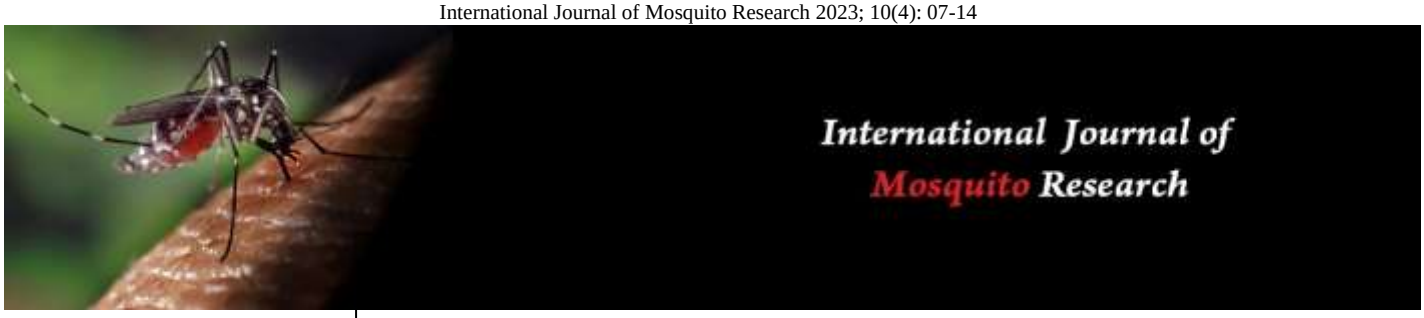

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In silico study on Hexadecanoic acid against the outer membrane protein transport protein of *Culex quinquefasciatus* and *Aeromonas hydrophila*

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Abstract
Culex quinquefasciatus, the "southern house mosquito" is a tiny to medium-sized mosquito that resembles *Cx. pipiens* Linnaeus, the "northern house mosquito." Where they overlap (36°–39° north latitude), they can interbreed, and intermediate species may result. Gram-negative, pleomorphic, non-spore-forming, and possessing a monotrichous flagellum, *Aeromonas hydrophila* is a bacillus. It is a facultative anaerobe that is frequently found in sewage as well as fresh water. In this investigation, the effectiveness of the chemical compound, Hexadecanoic acid (*Carica papaya*) was determined on the two species mentioned above. The insilico procedure was used to investigate Hexadecanoic acid's anti-microbial and insectide capabilities based on the comparison study. This study's technique included automated drug docking procedures and protein profiling research. All of the findings were shown in 3D. Hexadecanoic acid's effectiveness in binding to the Outer Membrane Protein Transport Protein of *Culex quinquefasciatus* and *Aeromonas hydrophila* was clearly demonstrated by the comprehensive molecular interaction analysis. Hexadecanoic acid was ultimately shown to have a better inhibitive effect on *Aeromonas hydrophila* than on *Culex quinquefasciatus*. Therefore, Hexadecanoic acid has the potential to treat illnesses brought on by *Aeromonas hydrophila*.

Keywords: *Aeromonas hydrophila, Culex quinquefasciatus* and drug docking

Introduction
According to Price (1977), parasites are organisms that live on or in their host at the expense of the host. Parasitism can have a variety of phenotypic and population-level effects on its hosts, including altered behaviour, reduced fitness, and increased mortality. For instance, *Plasmodium relictum* and *Leishmania* parasitic infection, respectively, modulate *Culex pipiens* survival and sandfly biting frequency, and these changes in vector biology significantly improve the efficiency of pathogen transmission (Beach *et al*. 1985 [1, 2, 3]. In the lab, *Culex pipiens* and *Culex quinquefasciatus* (Diptera: Culicidae) previously exposed to Plasmodium parasites showed an increase in mosquito biting frequency and altered host preference, which is thought to be similar to the effect of avian malaria on mosquito vector feeding behaviour [4]. Epidemiology and disease control continue to place a high priority on identifying variables that can affect pathogen transmission.
Because of their predominate role in West Nile virus (WNV) transmission and their primary role in the amplification of WNV in avian hosts due to their ornithophilic feeding behaviour, mosquitoes in the *Culex pipiens* complex (biotypes and hybrids of *Cx. pipiens* Linnaeus, *Cx. quinquefasciatus*) are the most significant vectors of mosquito-borne viruses in much of the United States [5, 6]. These mosquitoes frequently consume a variety of avian infections while feeding on birds [7-13], which may reduce their ability to transmit WNV. *Haemoproteus* spp. (Haemosporida: Haemoproteidae), avian haemosporidian parasites comparable to avian malaria, are one class of diseases frequently consumed.
Aeromonas species may be found in a variety of aquatic and environmental habitats, including estuaries, silt, seagrass, seaweed, drinking water, used water and food [14, 15]. Motile, gram-negative, bacilli or coccobacilli rods that are 1-3.5 m in length, do not produce spores, and have rounded ends are members of the Gamma proteobacteria genus Aeromonas.

They are facultative anaerobes that can convert nitrate to nitrite, contain the enzymes catalase, oxidase, and indol, and are often resistant to the vibrio static drug O/129. A. hydrophila is frequently found in the sediment of the Chesapeake Bay and its tributaries, where concentrations vary from 4.6 10 2 /g to 5 10 3 /ml, according to a microbiological analysis [16].

Material and Methods

Preparation of *C. Papaya* Leaf Extract: The collected *C. papaya* leaves were washed with running tap water to avoid surface contaminations and shade dried for 15 days. The dried leaves were cut into small pieces and macerated into a fine powder. The dried powder was soaked with different organics solvents such as aqueous, ethanol, methanol and was subjected to solvent extraction using the Soxhlet apparatus. The coarse powder was then stored in an air tight container and kept in a cool and dry place for further use.

Stock Preparation: 10 gm of *C. papaya* leaf extract was dissolved in 100 ml of different solvents in 250 ml capacity flask and the lid was closed with a silver foil and placed in shaking bath for 24 hrs for further study. 1 ml of each solvent was used as a standard for GCMS assays.

System for drug-protein retrieval: The NCBI GenPept database[7] (https://www.ncbi.nlm.nih.gov/protein/) was used to extract the amino acid sequence of the outer membrane protein transport protein (XP-038111716.1) from *Culex quinquefasciatus* and the outer membrane protein transport protein (WP_011706004.1) of *Aeromonas hydrophila* in FASTA format. The Carica papaya Hexadecanoic acid (CID: 985) extract was chosen from the NCBI PubChem Compound database [18] (https://www.ncbi.nlm.nih.gov/pccompound/). To conduct drug docking investigations, the 2D structure was transformed into a 3D model using Discovery Studio software.

Molecular Drug Docking: Using the H-Dock server [19] (http://hdock.phys.hust.edu.cn/), drug docking experiments against the chosen bacterial and mosquito protein sequences were conducted.

3. Results and Discussion

The outer membrane lipoprotein Blc with a nucleotide length of 597 bp (XP_038111716.1), is found in *Culex quinquefasciatus*. Its homologous amino acid sequence (WP_011706004.1) has a length of 198 aa. Similar to this, *Aeromonas hydrophila* has an outer membrane protein with a 432 amino acid sequence length (Figs. 1 and 2).

Initially discovered in eukaryotes and more recently in Gram-negative bacteria, lipocalins are a large family of proteins. Lipophilic substances that are carried by lipocalins serve a variety of cryptic and complex activities. The first lipocalin whose three-dimensional structure has been solved is a retinol-binding protein (RBP), which has a well-established role and transport mechanism. Pheromone and odorant molecule transporters are examples of other lipocalins whose structures are known.

An 8-stranded β-barrel is part of the lipocalin fold, which is followed by an α-helix at the C-terminus. In mammalian lipocalins, the number of disulfide bridges can range from zero to three. Lipocalins have extremely variable amino acid sequences, with the GXW motif at the N-terminus serving as their most distinctive sequence signature. Sequence comparisons and evolutionary investigations of lipocalins resulted in the identification of 14 clades in a taxonomy of these proteins. The bulk of eukaryotic lipocalins, including those from plants, invertebrates, and mammals, are located in the upper clades, whereas bacterial lipocalins are found in Clade 1 (The root clade) [20-29].

Although *A. hydrophila* has a great pathogenic potential, little is known about how it survives and reproduces when the host's immune system is engaged. It has been shown that mast cells, neutrophils, macrophages, eosinophils, and granulocytes may produce extracellular traps (ETs), which have a DNA backbone stabilised by antimicrobial chemicals, histones, and proteases. The main job of ETs is to halt and get rid of the virus right where it's infected, stopping it from spreading to other regions of the affected organism [30].

The most prevalent saturated fatty acid in the human body is palmitic acid (16:0, PA), which may be either through food or produced in the body from other carbohydrates, fatty acids and amino acids. In adipose triacylglycerols (TAG) and membrane phospholipids (PL), PA accounts for 20-30% of the total fatty acids (FA) [31]. A 70 kg guy typically contains 3.5 kg of PA. As the name implies, PA makes up a considerable portion of palm oil (44% of total fats), but it is also present in meat and dairy products (50–60% of total fats), cocoa butter (26%) and olive oil (8-20%) in significant levels. In addition, 20–30% of the total lipids in breast milk include PA [32]. Using Insilico procedures, the identified molecule, Hexadecanoic acid, was transformed from a 2D to a 3D structure. Using automated drug docking methods, the 2 chosen target proteins from *Culex quinquefasciatus* and *Aeromonas hydrophila* were introduced. The outcomes unequivocally showed that Hexadecanoic acid, the discovered chemical molecule, effectively inhibits proteins.

The 2D structure of Hexadecanoic acid is seen in Fig. 2 with coloured atom identifiers. The picture was taken from the NCBI PubChem compound database. The 3D structure of Hexadecanoic acid as shown by the Ball and Stick model is shown in Fig. 3 along with coloured atom labels. The 3D structure was visualised using Discovery Studio, a cutting-edge molecular visualisation software. The internal Vander Waals force allows the target protein, the outer membrane protein transport protein from *Culex quinquefasciatus*, to be bound to the chemical structure.

During the HDOCK server evaluation, homologous complexes having a sequence identity of less than 30% with the test instances were ignored. The input for the receptor, ligand, and protein-protein docking processes were the unbound structural sequences. The anticipated binding mode's acceptable accuracy in line with the CAPRI criteria was successfully identified during the assessment [33-38]. Our results are in agreement with past studies, which are shown in Fig. 23, 24, 25, 26, 27, 28, and 29. Comparing it to other drugs, Hexadecanoic acid with the outer membrane protein transport protein (*Culex quinquefasciatus*) has a greater negative value of -111.73 kcal/mol (Table 1). Similar to this, Hexadecanoic acid's drug scores for the outer membrane protein transport protein of *Aeromonas hydrophila* show a greater negative value of -110.17 kcal/mol (Table 1).

A greater negative value indicates a more advantageous binding interaction between the drug and the receptor, according to theoretical cheminformatics standards. Figures 4, 5, 6, 7, and 8 show how the outer membrane protein transport protein and Hexadecanoic acid bond to one another. Figures 9, 10, 11, 12, and 13 show similar hydrogen bond-based binding interactions between Hexadecanoic acid drugs and outer membrane protein transport proteins.

Interestingly, we discovered that Aeromonas hydrophila's outer membrane protein transport protein has a total length of 432 amino acids. The functional domain of the protein, OMPP1/FadL/TodX (IPR005017) [39], which is located in the amino acid region of 5-432 aa, is present in the protein sequence. The post-docking data made it abundantly evident which amino acid participates in the H-bond interactions involved inside these limits: PHE: 69, MET:430, SER:70, ARG:279, GLN:282, ASN:429, MET:430. All of the aforementioned findings were discussed.

In the same manner, we discovered that the outer membrane protein transport protein from Culex has a total length of 680 amino acids. The Lipocalin, ApoD type (IPR022271) [40] functional domain, which is located in the 6-197 amino acid region, is found in the outer membrane protein transport protein sequence. The post-docking data made it abundantly evident which amino acid participates in the H-bond interactions inside these limits: PHE: 115, PHE: 116, ASN: 124, SER: 114, ASN: 124 [41, 42].

Figures

>WP_011706004.1 outer membrane protein transport protein [Aeromonas hydrophila]

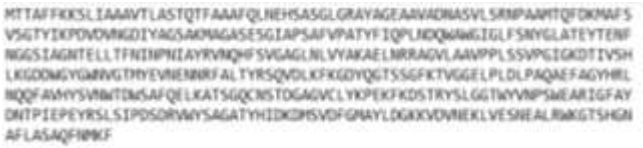

Fig 1: FASTA sequence of the outer membrane protein transport protein of *Aeromonas hydrophila*

>XP_038111716.1 outer membrane lipoprotein Blc [*Culex quinquefasciatus*]
MVKFPLLFSVLVLLGTVTVNAVLYDRPCRTDVPVVQN
FALTRYLGKWYELQRFEKDFQTNYDCVQAEYGL
LDPTTASVRNSAYSLVNETSIEAIGTAKFSFPEQDIVQA
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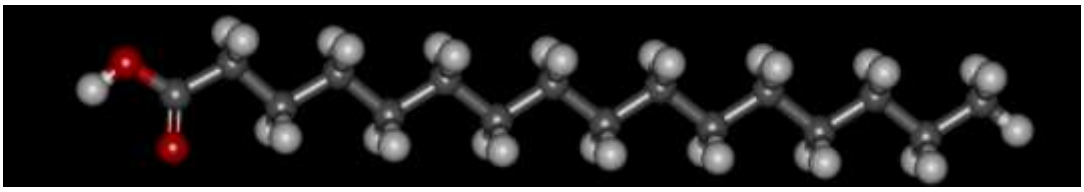
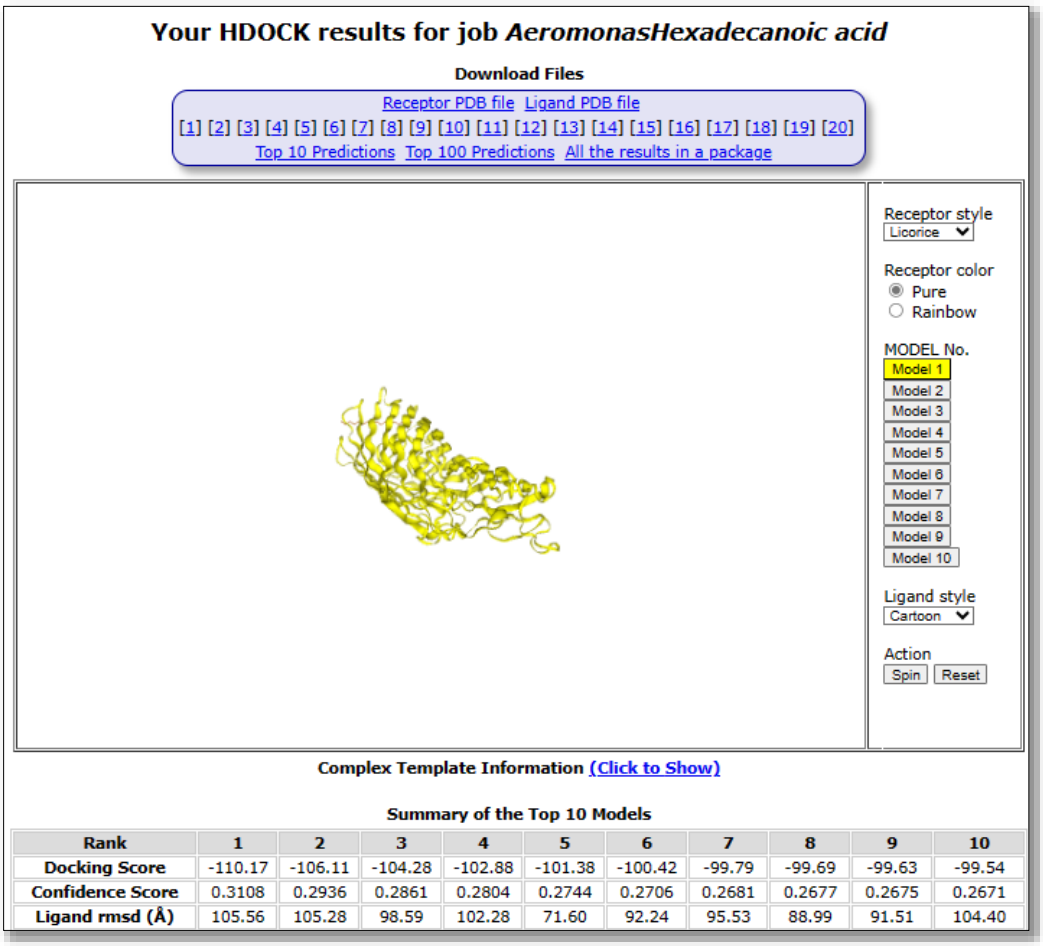


Fig 3: 3D structure of Hexadecanoic acid with respective coloured atoms viewed using Discovery studio software



Rank	1	2	3	4	5	6	7	8	9	10
Docking Score	-110.17	-106.11	-104.28	-102.88	-101.38	-100.42	-99.79	-99.69	-99.63	-99.54
Confidence Score	0.3108	0.2936	0.2861	0.2804	0.2744	0.2706	0.2681	0.2677	0.2675	0.2671
Ligand rmsd (Å)	105.56	105.28	98.59	102.28	71.60	92.24	95.53	88.99	91.51	104.40

Fig 4: Molecular docking studies on Hexadecanoic acid with *Aeromonas hydrophila* carried out using H-Dock server showing the respective binding scores

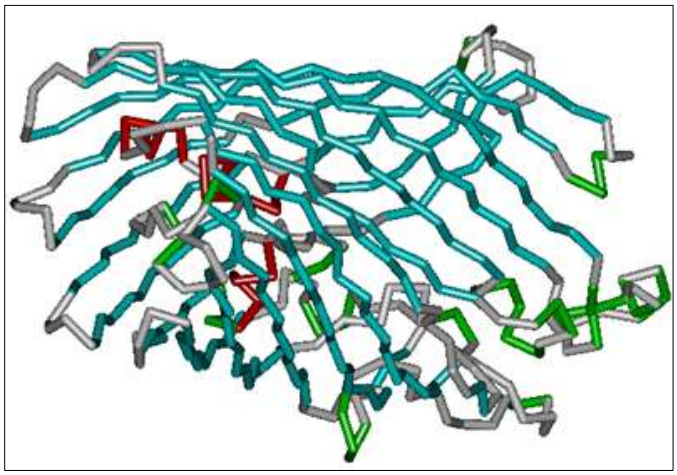


Fig 5: 3D structure of the Outer membrane protein transport protein of *Aeromonas hydrophila* viewed using Discovery Studio software

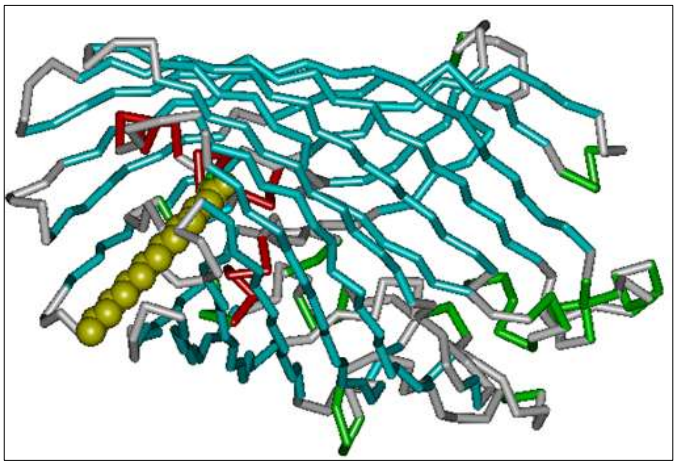


Fig 6: Complex 3D structure of the Outer membrane protein transport protein of *Aeromonas hydrophila* with Hexadecanoic acid viewed using Discovery Studio software

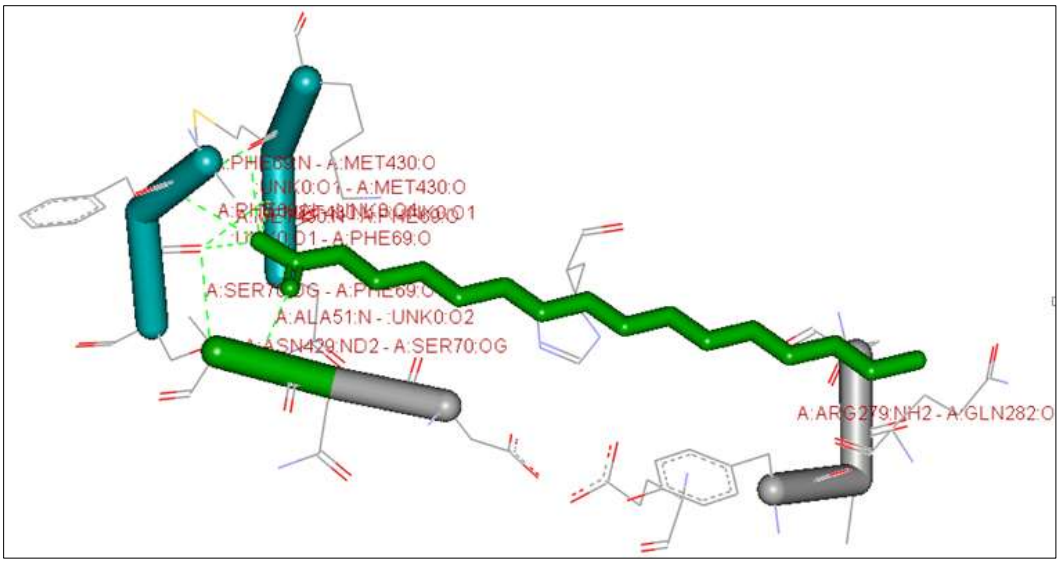


Fig 7: H-Bond interaction between the Outer membrane protein transport proteins of *Aeromonas hydrophila* with Hexadecanoic acid showing the respective amino acids viewed using Discovery Studio software

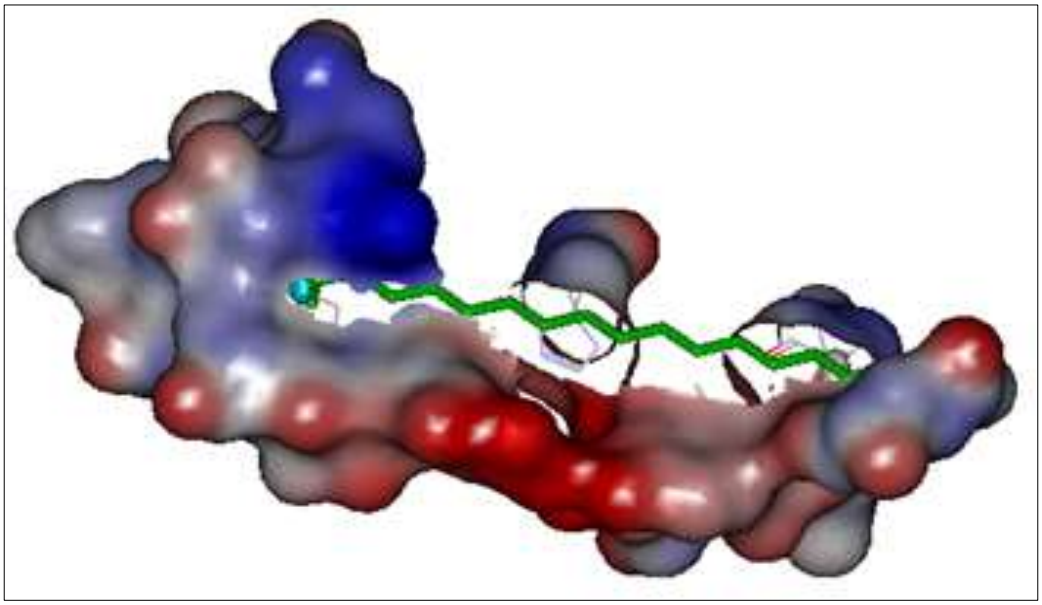
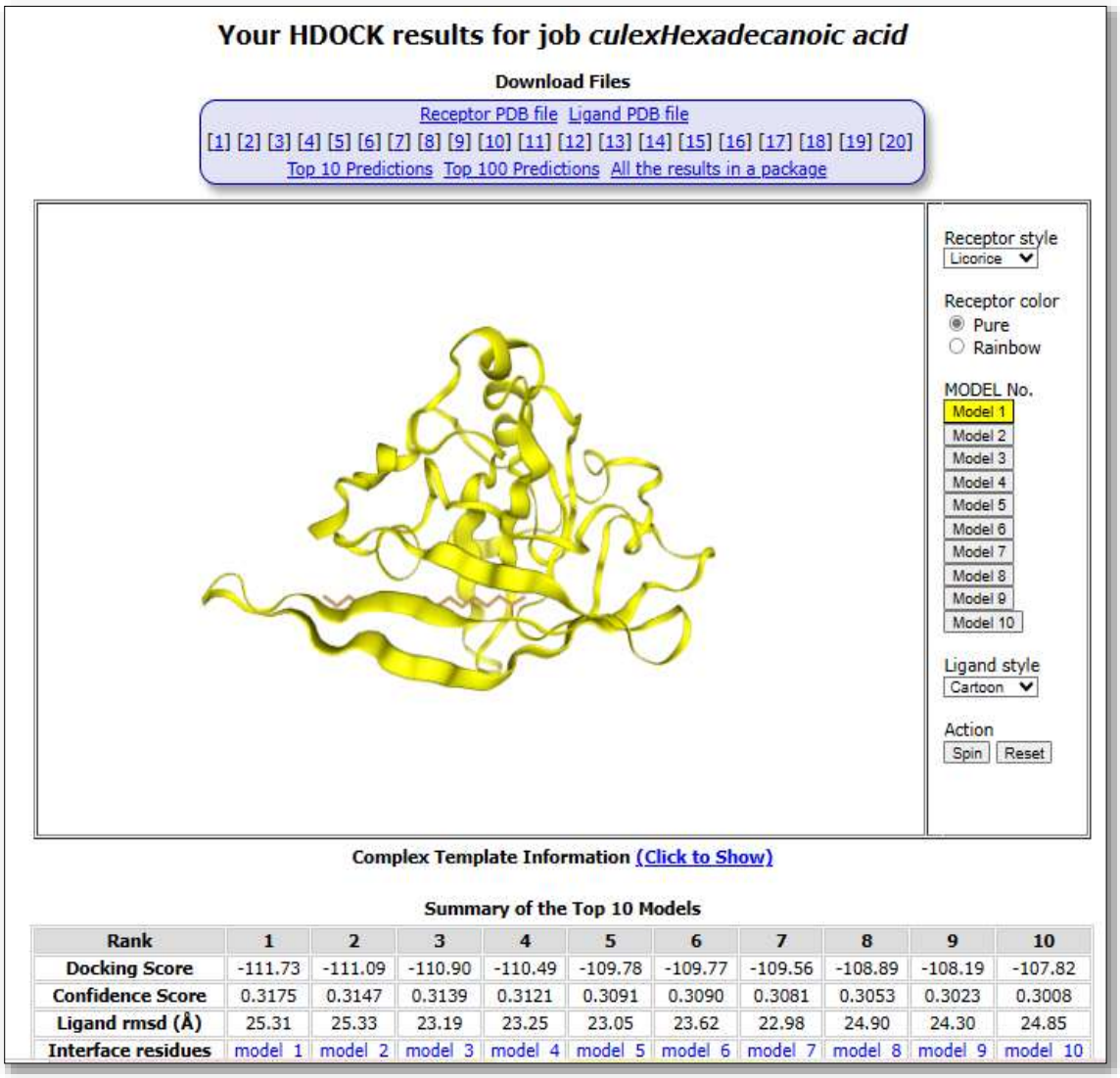


Fig 8: H-Bond interaction between the Outer membrane protein transport protein of *Aeromonas hydrophila* with Hexadecanoic acid in surface model view using Discovery Studio software



Rank	1	2	3	4	5	6	7	8	9	10
Docking Score	-111.73	-111.09	-110.90	-110.49	-109.78	-109.77	-109.56	-108.89	-108.19	-107.82
Confidence Score	0.3175	0.3147	0.3139	0.3121	0.3091	0.3090	0.3081	0.3053	0.3023	0.3008
Ligand rmsd (Å)	25.31	25.33	23.19	23.25	23.05	23.62	22.98	24.90	24.30	24.85
Interface residues	model 1	model 2	model 3	model 4	model 5	model 6	model 7	model 8	model 9	model 10

Fig 9: Molecular docking studies on Hexadecanoic acid with *Culex quinquefasciatus* carried out using H-Dock server showing the respective binding scores

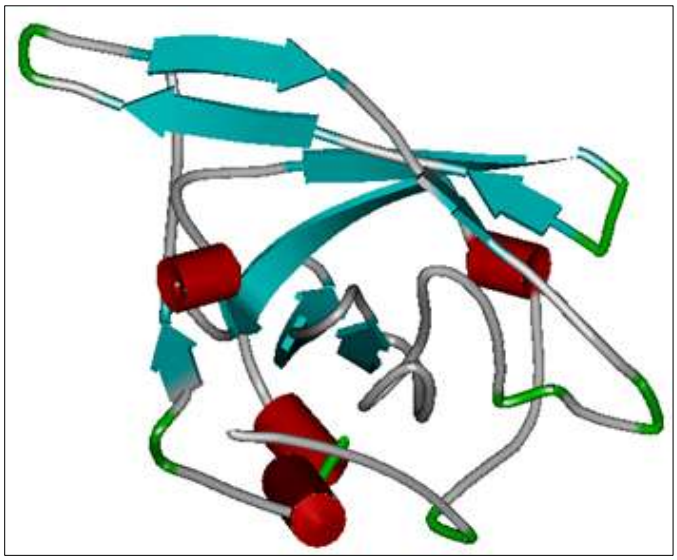


Fig 10: 3D structure of the outer membrane lipoprotein of *Culex quinquefasciatus* viewed using Discovery Studio software

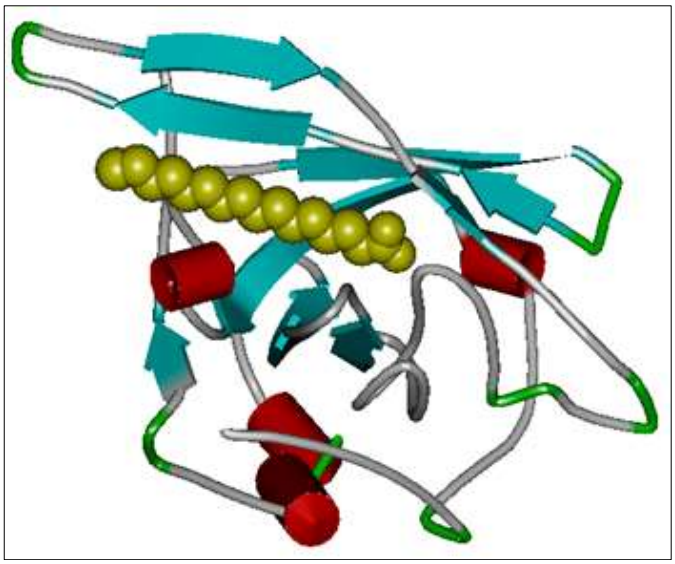


Fig 11: Complex 3D structure of the outer membrane lipoprotein of *Culex quinquefasciatus* with Hexadecanoic acid viewed using Discovery Studio software

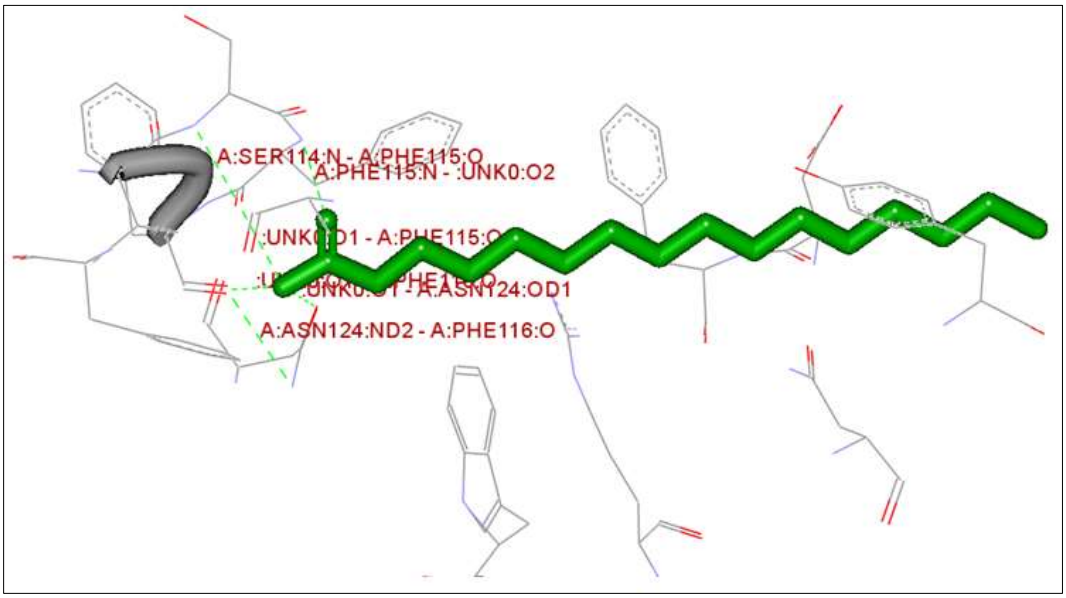


Fig 12: H-Bond interaction between the outer membrane lipoprotein of *Culex quinquefasciatus* with Hexadecanoic acid showing the respective amino acids viewed using Discovery Studio software

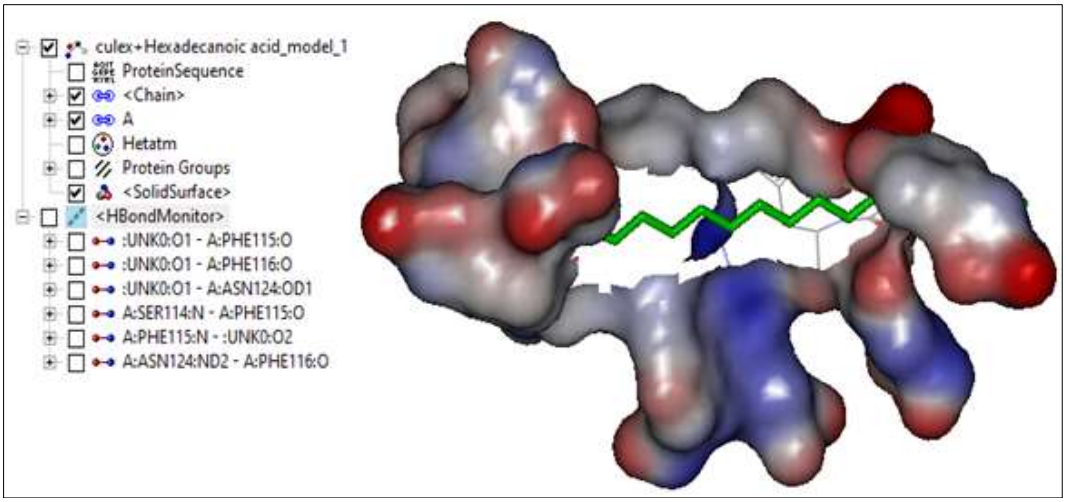


Fig 13: H-Bond interaction between the outer membrane lipoprotein of *Culex quinquefasciatus* with Hexadecanoic acid in surface model view using Discovery Studio software

Table 1: Drug docking summary

	Target 1	**Target 2**
Compound - Extract *Amla Phyllanthus emblica*	*Aeromonas hydrophila* (WP_011706004.1)	*Culex quinquefasciatus* (XP_038111716.1)
Hexadecanoic acid (CID:985)	-110.17 *kcal/mol*	-111.73 *kcal/mol*

4. Conclusion

It was discovered during this study examination that the papaya extract from *Carica papaya* contains Hexadecanoic acid. Utilising Insilico procedures, Hexadecanoic acid was verified to determine its effectiveness. Hexadecanoic acid was docked with the protein targets of the outer membrane protein transport proteins of *Aeromonas hydrophila* and *Culex quinquefasciatus*. The total findings made it abundantly evident that Hexadecanoic acid interacts more favourably with the outer membrane protein transport protein of *Aeromonas hydrophila* than it does with the outer membrane protein transport protein of *Culex quinquefasciatus*. Hexadecanoic acid possesses anti-microbial and pesticide qualities, as may be inferred from our work. Hexadecanoic acid, thus, is crucial to the study of *Aeromonas hydrophila* and *Culex quinquefasciatus*.

5. Acknowledgement

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