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RESEARCH ARTICLE

HOMOLOGY MODELLING OF *Culex quinquefasciatus* SALIVARY GLAND PROTEIN AND MOLECULAR DOCKING STUDY USING COMPOUNDS FROM *Capsicum annum* L.

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ABSTRACT

Salivary gland protein of *Culex quinquefasciatus* was obtained by Homology modelling technique. Ramachandran plot analysis showed that the portion of residues falling into the most favoured regions was (90.9 %). Predicted and validated structure is useful in structure based drug designing. Three compounds of *Capsicum annum* L. were selected through literature survey and Molecular docking studies were done using Schrodinger Mastro software. Results showed that out of three ligands taken from pub chem., Homocapsaicin 6442566 had glide score of -1.9 as compared to phytol 5280435 of -1.46. It was also observed that Capsanthin 5281228 had no glide score.

INTRODUCTION

Plants constitute major source of drugs for prevention and spread of wide range of pathogenic carriers and also treating various diseases of human beings. Modern people increasingly prefer drugs of natural origin mostly from plant origin due to abundant accessibility and fewer side effects. Whereas synthetic drugs and antibiotics often cause wide spread toxicity and harmful side effects to the end user other than targeted health condition / pathogen carrier. In search of novel active compounds from plant origin, and assess the efficient thereupatic properties with minimum side effects (Gaddaguti *et al*., 2012). Homology modelling is basically used for the prediction of protein structure and it constructs an atomic-resolution model of a protein from amino acid of query sequence. The quality of the homology model is dependent on the quality of the sequence alignment and template structure (Marti-Renom *et al*., 2000). Molecular Docking and Virtual Screening based studies on molecular level have become an integral part of many modern structure-based drug discovery efforts. Hence, knowledge of the protein and ligand interactions with the specific drugs may provide a significant insight into the binding interactions and relativeness of the drug (Brooijmans and Kuntz, 2003). The three-dimensional (3D) structure details of proteins are of major importance in providing insights into their molecular functions. Further analysis of 3D structures will help in the identification of binding sites and may lead to the designing of new drugs. Homology modelling is only a viable technique because it produces models that can be used for further research. Homology modelling helps in predicting the 3-D structure of a macromolecule with unknown structure (target) by comparing it with a known template (Malakar *et al*., 2011). Since there was no structure reported to this protein, the main aim of this study is to predict validated three dimensional structures from the protein sequence by comparative studies. Modelling tools and related structural data available on the online databases are used for structure prediction.

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MATERIALS AND METHODS

Molecular Modelling and Docking

Molecular docking of target proteins with inhibitory molecules

The receptor grids and the basic Glide settings for the ligand docking were specified. And specified a set of inhibitory molecules to dock and also specified the output options. The options in the remaining three tabs, Core, Constraints and Similarity can be left at their defaults for this exercise. Specifying Ligands to Dock and selected appropriated inhibitory molecules from workspace for docking with target proteins. Docked complex were examined with an emphasis on visual rather than numerical appraisal. Docked complex were visualized using XP visualizer.

Retrieval and Homology modelling of protein

The putative salivary gland protein sequence retrieved from NCBI database, it has no X-ray 3D structure hence homology modelling techniques adopted for predicting structure.

Template searching

An attempt was made to find a suitable template protein for the modelling of the target protein. The template protein was searched through Blast, which is an online tool for searching based on similar sequences and structure-wise similarity. From the homology searching, one template was selected. High-resolution X-ray crystallography structures of Chain A, *Anopheles Gambiae* Odorant-Binding [3L47A].

Sequence Alignment

Amino acid sequence alignment of target and template protein was derived using Bio edit programme. Default parameters were applied and the aligned sequences were inspected and adjusted manually to minimize the number of gaps and insertions.

3D structure prediction

A rough 3-D model was constructed from the sequence alignment between OBP and the template proteins using MODELLER 9v0 [10] (http://salilab.org/modeller/) with parameters of energy minimization value and five models were derived from modeller.

Evaluation of Refined Model

In the last step of homology modelling, the refined structure of the model was subjected to a series of tests for testing its internal consistency and reliability. Backbone conformation was evaluated by the inspection of the Psi/Phi Ramachandran plot obtained from PROCHECK, Verify3D and ERRAT has been performed.

Preparation of Protein

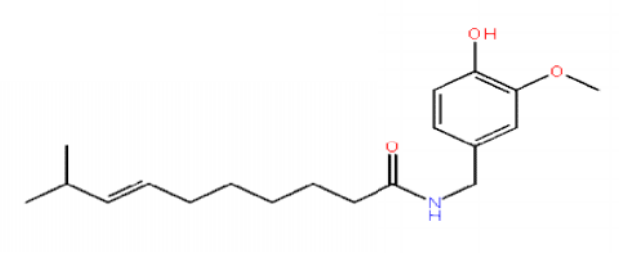
Three dimensional structures of proteins were imported into Maestro. Water molecules can be deleted and adjusted the protein, metal ions, and cofactors, Charges, bond orders, formal changes of co-factors and orientation were fixed. The prepared structures were examined. The orientation of water molecules and other groups, such as hydroxyls and amides were checked.

Grid generation

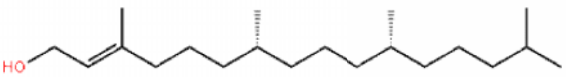
Grid files represent physical properties of a volume of the receptor (specifically the active site) that are searched when attempting to dock a ligand. The complex for this exercise is actually in two files, one containing the receptor and another containing the ligand. The prepared protein is displayed in the Workspace. The protein structure is displayed in ribbon representation. From the applications menu in the main window, select Glide > Receptor Grid Generation. The Receptor Grid Generation panel opened with the Receptor tab displayed. Dark green markers appear on the ligand. In the Vander Waals radii scaling section, we choose Scaling factor default value of 1.00 (no scaling.) The purple enclosing box represents the volume of the protein for which grids were calculated and made the enclosing box as small as consistent with the shape and character of the protein's active site and with the ligands to expect to dock.

Inhibitory molecules retrieval and preparation

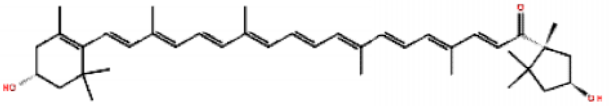
Inhibitory molecules were retrieved from pub chem database. Figure 1 shows the retrieved compounds in 3D SDF format (Pub chem id: CID_5280435, CID_5281228, CID_6442566). Retrieved molecules were prepared for docking using Ligand Preparation module from Schrodinger suit, this process consists of a series of steps that perform conversions, apply corrections to the structures, generate variations on the structures, eliminate unwanted structures and optimize the structures.



6442566 Homocapsaicin



5280435 Phytol



5281228 Capsanthin

Figure 1. Compounds retrieved from pub chem database

Molecular docking of target protein with inhibitory molecules

The receptor grids and the basic Glide settings for the ligand docking were specified set of inhibitory molecules to dock and output options also specified. The options in the remaining three tabs, Core, Constraints, and Similarity, can be left at their defaults for specifying ligands to dock. There are several methods for specifying ligand structures to be docked with receptor grids. We selected appropriated inhibitory molecules from workspace for docking with target proteins.

Visualization of docked complex

Docked complex were examined with an emphasis on visual rather than numerical appraisal. Docked complex were visualized using XP visualizer.

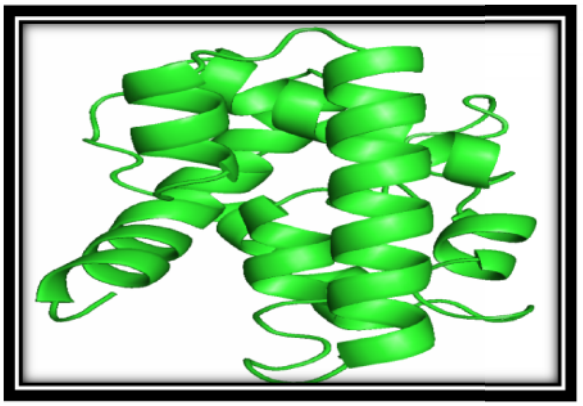


Figure 2. Modelled 3D structure of C. quinquefasciatus salivary gland protein

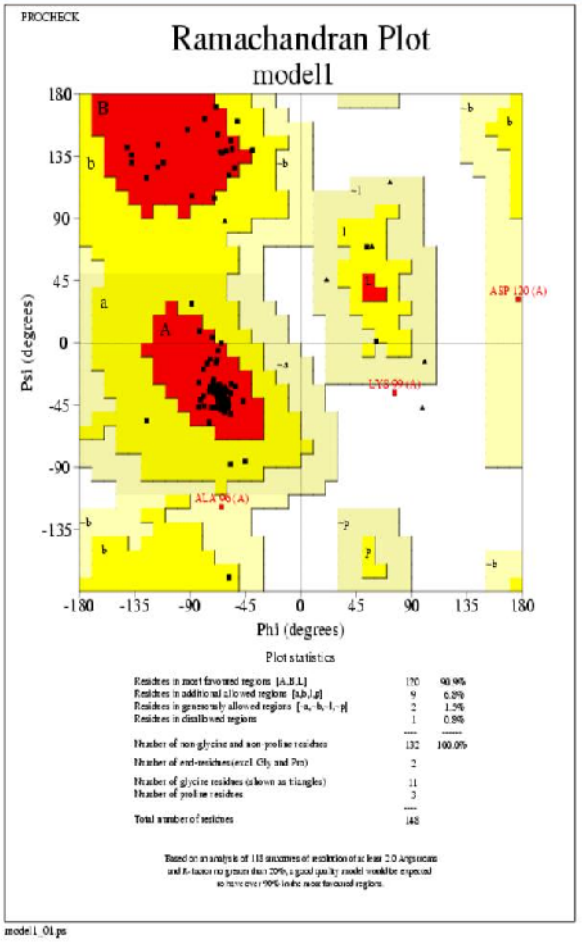


Figure 3. PROCHECK analysis of *Culex quinquefasciatus* salivary gland protein

RESULTS AND DISCUSSION

Protein 3D structure prediction and Validation

The three-dimensional (3D) structure details of proteins are of major importance in providing insights into their molecular functions. Further analysis of 3D structures will help in the identification of binding sites and may lead to the potential target. Homology modelling is only a viable technique because it produces model and sequence was obtained from sequence database and was submitted to blast-p search against PDB database. After the BLAST analysis, the appropriate template was selected. Modeller 9.v used for model the protein. Modelled 3D structure of Culex *quinquefasciatus* salivary gland protein showed in Figure 2. PROCHECK, Verify3D (Table 1) and ERRAT (Fig 4) were used to validate the model. The total energy values of the predicted 3-D model were calculated as 90.09 of Ramachandran plot (Fig 3).

Table 1. Validation of Culex quinquefasciatus salivary gland protein Using SAVS

Protein name	Procheck	Verify 3D	Errat
Culex quinquefasciatus Salaivary gland protein	90.09	83.22% of the residues had an averaged 3D-1D score > 0.2	39.286

Molecular docking of inhibitory molecules against Salivary gland proteins

Docking of inhibitory molecules against *C. quinquefasciatus* Salivary Gland

Totally 3 compounds has been selected for docking studies out of 3 compounds 2 compounds binding with Culex quinquefasciatus Salivary gland protein. The Glide score, number of H- bonds, Distance of H- Bonds, interacted residues and ligand atom showed in (Table 2) Compound 6442566 Homocapsaicin with (2H- bonds and Glide score, -1.9 shown in Fig 5) was high Followed by Homocapsaicin, compound 5280435 Phytol with(1H- bond and Glide score,-1.46 shown in Fig 6) interaction against target Salivary gland protein was low when compared to Homocapsaicin showed in (Fig1). Remaining compound 5281228 Capsanthin was not highly bound against Salivary gland protein and G Score was poor. Ryser *et al*. (2005) studied the four hydrogen bonds formed between 2-nitro-5-sulfosulfanyl-benzoic acid into PDI. Besides hydrogen bonding, van der Waals interactions were also taking part in the stabilizations of inhibitors binding with high frequency of residues such as Ala34, Trp36, Cys40, His39, Thr68 and Phe80 in PDI. The redox-inhibitory mode of all six inhibitors with PDI was consistent with the laboratory experimental results. Hence, in the present study 6442566 Homocapsaicin with (2H- bonds and Glide score, -1.9) was high Followed by Homocapsaicin, compound 5280435 Phytol with (1H- bond and Glide score,-1.46) interaction against target Salivary gland protein was low when compared to Homocapsaicin.

Table 2. Docking Score of inhibitory molecules against *C. quinquefasciatus* salivary gland protein

S. No	Compounds	Glide score	No of H bonds	Distance	Protein Residues	Ligand atom
1.	6442566	-1.9	2	2.163	HID 32: (H) HD1	(O)
				1.485	ALA6 : (O) O	(H)
2.	5280435	-1.46	1	1.900	ASP 29: (O) OD2	(H)
3.	5281228	-0.08	0	-	-	

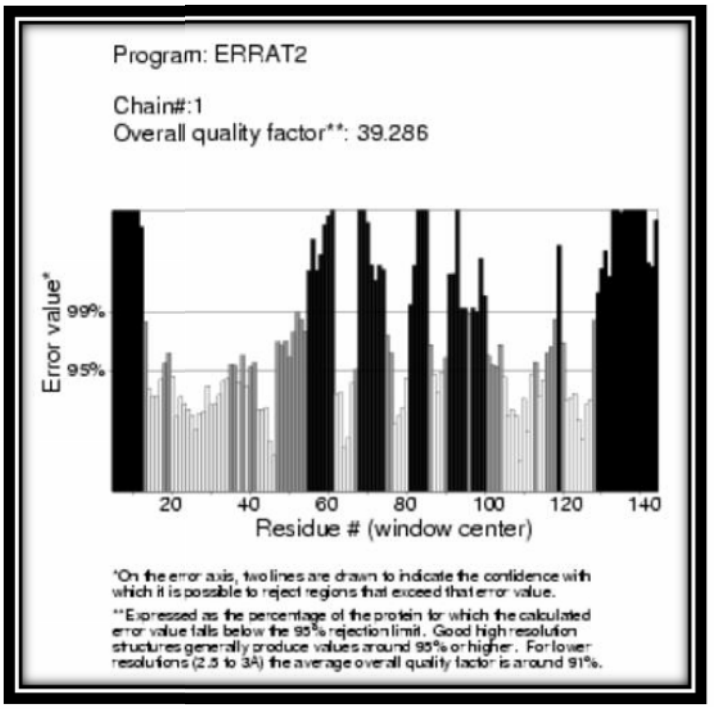


Figure 4. The graphical representation of ERRAT2 for *Culex quinquefasciatus* salivary gland protein

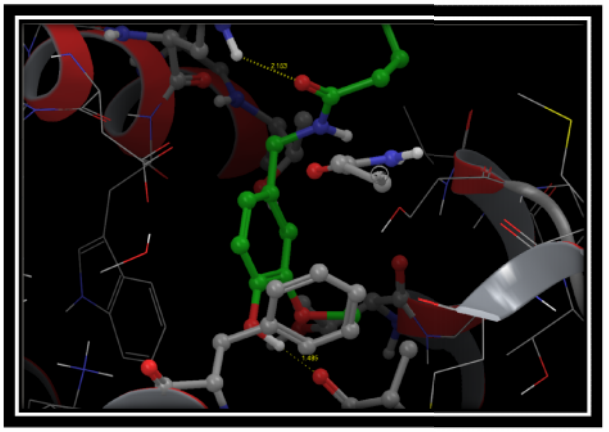


Figure 5. Compound 6442566 Docked against *Culex quinquefasciatus* Salivary gland protein

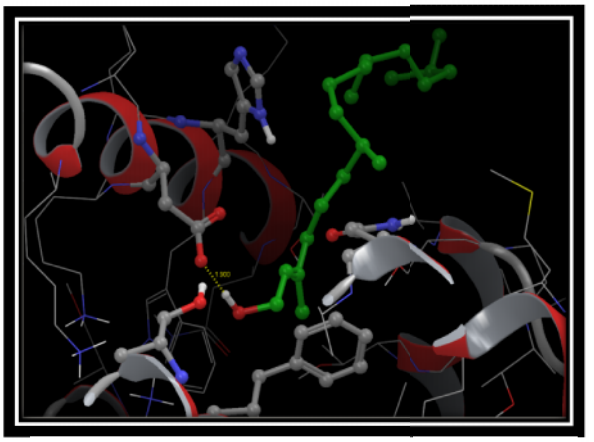


Figure 6. Compound 5280435 Docked against *Culex quinquefasciatus* Salivary gland protein

Conclusion

Chemical based interventions for mosquito control and repeated use of insecticides leads to resistance among vector hence its control has been explored by insilico molecular docking study between compounds of *Capsicum annum* L. and salivary gland protein of *C. quinquefasciatus*. Docking results showed that compounds acting on the target protein hence Homocapsaicin and phytol can be used as a candidate in vector control.

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