Uttar Pradesh Journal of Zoology

Volume 45, Issue 16, Page 334-344, 2024; Article no.UPJOZ.3052
ISSN: 0256-971X (P)

Isolation and Characterisation of Octa Siloxane from *Catharanthus roseus* Leaf Extract by Molecular Docking Analysis as Insecticide Against, *Aedes vittatus*, (Diptera: Culicidae)

Chandra Anjaiah [a*] and Madhavi [a]

[a] *Department of Zoology, University college of science. Osmania University, India.*

Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

Article Information

DOI: https://doi.org/10.56557/upjoz/2024/v45i164314

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://prh.mbimph.com/review-history/3052

Original Research Article

Received: 11/10/2023
Accepted: 13/12/2023
Published: 01/08/2024

ABSTRACT

Octa siloxane compound was isolated from *Catharanthus roseus*.and the sequences of three proteins (sterol Career protein, D7 protein and odorant - binding protein) were retrieved from Swissport database. the three-dimensional structure of these proteins was downloaded from PDB Database. The compound octa siloxane isolated and characterized from Catharanthus roseus, has been studied for use as a natural insecticide. The compound was previously evaluated for their bioactive and was found to possess potent larvicidal activity against the fourth instar larvae of Ae. *vittatus*. the inhibitor octa siloxane with pronounced larvicidal activity was subjected to

**Corresponding author: Email: anjaiahchandra202@gmail.com;*

Cite as: *Anjaiah, Chandra, and Madhavi. 2024. "Isolation and Characterisation of Octa Siloxane from Catharanthus Roseus Leaf Extract by Molecular Docking Analysis As Insecticide Against, Aedes Vittatus, (Diptera: Culicidae)". UTTAR PRADESH JOURNAL OF ZOOLOGY 45 (16):334-44. https://doi.org/10.56557/upjoz/2024/v45i164314.*

computational screening and was used as a tool to study the mode of action and molecular mechanism of the selected functional protein. The ligand (compound) structure was drawn using ACD Che Sketch and converted in to PDB format using Open Babel. The 3D structures of proteins were docked with the inhibitor octa siloxane using Patch dock tool. The 2D and 3D chemical structure of the ligand/compound isolated from *Catharanthus roseus,* was retrieved from PubChem data base.

Keywords: Larvicides; Aedes vittatus; octa siloxane; docking; ligands; filariasis.

1. INTRODUCTION

Molecular Docking is a key tool in structural biology and computer -aided drug design [1]. “The need for new mosquito larvicides has fuelled the use of computational prediction of potential larvicides by a method called docking which helps to investigate the detailed intermolecular interactions between the ligand and the target protein” [2]. “Grid-based ligand docking with energetic, searches for favourable interactions between one or more typically small ligand molecules and a typically larger receptor molecule, usually a protein is preferred” [3]. “An increasing number of protein crystallographic structures are available based on structural genomic projects” [4]. “Prediction of a potential lead and its potential target is a fundamental step in order to investigate the molecular recognition mechanisms of protein” [4]. “The three dimensional (3D) structural details of a protein are of major importance in providing insight into their molecular functions. Further analysis of 3D structures will help in the identification of binding sites which may lead to the designing of new drugs” [5]. mosquitoes are vectors for diseases such as malaria, dengue, chikungunya yellow fever.

Health challenge effecting 40% of the world’s population [6]. “The increasing incidences of malaria in tropical and subtropical countries reflects the development of drug resistant strains of Plasmodium and justify referring to malaria as a re-emerging disease” [7,8]. 92 Filariasis caused by W. bancroftian is transmitted by *C. Vishnui*, which infects 80 million peoples annually and about 40 million peoples suffer from lymphatic filariasis (Bowers et al., 1995). Dengue viruses transferred their pathogenicity to human by the vector Ae. vittatus. Molecular modelling techniques are used to predict how a protein (enzyme) interacts with small molecules (ligands) [9]. “The ability of protein (enzyme) to interact with small molecules plays a major role in the dynamics of the protein which may enhance/inhibit its biological function” [10]. The host seeking and feeding behaviour of mosquitoes are much affected by host odours (Takken and Knols, 1999). “For all blood feeding insects, olfaction is considered as the main sensory modality which helps in host recognition. This host seeking behaviour is true for all pathogen transferring mosquitoes which are able to locate their vertebrate host during scotophase” [11]. “Fundamental aspects of olfactory signal transduction at the peripheral level have revealed the involvement of olfactory receptors on maxillary palps and antennae” [12]. Many studies have been corroborated by field studies, mostly with *C. Vishnui*, which have shown that these insects are attracted to human volatiles from a distance [13]. “Repellents and synthetic agents have been used to control the contact between vector and man, but an obvious method for the control of contact between vector and human beings is by the use of repellents. Many synthetic agents 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 novel insecticides” [11]. “The reduced susceptibility status of malaria and filaria vectors to the recommended insecticides of choice has posed an alarming situation in public health” [14], (Carnevale et al., 2010; 93 Matowo, 2010). “The conventional chemical pesticides have resulted in the development of resistance, undesirable effects on nontarget organism and fostered environmental and human health concerns. An alternative approach for mosquito control is the use of natural products of plant origin” [15]. “Phytochemicals have province that they are potential mosquito control agents and also a toxic chemical and synthetic insecticides” [16]. “Molecular docking and virtual screening-based studies at molecular level have become an integral part of many modern structure-based drug discovery efforts” [17]. Molecular docking approaches are generally used in modern drug design process to understand the protein ligand interactions [5]. Hence, “knowledge of the protein and ligand interactions may provide a significant insight into the binding interactions and

effectiveness of the compound as a potent larvicidal agent" (Brooijmans and Kuntz, 2003). The three-dimensional structure of the protein-ligand composite could serve as a considerable source for understanding the way the protein interacts with one another and perform biological functions [17]. Blocking of target protein in mosquito physiology and in finding the potential inhibitors is considered as a promising approach in the control of mosquitoes.

2. MATERIALS AND METHODS

2.1 Selection of Plant Leaves for Current Research Study

Catharanthus roseus (Figs. 1 & 2) plant leaves were collected from Botanical Garden Were identified by Department of Botany. University college of science. Osmania University.

2.2 Selection of Mosquito Species for Current Research Study

Aedes vittetus adult (Fig. 4) and Larva (Fig. 5) is selected for current research study

This species was identified by Prof. Madhavi Head of the Department Zoology. And Chandra Anjaiah. Researcher. Division of Medical entomology laboratory.

2.3 D7 Salivary Protein of *Aedes vittatus*

"The D7 subfamily of salivary proteins is widespread in blood sucking Diptera and belongs to the super family of pheromone/odorant binding proteins. Although D7 proteins are most abundant salivary proteins in adult female mosquitoes and sand flies, their role in blood feeding remains elusive" [18]. "Adult female sand flies and mosquitoes feed on sugar meals to obtain energy for basal metabolism and flight activities, and on blood to produce an egg batch" [19], (Tesh and Guzman, 1996). "The salivary glands of mosquitoes produce enzymes that helps in the breakdown of sugars" [20], and anti-haemostatic compounds (anticlotting, antiplatelet and vasodilatory) that helps in blood feeding [21]. Anti-microbial agents such as lysozymes are found which prevents bacterial growth in the insect crop, where the sugar meal is stored [22], (Pimentel and Rossignol, 1990, Moreira-Ferro et al., 1999). "The first female specific cDNA cloned from the salivary glands of mosquito's codes for a protein named D7 which is abundantly expressed in Aedes vittatus" [23]. "More recently these D7-related proteins were found to belong to a larger family of proteins that include the odorant binding proteins with a characteristic fold structure" [24] and are adapted to binding small ligands. D7- related proteins from blood sucking insects play an anti-haemostatic role by trapping against of haemostasis [25].

2.4 Sterol Carrier Protein-2 (SCP-2) of *Aedes Vittatus*

"SCP- 2 demands that insects must have mechanism for its uptake, transport and storage of cholesterol which is required throughout their life cycle. Intracellular transportation of cholesterol in insects must meet two important biological needs, the necessity to absorb free cholesterol for the construction of cellular membranes and to provide cholesterol as precursors for biosynthesis. These two pathways most likely utilize the same intracellular transport proteins to metabolize cholesterol" [26]. In the yellow fever mosquito, *Aedes vittaus* an independent gene has been identified that is similar to vertebrates SCP – 2 (AeSCP-2). "This protein also has high levels of expression in the midgut of the larvae and high binding affinity to cholesterol" [27]. "The mosquito SCP-2 appears to represent the unique non- peroxisomal and low molecular weight protein in the SCP-2 gene family" [28]. However, "AeSCP-2 differs from the vertebrate SCP-2 in several aspects. In both cultured and *Ae. vittatus* cells in the larval midgut, AeSCP -2 localizes mostly in the cytosol, which is consistent with the fact that AeSCP-2 lacks the C - terminal peroxisome targeting sequences" [28]. "The coordination site for a ligand in AeSCP-2 is different from the vertebrate SCP-2, wherein, the hydrophobic moieties of these ligands are oriented at opposite ends of the protein" [26]. "AeSCP-2 seems to be a vital gene for the survival and development of mosquitoes, whereas, the vertebrate SCP-2 is not essential for its survival and fertility (Spates et al., 1998). Targeting cholesterol metabolism for the development of growth regulators in insects, to control the insect population, is one of goal of insect disease causing vector management" [29], (Haapalainen et al., 2001).

2.5 Odorant Binding Protein of *Aedes vittaus*

Odorant binding proteins are thought to be primary proteins involved in the transport of

odorant and pheromones to the olfactory receptors in insects [30]. “Members of these protein families have been identified and it helps in its host identification. This protein has been isolated from the female antennae of *Aedes vittatus* and is not detected in legs or in antennal extracts from males and is similar to that of the pheromone binding protein from Bombyx mori” (Pelletier et al., 2010). Previous studies report that, *Aedes Vittatus* OBP has the same overall six- helix structure as seen in other insects OBPs. There are two models for OBP- mediated signal transduction; (i) direct release of pheromone from man internal binding pocket in a pH- dependent fashion and (ii) detection of a pheromone- induced conformational change in the OBP pheromone complex [31]. “In silico modelling is the bypass for the traditional testing of compounds, synthesized in time consuming multi step process against biological screens. It is a new approach in clinical chemistry for the optimization, screening and testing by means of the observation of a particular compound” [32]. “The need for biological screening and chemical synthesis has increased in order to obtain the early information of absorption, distribution, metabolism, excretion and toxicity data” [33]. “The compound with successful lip ink rule of five parameters can be considered as a protein inhibitor of specific target protein and a good insecticidal compound” [34]

2.6 Databases Used in the Study Swissport

Knowledge base swiss port is the central access point for extensive curated protein information including function, classification and cross reference. It consists of two sections: Swiss port which is manually annotated and is reviewed and Uniport which is automatically annotated and is not reviewed. The UniProt Reference Clusters (UniRef) databases provide clustered sets of sequences from the Uniport and selected UniProt Archive records to obtain complete coverage of sequence space at several resolutions while hiding redundant sequences.

2.7 Protein Databank (PDB)

do The Protein Data Bank (PDB) is a repository for the 3-D structural data of large biological molecules, such as proteins and nucleic acids. The data, typically obtained by X-ray crystallography or NMR spectroscopy and are submitted by biologists and biochemists from around the world, are freely accessible on the Internet via the websites of its member organizations (PDBe, PDBj, and RCSB). The PDB is overseen by an organization called the Worldwide Protein Data Bank, wwPDB. The PDB is a key resource in areas of structural biology, such as structural genomics.

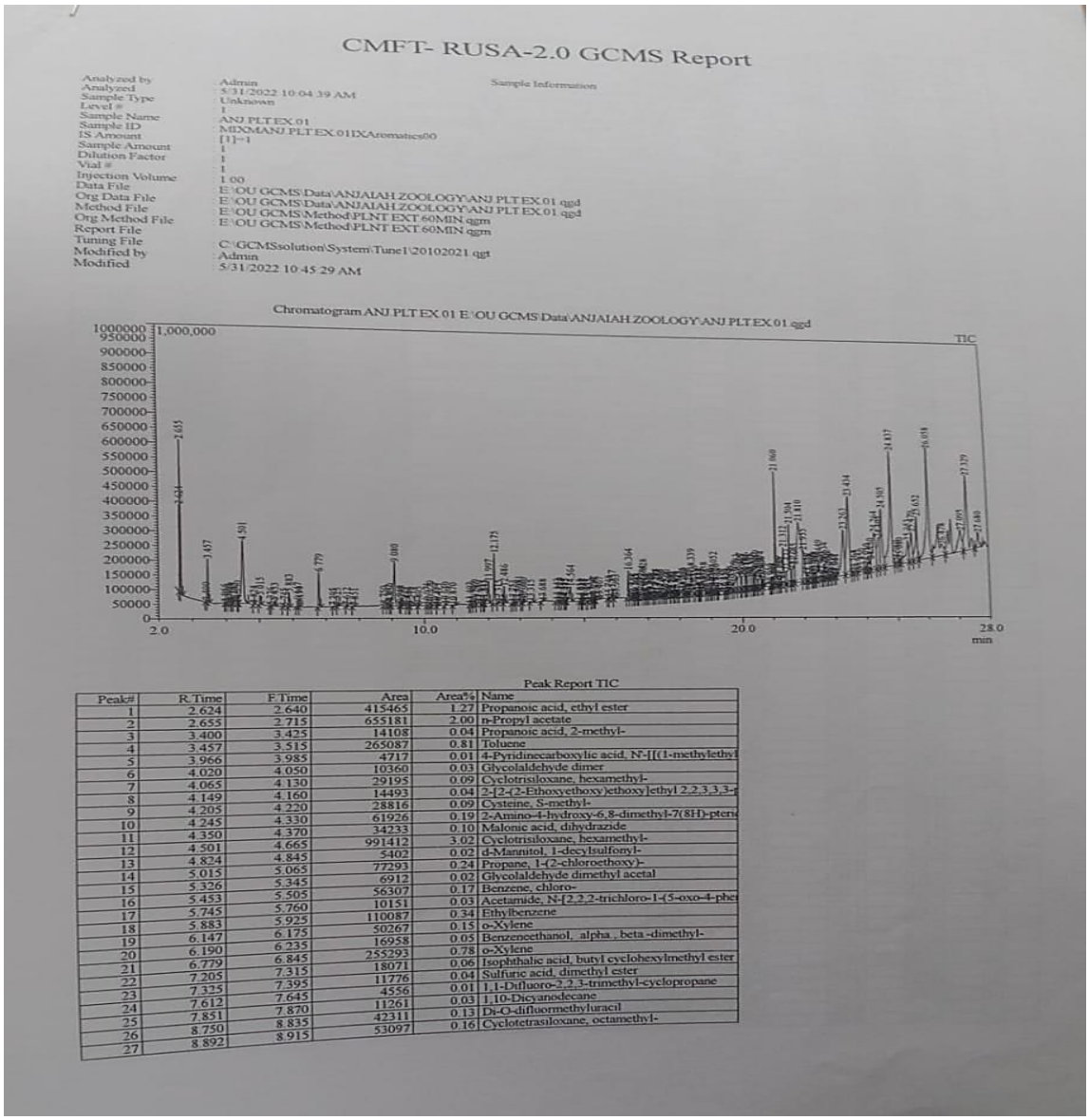
2.8 Tools Used in the Study

3D-Structure Visualization by RasMol

RasMol: RasMol is a computer program written for molecular graphic visualization and used primarily for the depiction and exploration of biological macromolecule structures, such as those found in the Protein Data Bank.

Fig. 1. *Catharanthus roseus* plant

Fig. 2. *C.roseus* leaves



CMFT- RUSA-2.0 GCMS Report

Analyzed by	Admin
Analyzed	5/31/2022 10:04:39 AM
Sample Type	Unknown
Level #	1
Sample Name	ANJ PLT EX 01
Sample ID	MIXMANJ PLT EX 01IXAromatics00
IS Amount	[1]=1
Sample Amount	1
Dilution Factor	1
Vial #	1
Injection Volume	1.00
Data File	E:\OU GCMS\Data\ANJAIAH ZOOLOGY\ANJ PLT EX 01.qgd
Org Data File	E:\OU GCMS\Data\ANJAIAH ZOOLOGY\ANJ PLT EX 01.qgd
Method File	E:\OU GCMS\Method\PLNT EXT 60MIN.qgm
Org Method File	E:\OU GCMS\Method\PLNT EXT 60MIN.qgm
Report File	
Tuning File	C:\GCMSsolution\System\Tune1\20102021.qgt
Modified by	Admin
Modified	5/31/2022 10:45:29 AM

Sample Information

Chromatogram ANJ PLT EX 01 E:\OU GCMS\Data\ANJAIAH ZOOLOGY\ANJ PLT EX 01.qgd

Peak Report TIC

Peak#	R.Time	F.Time	Area	Area%	Name
1	2.624	2.640	415465	1.27	Propanoic acid, ethyl ester
2	2.655	2.715	655181	2.00	n-Propyl acetate
3	3.400	3.425	14108	0.04	Propanoic acid, 2-methyl-
4	3.457	3.515	265087	0.81	Toluene
5	3.966	3.985	4717	0.01	4-Pyridinecarboxylic acid, N'-[[(1-methylethyl
6	4.020	4.050	10360	0.03	Glycolaldehyde dimer
7	4.065	4.130	29195	0.09	Cyclotrisiloxane, hexamethyl-
8	4.149	4.160	14493	0.04	2-[2-(2-Ethoxyethoxy)ethoxy]ethyl 2,2,3,3,3-
9	4.205	4.220	28816	0.09	Cysteine, S-methyl-
10	4.245	4.330	61926	0.19	2-Amino-4-hydroxy-6,8-dimethyl-7(8H)-pteri
11	4.350	4.370	34233	0.10	Malonic acid, dihydrazide
12	4.501	4.665	991412	3.02	Cyclotrisiloxane, hexamethyl-
13	4.824	4.845	5402	0.02	d-Mannitol, 1-decylsulfonyl-
14	5.015	5.065	77293	0.24	Propane, 1-(2-chloroethoxy)-
15	5.326	5.345	6912	0.02	Glycolaldehyde dimethyl acetal
16	5.453	5.505	56307	0.17	Benzene, chloro-
17	5.745	5.760	10151	0.03	Acetamide, N-[2,2,2-trichloro-1-(5-oxo-4-phe
18	5.883	5.925	110087	0.34	Ethylbenzene
19	6.147	6.175	50267	0.15	o-Xylene
20	6.190	6.235	16958	0.05	Benzeneethanol, alpha., beta.-dimethyl-
21	6.779	6.845	255293	0.78	o-Xylene
22	7.205	7.315	18071	0.06	Isophthalic acid, butyl cyclohexylmethyl ester
23	7.325	7.395	11776	0.04	Sulfuric acid, dimethyl ester
24	7.612	7.645	4556	0.01	1,1-Difluoro-2,2,3-trimethyl-cyclopropane
25	7.851	7.870	11261	0.03	1,10-Dicyanodecane
26	8.750	8.835	42311	0.13	Di-O-difluormethyluracil
27	8.892	8.915	53097	0.16	Cyclotetrasiloxane, octamethyl-

Fig. 3. Octa Siloxane Isolated by GC-MS Analysis of *Catharanthus roseus* leaf extract

Fig. 4. *Aedes vittatus*

Fig. 5. *Aedes vittatus* Larva (Control)

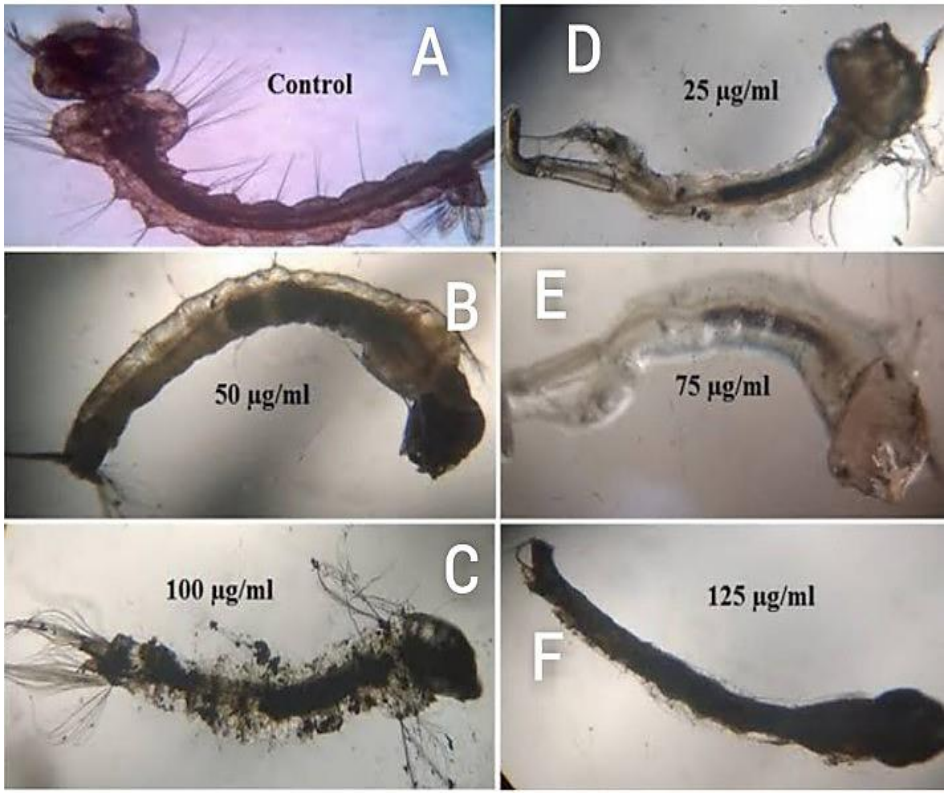


Fig. 6. Larvae treated with octa siloxane compound. A= control, BCDEF= treated

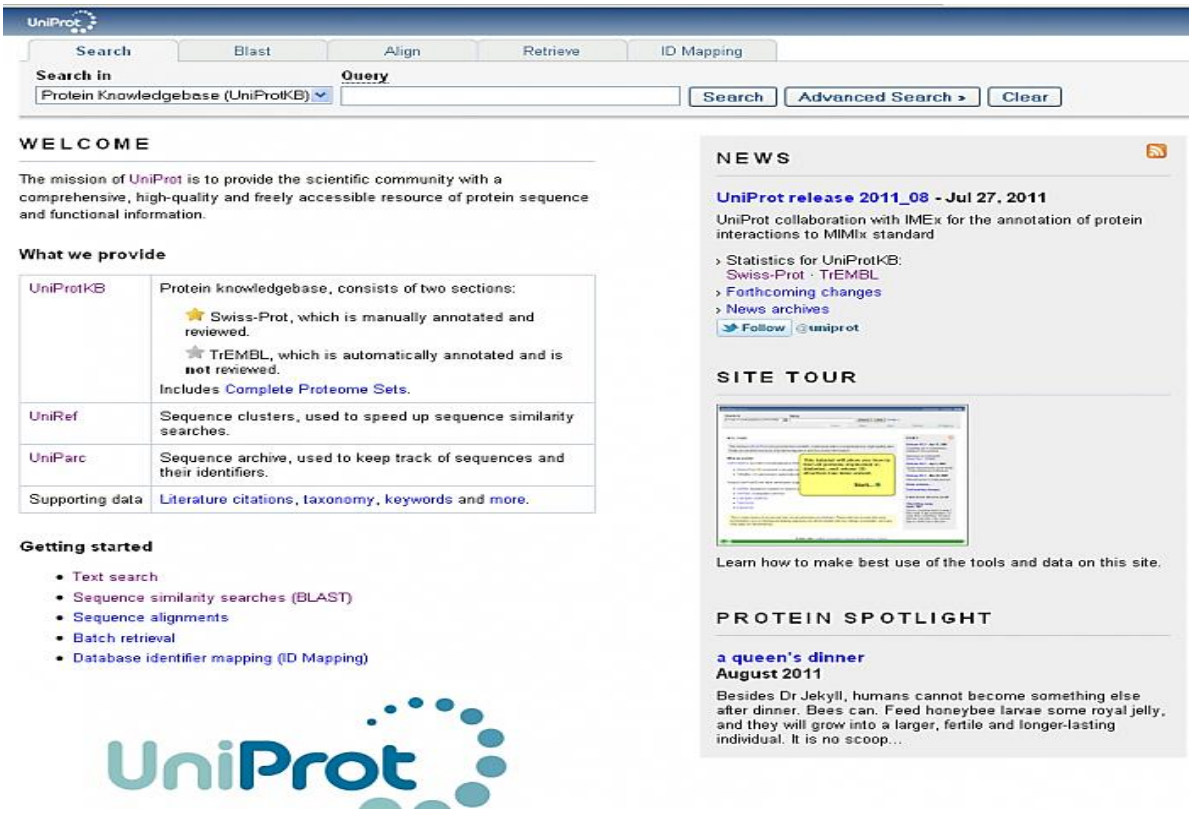


Fig. 7. Uniport Docking tool

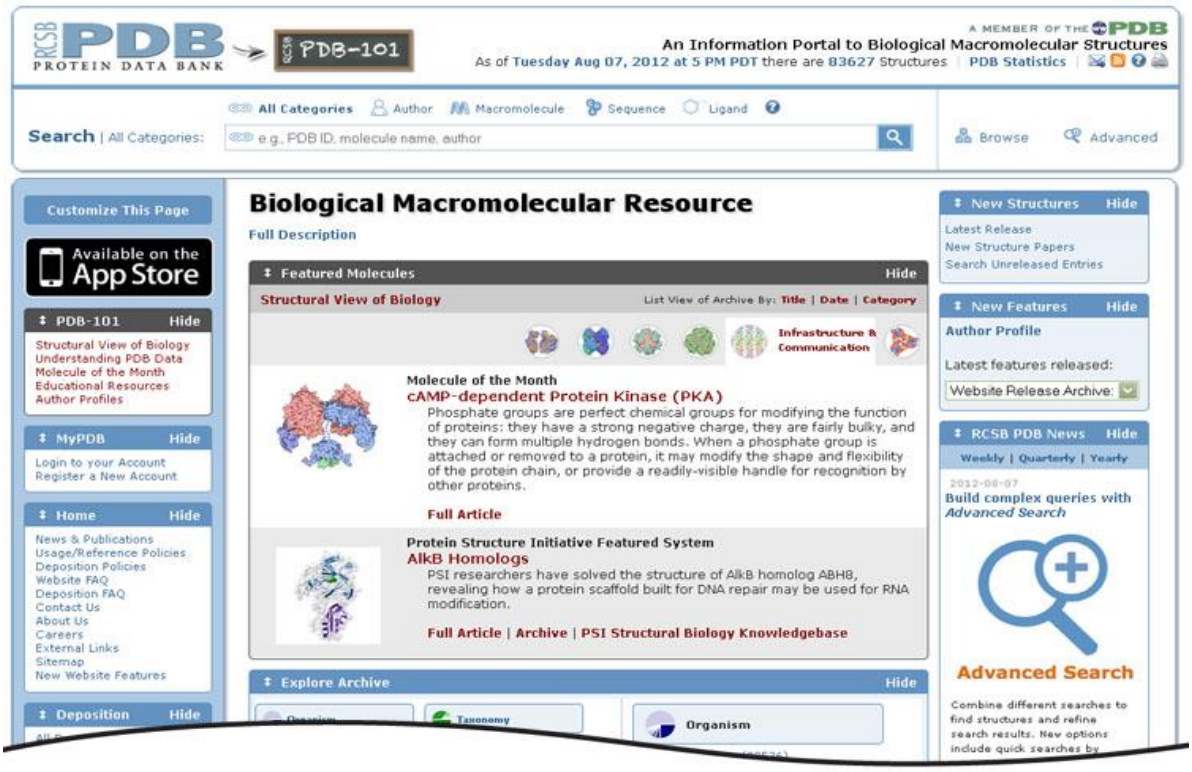


Fig. 8. PDB Docking tool

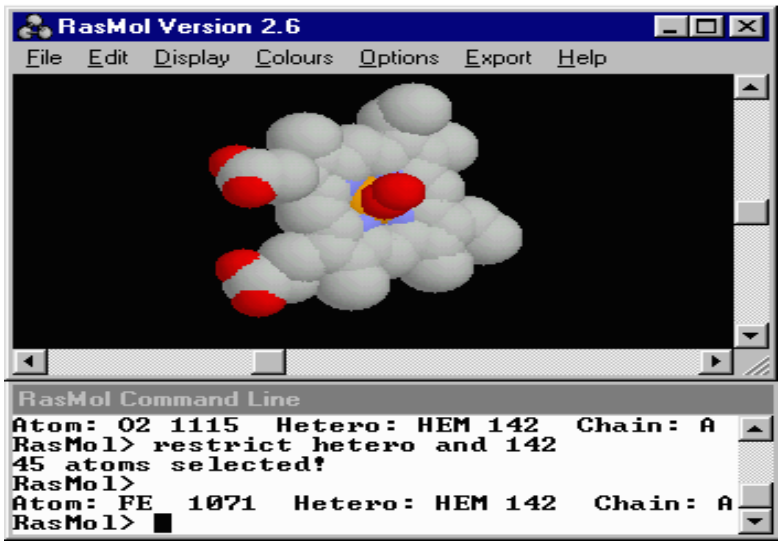


Fig. 9. Ras Mol Docking tool

Table 1. Geometric score and associate bonds of the compound octa siloxane

Name of the compound	Geometric score K cal/mol	Associate bonds
Octa siloxane	6270	2-H
Octa siloxane	6270	2-H
Octa siloxane	5388	1-H
Octa siloxane	5388	1-H
Octa siloxane	5066	2-H
Octa siloxane	5066	2-H

3. RESULTS

The sequences of three proteins (sterol career protein, D7 protein and Odorant-binding protein) were retrieved from Swissport database. The three-dimensional structure of these proteins was downloaded from PDB Database (Figs.1,2). The compound Octa siloxane isolated and characterized from Catharanthus roseus, has been studied for use as a natural insecticide. the compound was previously evaluated for their bioactivity and was found to possess potent larvicidal activity against the fourth instar larvae of *Ae. vittatus.* The inhibitor octa siloxane with pronounced larvicidal activity subjected to computational Screening and was used as a tool to study the mode of action and molecular mechanism of the selected functional protein. The ligand (compound) structure was drawn using ACD chem sketch and converted in to PDB formate using Open Babel. The 3D structures of proteins were docked with the inhibitor octa siloxane using Patch dock tool. The 2D and 3D chemical structure of the ligand/compound isolated from *Catharanthus roseus* was retrieved from PubChem data base (Table 1) The compound was prepared to dock with the sterol carrier protein of *Ae. vittatus*, D7 protein of *Ae.Vittatus*, and odorant binding protein of *Aedes Vittatus.* The docking results were analysed using PyMol visualization tool. The compound Octa siloxane was found to be binding with the receptor protein, AeSCP2 (PDB ID-IPZ5 Chain-A), OBP (ID-2L3C Chain-A) and D7 (PDB ID4NGV). The octa siloxane compound exhibited good geometric shape complementarity score (6370 Kcal/mol) when interacted with target protein SCP and formed 2-H bonds (Table 1) The amino acid isoleucine (ILE) position 99 of atom O binds with atom O of octa siloxane and the bond length is 3.55 Å and amino acid arginine (ARG) at position 15 of atom NH1 binds with the atom O of octa siloxane and the bond length is 3.28 Å. The ligand (octa siloxane) when docked with (OBP) showed a good geometric shape complementarity score (5488 Kcal/mol) and formed 1-H bond as depicted in (Table 1) The amino acid leucine (LEU) position 73 of atom O binds with atom O of octa siloxane and the bond length is 3.55 Å. the ligand when docked with target D7 protein showed a geometric shape complementary score (5068 Kcal/mol) and formed 2-H bonds as depleted in Table 1. The aminoacid lysine (LYS) position 256 of atom NZ binds with atom O of octa siloxane and the bond length is 2.44 Å and the amino acid glutamine (GLN) position 253 of atom NEZ binds with the atom O of octa siloxane and the bond length is 2.74 Å. The docking results clearly indicates that the ligand octa siloxane was highly binding with the target protein AeSCP2, OBP and D7, displaying a good geometric shape complementarity score of 6370 Kcal/mol, 5488Kcal/mol and 5068 Kcal/mol, respectively. The docking results confirmed that the compound octa siloxane was a best compound, as it exhibited a good geometric shape complementary score and also recorded the formation of hydrogen bonds. When the ligand binds with protein, the conformation of the protein structure is altered thereby the function of the specific protein is also changed. Therefore, the compound may have an ability to inhibit the contact between human and vector.

4. DISCUSSION

Inhibitors are useful tool for elucidating the mode of action and molecular mechanism of a functional protein (Kim et al., 2005; Kumar et al., 2010). “In the present study the ligand, Octa siloxane was allowed to dock against the mosquito proteins, AeSCP-2, OBP and D7. All insects lack the enzymatic pathway to synthesize their own cholesterol, highlighting the critical physiological process of cholesterol absorption and translocation” [35]. “Cholesterol is vital for growth, development and egg production of mosquitoes and is obtained from decomposed plants they eat during the larval stage, when living in shallow waters. Studies have indicated that the midgut and possibly the foregut as sites of cholesterol absorption in insects” (Langley et al., 1987; Komnick and Giesa, 1994; Jouni et al., 2002). “Plants make phytosterol which is converted to cholesterol in the mosquito’s gut. In order to transport it in a liquid medium, such as blood or cell fluids, the organisms must have a way to shield it from the watery environment through which it moves, which is studied typically in a carrier protein AeSCP-2” (Kumar et al., 2010). Divya and Manimghalai, (2014) reported the potential ability of the four selected repellent compounds, viz., Cis-ocimene, lutein, beta caryophyllene and piperidone of Targets erecta against the odorant binding protein of *Culex quinquefasciatus*. similar observation was reported by Gadagutti et al [17] in the in silico molecular docking studies of mosquito repellent compounds from Hyptis suave lens. Gaddaguti et al. [17] identified 13 compounds from methanol extracts of Hyptis suave lens docking analysis revealed the repellent potential of the compounds stigmast-5-en-3-ol, oleate, gamma-

sitosterol and Butyl 11-eicosenate against OBP of C. quinquefasciatus. “In malaria transmitting Anopheles mosquitoes, D7 salivary gland protein helps in the breakdown of sugar and is an anti-haemostatic compound which helps in blood feeding [36]. Inhibiting the function of D7 salivary protein can be considered as an attractive avenue for the development of insecticides” (Vijay et al., 2015). Observations of the present study reveal the inhibitory potential of the ligand Octa siloxane with D7 salivary protein. In silico docking studies of Dhivya and Manimegalai [11] established the high binding affinities of Cisocimene with a glide score of -3.7 against the odorant binding protein of *Culex quinquefasciatus*. Studies by Hetal et al., [37] suggested that the components of Tulsi and mamejarco components like apigenin, luteolin carvacol can be used as lead molecules in curbing the feeding behaviour of mosquitoes.

5. CONCLUSIONS

in silico docking studies and in vitro studies against the fourth instar larvae proves the compound ‘Octa siloxane’ as a potent and natural inhibitory agent to control mosquito borne diseases.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Morris GM, Lim-Wilby M. Molecular docking. Method in mol. Boil. 2008;443: 365-382.
2. Palleti DJ, Chitti S, Babu PA. Virtual screening and molecular docking analysis of Zap-70 Kinase Inhibitors. Inter. J. Chem. Anal. Science. 2011;2(9):1208-1211.
3. Balasubramanian S, Sasikumar P, Velmurugan D. Invitro evaluation and molecular docking calculation of tricyclic phthalimide quinoxaline analogues as novel, Int. J.Phar. Sci. Drug. Res. 2014; 6(1):60-66.
4. Abagyan R, Totrov M. Rapid boundary element solvation electrostatics calculations in folding simulations: successful folding of a 23-residue peptide. Biopolymers. 2001;60:24- 33
5. Annapoorani CA, Manimegalai K. Homology modelling of *Culex quinquefasciatus* salivary gland protein and molecular docking study using compounds from *Capsicum annum* L. Int. J. of curr.Res. 2013;5(10):3044-3047.
6. Greenwood B, Mutabingwa T. Malaria in 2002, Nature. 2002;415:670–672.
7. Sachs J, Malaney P. The economic and social burden of Malaria, Nature. 2002; 415:680-685
8. Kevin BJ. Chloroquine resistance in Plasmodium vivax, Antimicrob. Agents Chemother. 2004;98(41):4075–4083.
9. Yang HM, Boldrini JL, Fassoni AC, Freitas LFS, Gomez MC, Lima KKB, Andrade VR, Freitas ARR. PLoS One. 2016;24;11(3): e0152186.
10. Ojha PK, Roy K. Chemometric modeling, docking and in silico design of triazolo pyrimidine-based dihydroorotate dehydrogenase inhibitors as antimalarials. Euro. J. Medic. Chem. 2010;45:4645-4656.
11. Dhivya R, Manimegalai K. In silico Molecular docking and Molecular Dynamics Applications in the Designing of a new mosquito repellent from the plant Calotropis gigantean targeting the Odorant binding protein of *Culex quinquefasciatus*. Intern. J. Pharm. Phyto. Research. 2013; 3(2):134 -138.
12. Zwiebel LJ, Takken W. Olfactory regulation of mosquito–host interactions, Insect Biochem Mol Biol. 2004;34(7):645–652.
13. Costantini C, Gibson G, Sagnon N, Della Torre A, Brady J, Coluzzi M. Mosquito biolarvicidal compound gymnemagenol isolated from leaf extract of miracle fruit plant, *Gymnema sylvestre* (Retz) Schult against malaria and filariasis vectors. Parasitol. Res. 1996;109 (5):1373–1386.
14. Baleta A. Insecticide resistance threatens malaria control in Africa. Lancet. 2009;374(9701):1581- 582. *Capsicum annum* L. Int. J. of curr.Res. 2009;5(10): 3044-3047.
15. Arulpriya P, Lalitha P, Aarthi N. Larvicidal and pupicidal effect of ethanolic extract of the aerial roots of *Rhaphidophora aurea* intertwined over four different host

trees against *Culex quinquefasciatus* say, Res. J. Pharm. Bio. Chem.Sci. 2013;4(1): 18-27.
16. Remia KM. Larvicidal and pupicidal effect of *Spilanthes acmella* and *Andrographis paniculata* on the mosquito Aedes aegypti. Int. J. Inst.pharma. LifeSci. 2012;2(2):71-76.
17. Gaddaguti V, Jwala Mounika S, Sowjanya K, Rao TT, Krishna Chakravarthy MSR, Allu Prasada Rao R. GG - MS analysis and in silico molecular docking 5. studies of Mosquito repellent compounds from *Hyptis suaveolens* L. Inter. J. Bio. 2012;1(9):36-41.
18. Valenzuela JG, Charlab R, Gonzalez EC, de Miranda-Santos IK, Marinotti O, Francischetti IM, Ribeiro JM. The D7 subfamily of salivary proteins in blood sucking diptera. Insect Mol Biol. 2002; 11(2):149-55.
PubMed PMID: 11966880.
19. Clements AN. The Biology of Mosquitoes 1, Development, Nutrition and Reproduction. Chapman & Hall, London. 1992;509.
20. Lehane MJ, Msangi AR. Lectin and peritrophic membrane development in the gut of *Glossina* M. morsitans and a discussion of their role in protecting the fly against trypanosome infection. Med. Vet. Entomol. 1991;5:495– 501.
21. Riberio JMC. Role of saliva in blood feeding by arthropods. Ann. Rev. Entomol. 1987;32:463-378.
22. Rossignol PA, Lueders AM. Bacteriolytic factor in the salivary glands of Aedes aegypti. Comp. Biochem. Physiol. B. 986;83:819-822
23. James AA, Blackmer K, Marinotti O, Ghosn CR, Racioppi JV. Isolation and characterization of the gene expressing the major salivary gland protein of the female mosquito, Aedes aegypti. Mol.Biochem. Parasitol. 1991;44:245–253.
24. Graham LA, Tang W, Baust JG, Liou Y, Reid TS, Davies PL. Characterization and cloning of a Tenebrio molitor haemolymph protein with sequence similarity to insect odorant-binding proteins. Insect Biochem Mol Biol. 2001;31:691–702.
25. Arca B, Lombardo F, Lanfrancotti A, Spanos L, Veneri M, Louis C. Coluzzi M. A cluster of four D7-related genes is expressed in the salivary gland of the African malaria vector Anopheles gambiae. Insect Mol. Biol. 2002;11:47–55.
26. Dyer DH, Wessely V, Forest KT, Lan Q. 3-D structure/function analysis of sterol carrier protein-2-like2 reveals differences among scp-2 family members. J Lipid Res. 2008;49:644–653.
27. Ketamura T, Kobayasi S, Okada M. Regional expression of the transcript encoding sterol carrier protein x-relased thiolase and its regulation by homeotic genes in the midgut of Drosophila embryos. Dev. Growth. Differ. 1996;38: 373-81.
28. Krebs KC, Lan Q. Isolation and Expression of Sterol Carrier Protein-2- gene from the yellow fever mosquito, Aedes aegypti. Insect. Mol. Biol. 2003;12:51-60.
29. Fuchs M, Hafer A, Muanch C, Kannenberg F, Teichman S, Scheibner J. Disruption of the Sterol carrier protein 2 gene in mice impairs lipid and hepatic cholesterol metabolism. J. Biol.Chem. 2001;276: 48058-65.
30. Vogt RG, Callahan FE, Rogers ME. Dickens JC. Odorant binding protein diversity and distribution among the insect orders, as indicated by LAP, an OBP-related protein of the true bug Lygus lineolaris (Hemiptera, Heteroptera). Chem. Senses.1999;24:481-495.
31. Thode AB, Kruse SW, Nix JC, Jones DNM, The role of multiple hydrogenbonding groups in specific alcohol binding sites in proteins: Insights from structural studies of LUSH. J. Mol. Biol. 2008;376:1360–1376.
32. Waterweed HV, Gifford, Eric. “Admit in silicon modelling: towards prediction paradise?” Nature 2003;2:192-204.
33. Khamkar T, Abhyankar M, Tendulkar G, Gopiesh Khanna V, Kannabiran K. *In silico* Molecular Docking of Marine Drugs Against Cancer Proteins. Advances in Chemical Science. 2013;2(2).
34. Komala S, Ravi S, Usnantaj P, Shanmugam B, Venkata Subbaiah G, Sathyavelu Reddy K, *In silico* Molecular Modelling and Docking Studies on Therapeutic Target Non Sturctural Protein 3 (NS3) of Dengue Virus, Int. J. Phram. Sci. Rev. Res. 2014;29(1):08,37-41.
35. Zdobnov EM, VonMering C, Letunic I, Torrent D, Suyama M, Copley RR. Comparitive genome and proteome analysis of Anopheles gambiae and Drosophila melanogaster. Science. 2002; 298:149-59.
36. Sor-suwan S, Jariyapan N, Roytrakul S, Paemanee A, Phumee A, Phattanawiboon

B, Intakhan, N, Chanmol W, Bates A, Saeung A, Choochote W. Identification of Salivary Gland Proteins Depleted after Blood Feeding in the Malaria Vector Anopheles campestris-like Mosquitoes (Diptera: Culicidae). PLOS ONE;2016. DOI:10.1371 /journal.pone.0163810-2;14.

37. Hetal KP, Ratna AT, Pretibha BD. Docking studies of components of Tulasi and Mamejavo against Plasmodium Lactate Dehydrogenase, Inter. J. Biol. Scie. 2013; 2(2):8-22.

Peer-review history:
The peer review history for this paper can be accessed here:
https://prh.mbimph.com/review-history/3052