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Research Article

In silico Molecular Docking and Molecular Dynamics Applications in the Designing of a New Mosquito Repellent from the Plant *Calotropis gigantea* Targeting the Odorant Binding Protein of *Culex quinquefasciatus*

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Abstract

Mosquitoes are one of the most medically significant vectors and they transmit parasites and pathogens which continue to have devastating impact on human beings. An obvious method for the control of contact between vector and human beings is the use of repellents and many synthetic agents that have been developed and employed successively. But the growing toxicity problem, together with the incidence of insect resistance, has called attention for the search of insecticides. In the present investigation, biological activity prediction, molecular docking and molecular dynamics (MD) simulation experiments were performed on potential ligands of the plant *Calotropis gigantea* to the Odorant Binding Protein of the mosquito *Culex quinquefasciatus*. To explore the binding mechanism of the phytochemicals with the target protein, the mosquito OBP (PDB id 2L2C) with known attractive activities was docked with the important secondary metabolites of the plant *C.gigantea* with excellent repellent activities. Results suggested that among the three phytochemicals selected [di(2-ethylhexyl) phthalate, beta amyrin and alpha amyrin] beta amyrin was found to be highly binding with the OBP producing a good glide score and hydrogen bonds than the other two and hence can be used to design new and more efficient natural mosquito repellent.

1 INTRODUCTION

Mosquito are a serious threat to public health through which several dangerous diseases are transmitted in both animals and human beings[1]. Vector control is a global problem. Control measures may be directed against the immature or adult stages of mosquitoes. The problems of vector control differ from country to country and may not be similar even in different areas of the same country[2]. Mosquito-borne disease not only cause high levels of morbidity and mortality, but also inflict great economic impact, including loss in commercial and labor output, particularly, in tropical and subtropical countries. However, no part of the world is free from these diseases[3]. The residual spraying of insecticides is the most common method of vector control, but usefulness of insecticides in the control of vector-borne diseases is limited[4].

Repeated use of chemical insecticides is harmful to human health and environment. Even DEET the world's most popular and efficient repellent is now reported to be non protective against some dangerous mosquito species and it requires frequent applications[5]. Low irritancy may represent a serious risk against personal protection and in some cases airway irritation have been reported with the use of these products in indoor application[6]. Multiple preparations from naturally occurring sources are repellent to certain insects. The use of scientifically proven non-chemical methods and limited use of drug is being considered as safety to environment and human health[7,8]. Numerous plant products have been reported as insect antifeedants and repellents[7].

Application of repellents to the skin is a common practice of personal protection[9]. In recent years interest in plant-based products has been revived because of the development of resistance, cross-resistance and possible toxicity hazards associated with synthetic insecticides and the rise of their cost. Phytochemicals which are obtained from huge diversity of plant species are important source for safe and easily biodegradable chemicals, which can be screened for their mosquito repellent and insecticidal activities and tested for mammalian toxicity[10]. The increasing need for a new drug has led to the computational prediction of potential drugs by the process of drug docking. The process involves the prediction of ligand conformation and orientation within the targeted binding site[11]. Odorant binding proteins are thought to be the primary proteins involved in the transport of odorants and pheromones to the olfactory receptors in insects[12,13]. Members of these protein families have been identified in a number of insect species including *Culex quinquefasciatus*[14] and it helps in its host identification.

Molecular recognitions including drug-protein interactions play important roles in many biological processes such as signal transduction, cell regulation, and other macromolecular assemblies. Therefore, determination of the binding mode and affinity between the constituent molecules in molecular recognition is crucial in understanding the interaction mechanisms and to design therapeutic interventions. Due to the difficulties and economic cost of the experimental methods for determining the structures of complexes, computational methods such as molecular docking are desired for predicting putative binding modes and affinities. [15-21]. Keeping this in view the present investigation was carried to understand the interaction mechanism of the Odorant Binding Protein of *Culex quinquefasciatus* mosquito with the selected important ligands of the plant *Calotropis gigantea*.

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2 MATERIALS AND METHODS

2.1 Selection of Ligands from *Calotropis gigantea*

The important phytochemicals of the plant *Calotropis gigantea* selected from the previously published literatures such as di(2-

ethylhexyl) phthalate[22,23], beta amyrin[24-26] and alpha amyrin[27,28] were used in the present investigation for the computational prediction of potential drugs from it by the process of molecular docking.

2.2 Molecular Docking Studies

2.2.1. Target Protein Retrieval and Preparation

Three dimensional NMR structure of mosquito Odorant Binding Protein (PDB id: 2L2C) was obtained from PDB databank (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.

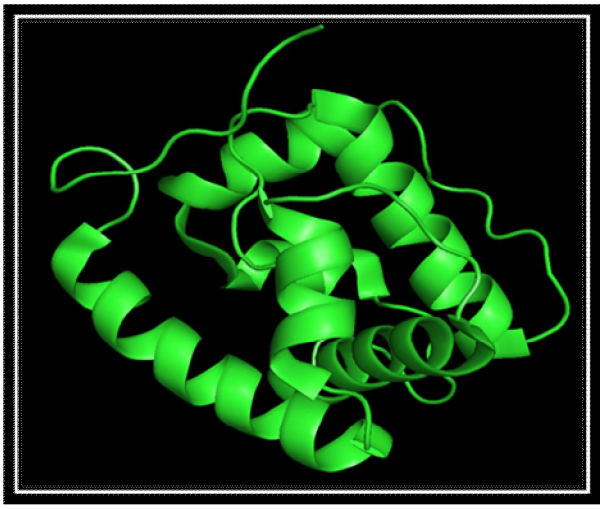


Fig.1: Three dimensional structure of mosquito Odorant Binding Protein (PDB id 2L2C)

2.2.2. 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.

2.2.3. Ligands Retrieval and Preparation

Ligand molecules were retrieved from pubchem database. The following compounds were retrieved in 3D SDF format (Pubchem id: CID_8343, CID_201783, CID_73170). All the three compounds were processed, unwanted structures were eliminated and optimized using LigPrep module from Schrodinger.

2.3 Biological Activity Prediction

The activities of processed three secondary metabolites were predicted using PASS (Prediction of Activity Spectra for Substances) online server. The PASS software product, which predicts more than 300 pharmacological effects and biochemical mechanisms on the basis of the structural formula of a substance, may be efficiently used to find new targets (mechanisms) for some ligands and, conversely, to reveal new ligands for some biological targets[29]. The mean accuracy of prediction with PASS is about 86% in LOO cross-validation[30]. The tool uses the descriptors to predict the activity of a substance.

2.4 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. All the three 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.

2.5 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.

Table 1: Activity of Di (2-ethylhexyl) phthalate

Pa	Pi	Activity	Pa	Pi	Activity
0,973	0,002	Eye irritation, inactive	0,773	0,004	Phenol O-methyltransferase inhibitor
0,949	0,003	Skin irritation, inactive	0,777	0,010	5-O-(4-coumaroyl)-D-quinate 3'-monooxygenase inhibitor
0,927	0,002	Cutinase inhibitor	0,771	0,005	Anesthetic general
0,888	0,005	Sugar-phosphatase inhibitor	0,771	0,010	Carboxypeptidase Taq inhibitor
0,876	0,008	Alkenylglycerophosphocholine hydrolase inhibitor	0,772	0,012	Arginine 2-monooxygenase inhibitor
0,847	0,004	Lipid metabolism regulator	0,793	0,036	Aspulvinone dimethylallyltransferase inhibitor
0,854	0,016	Ubiquinol-cytochrome-c reductase inhibitor	0,764	0,009	IgA-specific serine endopeptidase inhibitor
0,836	0,004	Acetylesterase inhibitor	0,768	0,017	Sphinganine kinase inhibitor
0,848	0,017	Phobic disorders treatment	0,749	0,008	Lipoprotein lipase inhibitor
0,824	0,004	Gluconate 5-dehydrogenase inhibitor	0,769	0,029	CYP2J substrate
0,831	0,014	Acrocylindropepsin inhibitor	0,750	0,012	Exoribonuclease II inhibitor
0,831	0,014	Chymosin inhibitor	0,768	0,044	CYP2C12 substrate
0,831	0,014	Saccharopepsin inhibitor	0,744	0,023	CYP2J2 substrate
0,820	0,004	All-trans-retinyl-palmitate hydrolase inhibitor	0,723	0,002	Sclerosant
0,829	0,015	Polyporopepsin inhibitor	0,727	0,010	Macrophage colony stimulating factor agonist
0,813	0,007	Pullulanase inhibitor	0,730	0,016	Alkylacetylglycerophosphatase inhibitor
0,819	0,015	Antiseborrheic	0,721	0,013	Lysine 2,3-aminomutase inhibitor
0,803	0,014	Pro-opiomelanocortin converting enzyme inhibitor	0,711	0,005	Anthranilate-CoA ligase inhibitor
0,785	0,004	Spasmolytic, Papaverin-like	0,705	0,008	Cholesterol antagonist
0,784	0,012	Membrane permeability inhibitor	0,702	0,006	Poly(beta-D-mannuronate) lyase inhibitor
0,787	0,016	5 Hydroxytryptamine release stimulant	0,704	0,008	Poly(alpha-L-guluronate) lyase inhibitor
0,797	0,026	Testosterone 17beta-dehydrogenase (NADP+) inhibitor	0,703	0,021	Fibrinolytic

(Pa – probability of active, Pi – probability of inactive)

Table 2: Activity of Beta amyrin

Pa	Pi	Activity	Pa	Pi	Activity
0,974	0,002	Caspase 3 stimulant	0,816	0,001	ICAM1 expression inhibitor
0,957	0,002	Insulin promoter	0,823	0,015	Alkenylglycerophosphocholine hydrolase inhibitor
0,938	0,002	Hepatoprotectant	0,808	0,002	Antinociceptive
0,934	0,001	Transcription factor NF kappa B stimulant	0,806	0,004	Antineoplastic (lung cancer)
0,934	0,001	Transcription factor stimulant	0,790	0,004	Antiulcerative
0,912	0,005	Antineoplastic	0,794	0,027	Testosterone 17beta-dehydrogenase (NADP+) inhibitor
0,906	0,002	Antiviral (Influenza)	0,766	0,003	Chitinase inhibitor
0,903	0,002	Oxidoreductase inhibitor	0,763	0,004	Lipid peroxidase inhibitor
0,899	0,004	Apoptosis agonist	0,754	0,005	Phosphatase inhibitor
0,894	0,004	Lipid metabolism regulator	0,751	0,003	Nitric oxide antagonist
0,879	0,001	Caspase 8 stimulant	0,769	0,029	CYP2J substrate
0,880	0,003	Membrane integrity antagonist	0,736	0,007	Antisecretoric
0,875	0,006	Mucomembranous protector	0,723	0,001	DNA ligase (ATP) inhibitor
0,860	0,003	Hepatic disorders treatment	0,716	0,004	Gastrin inhibitor
0,855	0,003	Chemopreventive	0,713	0,004	Wound healing agent
0,850	0,005	Antiinflammatory	0,714	0,005	Antineoplastic (breast cancer)

(Pa – probability of active, Pi – probability of inactive)

Table 3: Activity of Alpha amyrin

Pa	Pi	Activity	Pa	Pi	Activity
0,934	0,002	Insulin promoter	0,835	0,002	Nitric oxide antagonist
0,926	0,002	Hepatoprotectant	0,826	0,019	Testosterone 17beta-dehydrogenase (NADP+) inhibitor
0,911	0,004	Apoptosis agonist	0,808	0,003	Lipid peroxidase inhibitor
0,901	0,005	Antineoplastic	0,808	0,017	Alkenylglycerophosphocholine hydrolase inhibitor
0,897	0,002	Transcription factor NF kappa B stimulant	0,793	0,003	Antiviral (Influenza)
0,897	0,002	Transcription factor stimulant	0,789	0,002	Caspase 8 stimulant
0,890	0,003	Chemopreventive	0,788	0,002	Antinociceptive
0,889	0,004	Antiinflammatory	0,781	0,011	Alkylacetylglycerophosphatase inhibitor
0,885	0,003	Oxidoreductase inhibitor	0,773	0,004	Phosphatase inhibitor
0,878	0,003	Antiprotozoal (Leishmania)	0,772	0,003	Wound healing agent
0,876	0,003	Hepatic disorders treatment	0,782	0,015	Acylcarnitine hydrolase inhibitor
0,864	0,004	Caspase 3 stimulant	0,753	0,001	DNA ligase (ATP) inhibitor
0,865	0,007	Mucomembranous protector	0,755	0,006	Antisecretoric
0,851	0,005	Hypolipemic	0,731	0,004	Gastrin inhibitor
0,840	0,003	Antiulcerative	0,756	0,033	CYP2J substrate
0,839	0,005	Membrane integrity antagonist	0,716	0,005	Antineoplastic (lung cancer)

(Pa – probability of active, Pi – probability of inactive)

3 RESULTS AND DISCUSSION

3.1 Biological Activity Prediction

The 3D SDF structures of the processed three secondary metabolites were given as an input for PASS server. The PASS server provides all the possible activities of the given secondary metabolites. PASS can be effectively applied to predict biological potential of compounds and to analyze large chemical databases. The activity of the compounds di(2-ethylhexyl) phthalate is presented in the Table 1. It exhibits a number of biological activities. The structural activity of beta amyrin and alpha amyrin provided by the PASS server are presented in the Table 2 and 3 respectively. PASS predicted search results show all the available information on the pharmacological and toxicological activity of all the three compounds analysed.

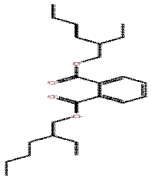
Similar observation in accordance with the present study using PASS server was already reported by many researchers. De Britto *et al*[31] used PASS to predict the biological activity profile of seven secondary metabolites and successfully compared the PASS predictions with the available information on the pharmacological and toxicological activity of these compounds. The antiviral activities of selected seven compounds were confirmed in another work by Narayanan and Velmurugan[32]

The result suggests that the compounds di(2-ethylhexyl) phthalate, beta amyrin and alpha amyrin are highly active exhibiting a number of biological activities. Pa and Pi are the estimates of probability to be active and inactive respectively from the biological activity spectrum. Their values vary from zero to one.

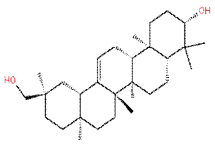
Each active compound possesses a number of biological activities. Its specificity of action is always relative and is defined by the peculiarities of object, dose, route, etc. Biological activity spectrum of compound can be predicted on the basis of structure-activity relationships found by the analysis of the known data from the training set. Based on the analysis of large training set consisting of tens of thousands of the known biologically active compounds, computer program PASS provides the means to evaluate any new compound in huge chemical-pharmacological space.

3.2 Molecular Docking

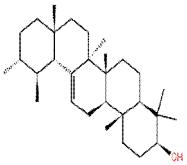
All three compounds were prepared to dock with the mosquito Odorant Binding Protein 2L2C (Fig.1). The chemical structure of the three important ligands selected from *Caloptropis gigantea* were retrieved from Pubchem database and were shown in Fig.2.



Di (2-ethylhexyl) phthalate



Beta amyrin



Alpha amyrin

Fig. 2: 2D structure of ligands retrieved from Pubchem database

Table 4: Docking Score and H-bond interaction of ligands against mosquito Odorant Binding Protein (PDB id 2L2C)

Sr. No	Name of compound	Compound id	G score	No. of H bonds	Distance	Protein residues	Ligand atom
1	Di(2-ethylhexyl) phthalate	8343	-8.66	-	-	-	-
2	Beta amyrin	201783	-6.73	1	2.308	HIS111:(N) NE2	H
4	Alpha amyrin	73170	-5.7	-	-	-	-

All the three compounds were found to be binding with the mosquito OBP (PDB id 2L2C) and were used for further docking studies. The glide score, number of H-bonds, distance of H-bonds, interacted residues and ligand atom of docked compounds are exhibited in Table 4.

Compound id 201783 exhibited good glide score (-6.73A^0**)** and formed 1 H-bond with target OBP. The other two compounds di (2-ethylhexyl) phthalate and alpha amyrin were highly binding with the mosquito OBP. Di (2-ethylhexyl) phthalate, an important component of *C.gigantea* displayed an excellent glide score of (-8.66 A^0) when it is docked with the mosquito OBP, but didn't produce any hydrogen bond. The compound alpha amyrin, when docked with the mosquito OBP (PDB id 2L2C) recorded a glide score of (-5.7 A^0). In accordance with the present investigation similar study of *in silico* docking analysis was carried out by Suresh *et al*[33] to assess the mosquito larvicidal potential of three terpene compounds isolated from *C.gigantea* which revealed the potential of *α*- amyrin against AeSCP-2.

From the above results it's confirmed that the compound beta amyrin with compound id 201783 was found to be best among the three compounds selected for the study, as it recorded the formation of one hydrogen bond and also exhibited a good glide score. Therefore compound id 201783 was carried forward for further molecular dynamics simulation studies. The diagrammatic representation of the compound id 201783 docked against mosquito Odorant Binding Protein (PDB id 2L2C) is given in Fig. 3.

The results of the present study were in concordance with the early reports of many researchers. In the present investigation beta amyrin was found to be highly binding as well as interacting with the mosquito OBP, therefore it may have an ability to suppress human seeking behavior of mosquitoes and thereby preventing the man-vector contact. Similar observation was reported in the study of *in silico* molecular docking of mosquito repellent compounds from *Hyptis suaveolens* by Gaddaguti *et al*[34]. The result revealed that gamma sitosterol isolated from the methonolic extracts of *H.suaveolens* has high binding affinity than the known predominant odour binding protein compounds decanol and can be further used for designing of potential natural mosquito repellent.

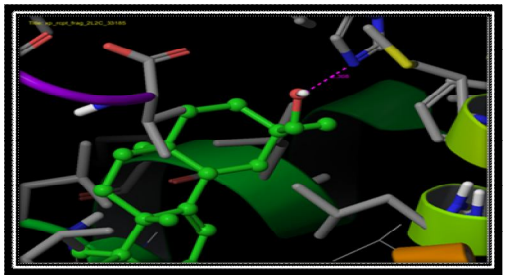


Fig. 3: Compound 201783 docked against mosquito odorant binding protein (PDB id 2L2C)

3.3 Molecular Dynamics

The MD simulation was carried out for the complex of compound 201783 - mosquito odorant binding protein (PDB id 2L2C) to evaluate the structural stability. The final trajectory files were taken for calculating the Random Mean Square Deviation (RMSD) of the complex structures. Molecular dynamics will help in the better understanding of the ligand binding site of the mosquito Odorant Binding Protein 2L2C.

While running MD simulation for 201783-2L2C complex for 100 ps, the RMSD plot shows the stability of the complex structures at 90ps (Fig. 4 a). Graphical representation of Time vs. Potential energy map for 201783 - 2L2C complex structure during molecular dynamics simulation for 100ps is shown in Fig. 4 b. Similar study was carried out by Affonso *et al*[35] in which docking and molecular dynamics performed on potential ligands to the Odorant Binding Protein of the mosquito *Anopheles gambiae* (*Ag*OBP1) suggested eugenyl acetate as a better repellent than DEET against the main vector of malaria.

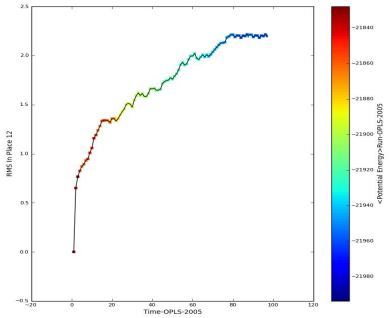


Fig. 4 a: Graphical representation of Time vs. RMS map for 201783 - 2L2C complex structure during molecular dynamics simulation for 100ps

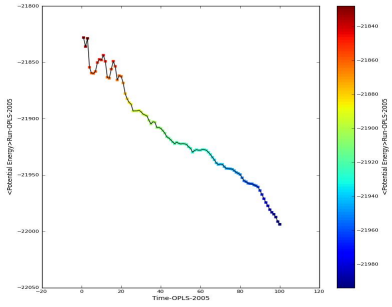


Fig. 4 b: Graphical representation of Time vs. Potential energy map for 201783 - 2L2C complex structure during molecular dynamics simulation for 100ps

The results of the RMSD plot revealed that the ligand structures perfectly occupied within the binding sites of the protein during Molecular Dynamic simulation studies and also thereby confirmed the stability of the bonds and the results that were obtained by molecular docking.

4.0 CONCLUSION

The protein-ligand interaction plays a significant role in structure based drug designing. The plant *C.gigantea* is easily available in most of the agricultural and non-agricultural fields and the usage of this plant for medicinal purpose was reported by several researchers. Although *C.gigantea* was used as a very famous traditional folk medicine by many cultures, and also has been subjected to extensive phytochemical and bioactive investigations, the computational prediction of potential drugs from it by the process of drug docking have not been completely investigated yet. In the present work, three phytochemicals namely di(2-ethylhexyl) phthalate, beta amyrin and alpha amyrin from the plant were selected for screening against the Odorant Binding Protein 2L2C of *C. quinquefasciatus* mosquito. The results suggested that all the three were highly binding with the OBP, but beta amyrin was found to be interacting better than the other two producing a good glide score and the bonds showed good structural stability in molecular dynamics simulation experiments and therefore it may have an ability to suppress human seeking behavior of mosquitoes. From these results, it can be concluded that since the identified compound beta amyrin is a natural compound with repellent activity, the compound may be a better option to be designed as an efficient mosquito repellent than the existing harmful synthetic mosquito repellents such as DEET and other known mosquito repellents.

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