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Maithreyee S
Research Scholar, Department of Biochemistry, DKM College for Women, Vellore, Tamil Nadu, India

Prabha V
Assistant Professor and H.O.D, Department of Biochemistry, DKM College for Women, Vellore, Tamil Nadu, India

Corresponding Author:
Maithreyee S
Research Scholar, Department of Biochemistry, DKM College for Women, Vellore, Tamil Nadu, India

Study on the cytochrome B protein of *Culex quinquefasciatus* with Malic acid using automated *Insilico* docking protocols

Maithreyee S and Prabha V

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Abstract
Culex quinquefasciatus is a major threat faced by people in India. The nematode worm, *Wuchereria bancrofti* causes Lymphatic filariasis which is transmitted by *Culex quinquefasciatus* acting as a vector. The aim of the present *Insilico* research work is to validate how malic acid interacts with the protein of the mosquito species, *Culex quinquefasciatus* using Bioinformatic tools. The target protein molecule is Cytochrome b mitochondrion protein of the mosquito species. The structure of Malic acid is retrieved from the generalized database, NCBI (National Center for Biotechnology Information). Drug docking studies are performed using an automated drug docking server. The chemical molecule is converted from 2D to 3D using an advanced molecular visualization software. The result obtained from docking clearly shows that Malic acid binds with the functional domain region of Cytochrome b protein of the mosquito sps. All the results were clearly elucidated using 3D visualization techniques on the binding interaction between malic acid and the protein of the mosquito sps. It was thus proved that Malic acid is an efficient agent for controlling *Culex quinquefasciatus.*

Keywords: *Culex quinquefasciatus*, cytochrome b, drug docking

1. Introduction
Zika virus (ZIKV) epidemic that occurred in the Americas from 2015 to 2016 [1] has brought fresh attention to the burden of diseases brought on by these infections. Emerging and reemerging arboviruses have long been recognised as a danger. Hundreds of thousands of cases of ZIKV were reported in Brazil alone when it was first introduced there in 2014, and by early 2016 it had spread to 28 other countries [2, 3]. This was due to the virus' quick dissemination among the naive American population. Regarding the unusual spread and pathogenesis of ZIKV in the Americas, a number of theories have been put forth. These include the lack of immunity in the American population, virus mutation and adaptation during its geographic spread, and increased ZIKV transmission because of a more vulnerable and/or plentiful mosquito vector population.

When a female mosquito feeds on the blood of an infected host, she picks up infectious virions, making her a "competent vector," or mosquito that spreads arboviruses. The virus then adheres to and enters midgut epithelial cells, where it multiplies and creates contagious viral particles that spread into the mosquito's hemocoel. Before virions are injected in the saliva during subsequent blood feeding, the virus must adhere to and enter the epithelial cells of the salivary glands, where another cycle of virus replication takes place. "Barriers" exist at the levels of infection and dissemination for each transmission-relevant tissue, which significantly limit or prevent viral transmission in resistant or refractory mosquito species or strains [4, 5]. In-depth research has been done on the obstacles that lower the severity of viral infection in capable vectors [6]. Understanding these relationships in non-competent mosquito species has received less attention. Knowledge of the essential elements for vector competence has substantially improved as a result of studies into various types of host-pathogen interactions. [7, 8, 9, 10, 11]. The transmission of parasites and virus through mosquitoes should be arrested, especially, the spread of *Wuchereria bancrofti* through *Culex quinquefasciatus*. Natural products are an effective way of controlling these mosquitoes as they have no harmful effects on human beings.

2. Material and Methods

Drug - Protein retrieval system: The target protein sequence (YP_003934133.1 cytochrome b (mitochondrion) *Culex quinquefasciatus*) was retrieved from NCBI GenPept database [12] (https://www.ncbi.nlm.nih.gov/protein/) in FASTA format. Malic acid (CID: 525) was selected from NCBI PubChem Compound database [13] (https://www.ncbi.nlm.nih.gov/pccompound/). The 2D structure was converted into 3D form using Discovery studio software in order to perform drug docking studies.

Molecular Drug Docking: The selected mosquito protein sequence and the predicted 3D structure of Malic acid were subject to drug docking studies in order to find out the binding efficiency of malic acid with Cytochrome b of *Culex quinquefasciatus*. The molecular drug docking server, HDOCK server [14] (http://hdock.phys.hust.edu.cn/) was used for docking studies.

3. Results and Discussion

The selected mosquito sps, *Culex quinquefasciatus* has mitochondrion cytochrome b whose nucleotide length is 1135 bp (NC_014574.1) and the length of its corresponding amino acid sequence is 378 aa (YP_003934133.1) (Fig 1). Bioinformatics and experimental methods similar to the above were chosen to identify the mtDNA sequences from the trace file database of *C. quinquefasciatus* at NCBI and to assemble the whole mitochondrial genome *of C. quinquefasciatus* (accession no. GU188856).

As per endosymbiotic theory, mitochondria present in animals are considered to be derived from α-proteobacteria. The size of the genome of proteobacteria is ~4000–6000 kb. However, eukaryotic mitochondrial DNA present today is just ~15 to 40 kb. This is due to reduction in the genome size of mitochondria over time which might have occurred by the progressive transfer of mitochondrial genes to the nuclear genome (Thorsness, Weber 1996; Timmis *et al*. 2004). In several eukaryotes, copies of the mitochondrial sequences transferred to the nuclear genome have been identified in several eukaryotes [15, 16]. They are called as nuclear mitochondrial sequences (NUMTs).

The occurrence of mitochondrial sequences present in nuclear genomes were primarily reported in mice [17]. There are several reasons to explain the fact why the identification of NUMTs is primordial. As the mtDNA sequences transferred continue to exist as “molecular fossils” in the nuclear genome, identifying them may help in the better understanding of the confounding effects in the phylogenetic inferences of organisms [18]. Besides, NUMT insertion loci can be employed for predicting common ancestry for a specific lineage and for determining the phylogenetic branching orders of several organisms [19]. In addition, within species variation of NUMT copy number may even be made use of as a population genetics tool [20].

In this *Insilico* research study, we focus on how Cytochrome b protein is involved in drug interactions against the selected chemical compound, malic acid.

During the past few decades, there has been augmenting concerns on the depletion of fossil fuel and uncontrolled emission of CO_2 which have given rise to comprehensive basic studies and industrial tests on chemical production of microbes. L-malic acid, as a precursor or an additive, has been identified to exhibit specific properties in the pharmaceutical, food and daily chemical industries. L-malic acid is, at present, manufactured mainly by means of a biocatalytic conversion route based on fumarate hydratase, where fumaric acid derived from petroleum acts as a substrate [21].

Fig 2 clearly shows the 2D structure of Malic acid with coloured atom labels. The picture is obtained from NCBI PubChem compound database. Fig 3 represents the 3D structure of Malic acid viewed in Ball and Stick model with coloured atoms labels. The 3D structure was visualized using an advanced molecular visualization software, Discovery Studio. The chemical structure based on the internal Electrostatic force, it binds to the target protein of cytochrome b of *Culex quinquefasciatus*.

During the evaluation of HDOCK server, homologous complexes which have a sequence identity ≥30% with the test cases were eliminated. In the process of protein–protein docking, the unbound structure sequences were made use of as input for receptor and ligand as well. While assessing, it was successfully defined that the predicted binding mode has an admissible precision or better as per the CAPRI criteria [22]. Our results coincide with the previously proved research work [23, 24, 25, 26, 27, 28, 29]. In our drug scores between the malic acid with cytochrome b shows the higher negative value is –104.67 *kcal/mol* (Table: 1).

According to theory, higher negative value indicates that the binding interaction between receptor and drug is good. Fig. 4,5,6,7 show how binding interaction takes place between cytochrome b and Malic acid drug at the hydrogen bonds. The results reveal the complete interaction along with the corresponding amino acids labels in 3D view using Discovery Studio Software. Fig 8. clearly reveals the interaction between the acceptor and donor, that is, how malic acid inhibits the functional part of Cytochrome b protein.

Interestingly, we found out that the total length of the protein, cytochrome b of *Culex quinquefasciatus* was 378 aa. Within this length, various functional domains, such as, (PS51002) CYTB_NTER [30,31,32] *Cytochrome b/b6 N-terminal region profile* (1-210), (PS51003) CYTB_NTER *Cytochrome b/b6 N-terminal region profile* (211-378), (PS00005) PKC_PHOSPHO_SITE [33] *Protein kinase C phosphorylation site* (310-312), are present. The binding interaction of Malic with cytochrome b protein takes place at the trans-membrane helical regions (309aa, 370aa) and at the protein kinase site (311aa). All the results clearly indicate that malic acid binds at the functional part of the protein, thereby, inhibiting it.

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>YP_003934133.1 cytochrome b (mitochondrion)
[Culex quinquefasciatus]
MNKPLRKSHPLISIANNALVDLPAPSNISAWWNFGSLLGLCLVIQIL
TGLFLAMHYTADIETAFNSVNHI
YRDVNNGWFLRICHANGASFFFACLFIHVGRGVYYNSYLYIPTWMIG
VIILFMVMATGFLGYVLPWGQMS
FWGATVITNLLSAVPYLGTDLVQWIWGGFAVDNATLTRFFTFHFILP
FIVLALTMIHLLFLHQTGSNNPL
GLNSNVDKIPFHPYFVYKDIVGFIIFMWILIGFIWKFNYLLMDPENF
IPANPLVTPVHIQPEWYFLFAYA
ILRSIPNKLGGVIALVLSIAILMILPFTHTSKFRGLQFYPLNQILFW
NMVIVASLLTWIGARPVEDPYVL
TGQILTVLYFSYFIINPLMSKYWDKLLN
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Fig 1: FASTA sequence of mosquito protein was retrieved from NCBI database

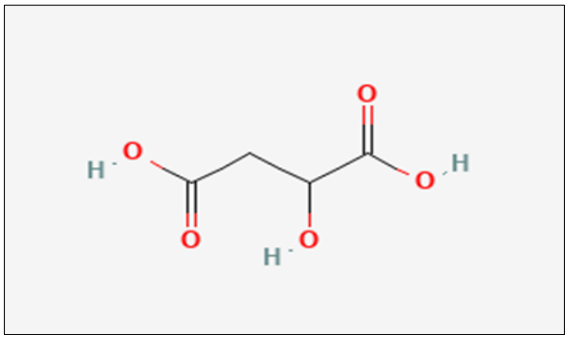

Fig 2: 2D structure of Malic acid with respective atoms retrieved from NCBI PubChem compound database

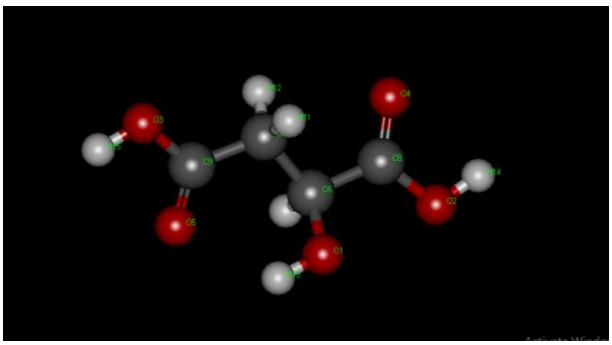

Fig 3: 3D structure of Malic acid with respective atoms in Ball and Stick model viewed using Discovery Studio software.

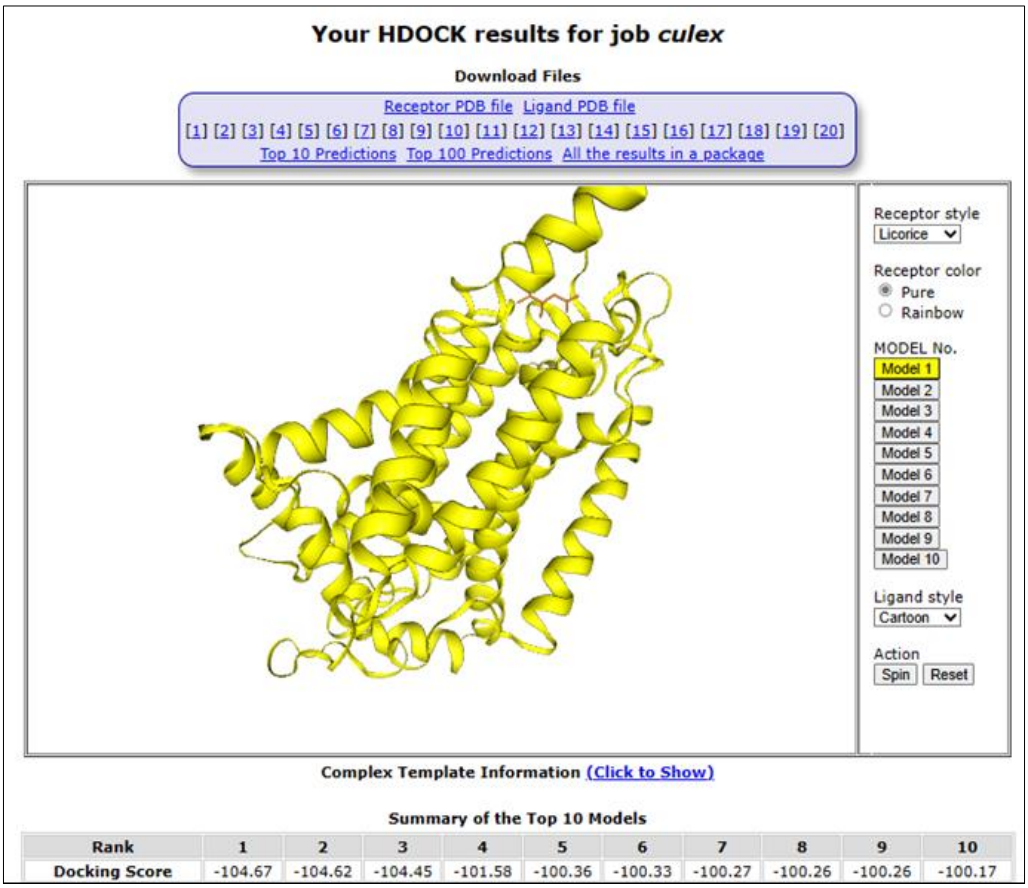

Fig 4: The above picture shows the docking results of *Cytochrome b* with Malic acid with the respective docking score of -104.67 kcal/mol viewed using Discovery studio software

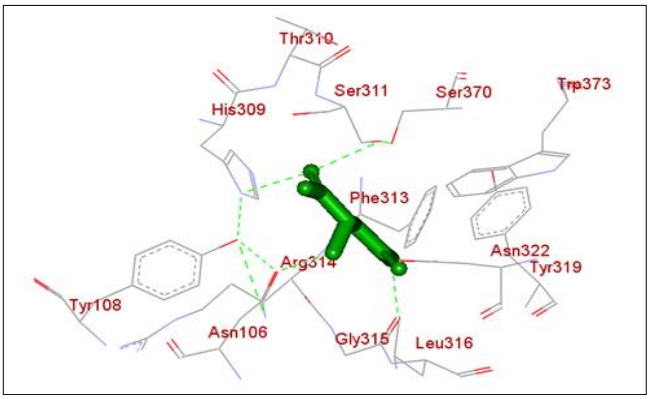

Fig 5: The above picture shows the H-bond interaction between Cytochrome b and Malic acid showing the amino acids present at the H-bond interacting sites viewed using Discovery studio software

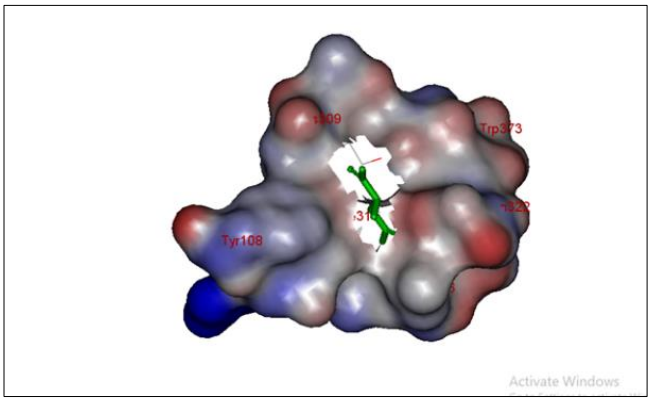

Fig 6: The above picture shows the electrostatic interaction between Cytochrome b and Malic acid in surface model viewed using Discovery studio software

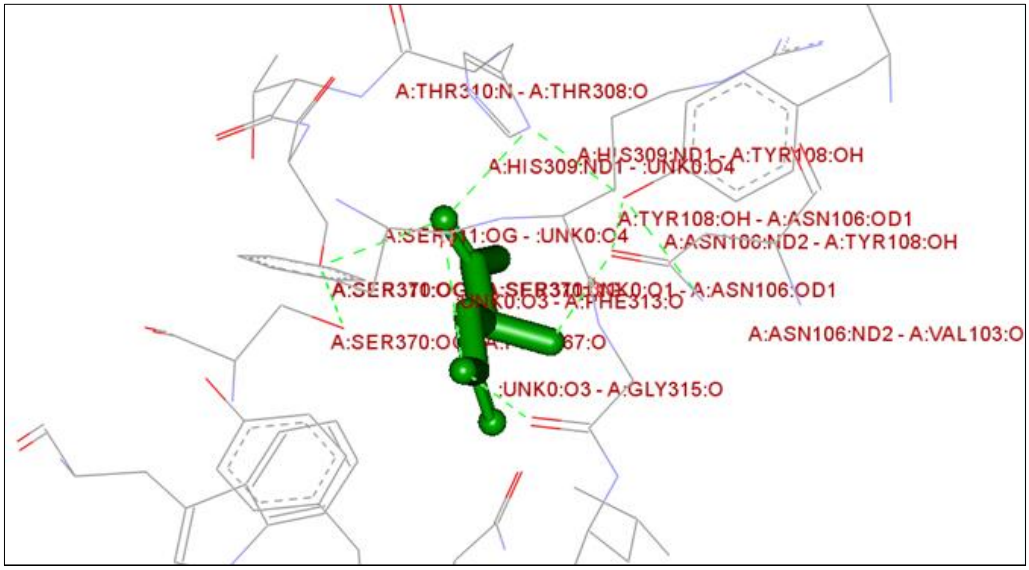

Fig 7: The above picture shows the electrostatic interaction between Cytochrome b and Malic acid with acceptor and donor amino acid labels viewed using Discovery studio software

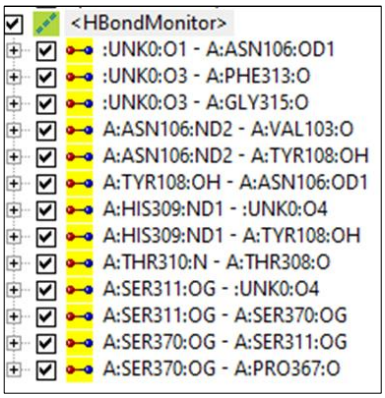

Fig 8: The above picture shows the list of acceptor and donor amino acids interacting at the H-bonds viewed using Discovery studio software

Table 1: Molecular Drug Interaction summary of drug and receptor with the binding score along with units

	Drug
Receptor	Malic acid - *alpha hydroxy acid* (CID:525)
YP_003934133.1 cytochrome b (mitochondrion) [*Culex quinquefasciatus*]	-104.67 *kcal/mol*

4. Conclusion

Culex quinquefasciatus is primarily involved in causing filariasis in Human beings. In this research investigation, it has been clearly elucidated that Cytochrome b protein of *Culex quinquefasciatus* directly binds with the selected chemical compound, Malic acid. Malic acid is naturally present in various vegetables and fruits. Our present investigation aimed at controlling mosquitoes using naturally-derived products such as Malic acid which are in no way harmful to human beings. The overall research clearly shows that malic acid directly binds with the functional part of Cytochrome b protein of *Culex quinquefasciatus* and thus downregulates it. Hence, Malic acid can be used as a potential agent for controlling mosquitoes.

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