vector of diseases. The vector-borne diseases caused by mosquito are one of the major health problems in most of the countries. It is affecting the socioeconomical status of many nations and it is an important

pest against human causing allergy, which includes a local skin reaction [1]. Several mosquito species of the genera *Anopheles*, *Culex* and *Aedes* are vectors of various human diseases [2]. They transmit parasites and pathogens which continue to have disadvantageous impact on human beings [3]. Even though chemical vector program has been carried on for long time, mosquito vectors remain because the repeated use of synthetic products, house hold spray, and insecticides has resulted in the development of resistance among the mosquitoes [4].

One of the approaches for controlling mosquito borne diseases is the interruption of disease transmission through mosquito control or avoiding mosquito bites. Plant products as potential insecticides or repellents can play an important role in the interruption of the transmission of mosquito-borne diseases at the individual as well as at community level[5]. Nevertheless, the discovery and use of synthetic persistent chemicals not only overshadowed the use of plant products but also become the major tactic for mosquito control. However, the extensive and indiscriminate uses of pesticides have resulted in serious draw backs such as toxicity hazards to man, livestock and wild life [6]. The residues of some persistent chemicals in the environment have subsequently disturbed the ecosystem [7]. Botanical biocides are relatively harmless to non-target organisms and present little risks to users and consumers [8]. As an alternative to the use of insecticides, botanical mosquito repellent are convenient, inexpensive and afford advantages in protection against a wide range of vector [9]. Repellents can be used by individuals for personal protection and thus helps in prevention of the disease transmission [10]. They are also the primary means of mosquito-borne disease prevention available in areas, where vector control is not practical [11, 12]. Based on the above mentioned and many other drawbacks of pesticides, researchers all over the world are working hard to find environmentally safe alternatives. They resorted again to plant extracts as potent sources of natural biocides [13]. Thus the need for new drugs has fueled the use of computational prediction of potential drugs by a method called docking which helps to investigate the detailed intermolecular interactions between the ligand and the target protein. It performs grid-based ligand docking with energetics and searches for favorable interactions between one or more typically small ligand molecules and a typically larger receptor molecule, usually a protein [14]. Molecular Docking is a key tool in structural biology and computer-aided drug design [15]. The host seeking and feeding behaviors of mosquitoes are much affected by host odors [16]. Fundamental aspects of olfactory signal transduction at the peripheral level have revealed the involvement of olfactory receptors on maxillary palpi and antennae. Many studies have been corroborated by field studies, mostly with *C. quinquefasciatus*, which have

shown that these insects are attracted to human volatiles from a distance [17]. Keeping this in view the present study has been conducted to understand the potential ability of the repellent compounds from the plant *Tagetes erecta* to block the odorant binding protein of mosquito *C. quinqufasciatus* and also to understand their key role in affecting the host seeking and feeding behaviors of the mosquitoes by *in silico* approaches.

MATERIALS AND METHODS

Selection of Ligands from *Tagetes erecta*

Potential bioactive compounds of the plant *Tagetes erecta*, viz., Cis-ocimene[18], Lutein[19], Beta caryophyllene[20] and Piperitone[21] selected with the help of previously published literatures were used in the present investigation for the computational prediction of potential drugs from it by the process of *in silico* molecular docking.

Molecular Docking Studies

Target Protein Retrieval and Preparation

Three dimensional NMR structure of mosquito odorant binding protein (PDB id: 2L2C) was obtained from Protein Data Bank (PDB) (Fig 1). The preparation of a protein involves importing of the mosquito odorant binding protein structure. The water molecules have been deleted but water that bridge between the ligand and the protein were retained, charges were stabilized, missing residues were filled in and side chains were generated according to the parameters available.

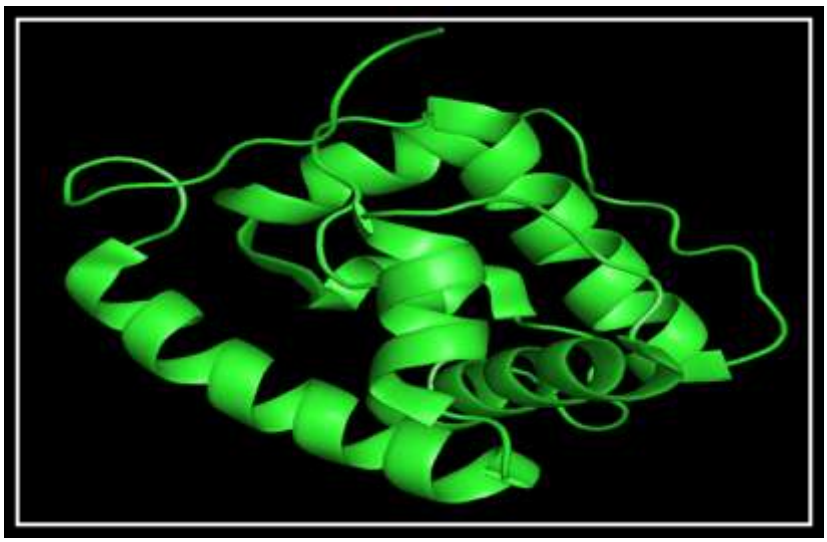


Fig 1: Three Dimensional Structure of Mosquito Odorant Binding Protein (PDB Id 2L2C)

Grid Generation

Glide was used for receptor grid generation. The prepared mosquito odorant binding protein was displayed in the Workspace. The volume of grid was calculated. The entire complex was shown with several types of markers. The enclosing box was made small so that it will be consistent with the shape and character of the protein's active site and with the ligands that were expected to be docked.

Ligands Retrieval and Preparation

Ligand molecules were retrieved from PubChem database. The following compounds were retrieved in 3D SDF format (PubChem id: CID_5369951, CID_5281243, CID_5281515 and CID_6987). The four compounds were processed, unwanted structures were eliminated and optimized using LigPrep module from Schrodinger.

Molecular Docking of Target Protein with Ligands

In order to explore the binding mechanism of phytochemicals with the target proteins, molecular docking studies have been performed. The two ligands were docked against mosquito odorant binding protein (2L2C). When the ligand binds with protein, the conformation of the protein structure will change and therefore the function of the protein will alter automatically. The entire docked complex was visualized by using XP visualizer. The hydrogen bonding interaction between the receptor and the ligands were also visualized.

Molecular Dynamic Simulation of Docked Complex

In order to confirm the docking results, Molecular Dynamics simulation study was carried out. Molecular Dynamics simulation was done using Macro Model. It is a general purpose, force-field-based molecular modeling program with applicability to a wide range of chemical systems. Macro Model provides researchers with multiple advanced methods to understand the chemical structures, energetics, and dynamics. Best docked complex was carried for Molecular Dynamics. 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. The entire coordinate file was saved every 0 ps up to 100 ps and the result was analyzed by Scatter Plot.

RESULTS AND DISCUSSION

Molecular Docking

In a rational drug design, the process begins with knowing the structure of the target protein and then forms a database that contains a collection of compounds that are expected to interact with the target protein. To determine which compounds that have the best interaction with protein target and become candidates for drug synthesis, a series of analyzing techniques is performed by using computer-assisted tool. Two of the most well known computational techniques in drug design process are docking and molecular dynamics simulations [22]. The three dimensional structure of Odorant Binding Protein of *C. quinquefasciatus* (2L2C) was collected from PDB (Fig 1). The three dimensional SDF structures of the processed four secondary metabolites of the plant *Tagetes erecta* were retrieved from PubChem database (Fig 2) and were prepared to dock with the mosquito odorant binding protein 2L2C. Glide calculations were performed with Schrodinger. It performs grid-based ligand docking with energetics and searches for favorable interactions between one or more typically small ligand molecules and a typical receptor molecule (usually a protein). Molecular docking simulation was conducted in such a way that the protein was made rigid and ligand was left free to find the most suitable conformation.

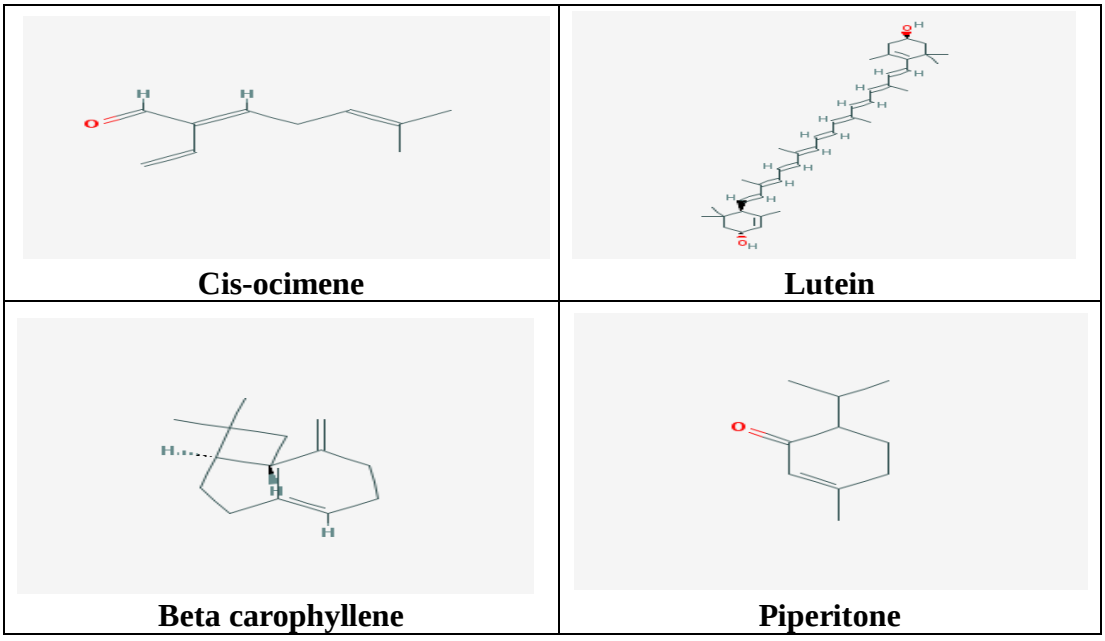


Fig 2: 2d Structure of Ligand Compounds Retrieved From Pubchem Database.

Prepared ligands were docked against the target Odorant Binding Protein (PDB ID 2L2C). The results of the docking study showed that the four compounds were highly binding with the target protein. The entire docked complex was visualized by using XP visualizer.

Molecular docking results based on the G-score, H-Bond and residue interaction shows binding affinity of the ligands towards protein 2L2C and are depicted in Table 1. On the basis of these parameters the binding affinity of ligand towards the odorant binding receptor are discussed. The more negative value of G-score indicates good binding affinity of the ligand with odorant binding receptor. More hydrogen bonds in the structure show the ligand having good binding mode to the receptor. Residual interaction shows where the ligand exactly binds to particular amino acid of the protein[23].

Table 1: Docking Score and H-Bond Interaction of Ligands against Mosquito Odorant Binding Protein (PDB Id 2L2C)

Sl. No	Name of compound	Compound id	G score	No. of H bonds	Distance	Protein residues	Ligand atom
1	Cis-ocimene	5369951	-3.7	1	1.986	TRP 114: (H)HE1	O
2	Lutein	5281243	-6.12	-	-	-	-
3	Beta caryophyllene	5281515	-5.61	-	-	-	-
4	Piperitone	6987	-4.19	-	-	-	-

The results of molecular docking studies depicted in table 1 show that the selected four bioactive compounds bound with the target Odorant Binding Protein producing good glide scores. Compound id 5369951 (Cis-ocimene) exhibited good glide score (-3.7) and formed 1 H-bond with target OBP. The distance was recorded to be as 1.986. The protein residue was observed to be TRP 114: (H) HE1. If Glide score is more, the binding affinity of the ligand is higher. In concordance with the present study similar observations were recorded by Gaddaguti *et al* [24] in which compounds which are structurally similar to the DEET were docked with Schrodinger mastero software. N-Hexa decanoic acid and, 4H-1-Benzopyran-4-One,5-Hydroxy-6,7-Dimethoxy-2-(4-Methoxyphenyl) showed good docking scores with mosquito odorant binding protein 3N7H. The diagrammatic representation of the ligand Cis-ocimene docked against mosquito odorant binding protein (PDB id 2L2C) is shown in Fig. 3.

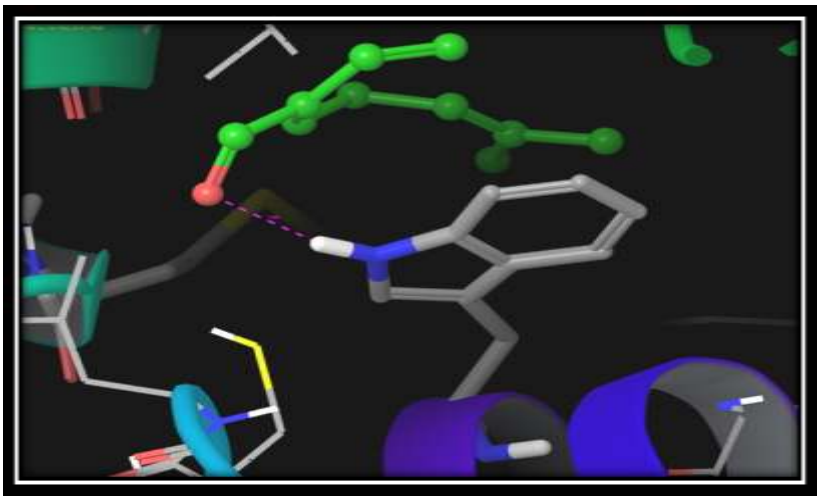


Fig 3: Compound Cis-Ocimene Docked Against Mosquito Odorant Binding Protein (PDB Id 2L2C)

The other compound id 5281243 (Lutein) were also binding with OBP but didn't produced any hydrogen bond. But the compound Lutein when docked with the Odorant Binding Protein produced an excellent glide score of -6.12. The compound id 5281515 (Beta carophyllene) and the compound id 6987 (Piperitone) were also highly binding with the mosquito OBP 2L2C, but didn't produce any hydrogen bonding with the protein. The results clearly established high binding affinities of Beta-carophyllene and Piperitone with glide scores of -5.61 and -4.19 respectively. In another study docking results of various chemical components of tulsi and mamejavo by Argus lab and Swissdock was carried out by Hetal *et al* [25]. Their results suggested that tulsi and mamejavo components like apigenin, luteolin, carvacrol, rosmarinic acid, ajmalicine and swertiamarin can be used as a lead molecule against *Plasmodial LDH* enzyme for performing *in vitro* and *in vivo* study.

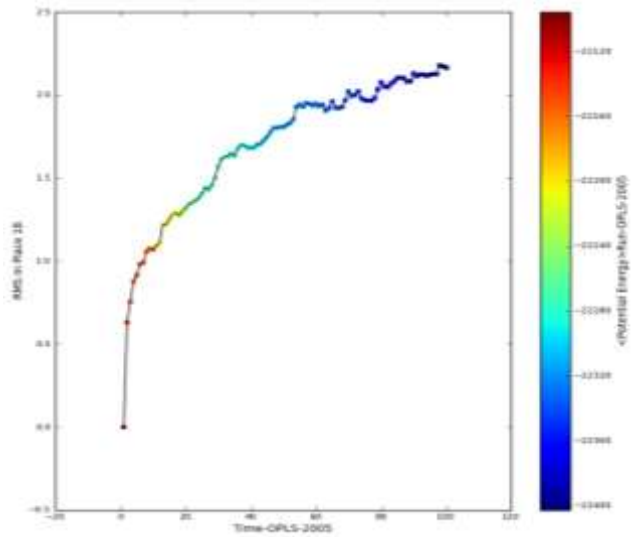
Molecular Dynamics

Besides getting the most reliable conformation of complex PDB id 2L2C with ligand Cis-ocimene, Molecular Dynamics Simulation (MDS) were also conducted as a refinement from docking result. There are several ways to analyze Molecular dynamics simulation result. In this study the Root Mean Square map and total potential energy plot of complex conformation and ligand interaction between mosquito OBP (PDB id 2L2C) and compound 5369951 (Cis-ocimene) were reviewed.

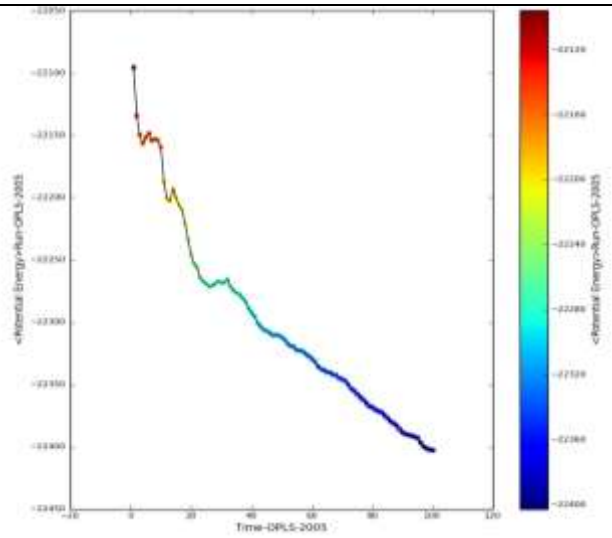
The MD simulation was carried out for the complex of compound 5369951 (Cis-ocimene) with the mosquito OBP (PDB id 2L2C). The final trajectory files were taken for calculating

the Root Mean Square Deviation (RMSD) of the complex structures. While running MD simulation for 5369951 (Cis-ocimene)-2L2C complex for 100 ps, the RMSD plot shows the stability of the complex structures at 90ps (Fig 4a). Graphical representation of Time vs. Potential energy map for 5369951 (Cis-ocimene)-2L2C complex structure during molecular dynamics simulation for 100ps is exhibited in figure 4b.

Fig 4: Graphical Representation of Molecular Dynamics Simulation Studies For Docked Complex.



a)Time vs. RMS map for compound 5369951 (Cis-ocimene) - 2L2C complex structure for 100ps



b)Time vs. Potential energy map for compound 5369951 (Cis-ocimene) - 2L2C complex structure for 100ps

Similar study by Tambunan *et al* [26] in which the interaction of ligands as inhibitors for protein in solvent explicit condition was carried out by performing molecular dynamics simulation at 300 and 312 K. The results provide conformational changes of enzyme-inhibitor complex that is shown by RMSD values and the complex was stable during simulation at both temperatures. Total potential energy plot could be used to overview system conformation changes during simulation.

The results of molecular dynamics showed that the bonding between the target protein and the ligands were stable and from total potential energy plot, it can be inferred that the ligand Cis-ocimene inhibited OBP 2L2C by interacting with its important residues without affecting the stability of the protein. Molecular dynamics confirmed the results of molecular docking.

CONCLUSION

Molecular dynamics simulation is a computation approach in which atoms and molecules are allowed to interact with each other during a certain time period so that system behavior can be observed. Fast and inexpensive docking protocols can be combined with accurate but more costly MD techniques to predict more reliable protein ligand complexes. The strength of this combination lies in their complementary strengths and weaknesses. From the results of docking interactions along with its confirmation by molecular dynamics simulation in the present investigation, the potentiality of the compound Cis-ocimene in inhibiting the host seeking and feeding behavior of the *C. quinquefasciatus* is clarified. As Cis-ocimene is a natural compound of plant origin, it may play a crucial role in being designed as an effective mosquito repellent. Therefore, to assess the efficient therapeutic properties with minimum side effects, application of advanced methods like computational techniques play a crucial role in designing and development of drug of interest.

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