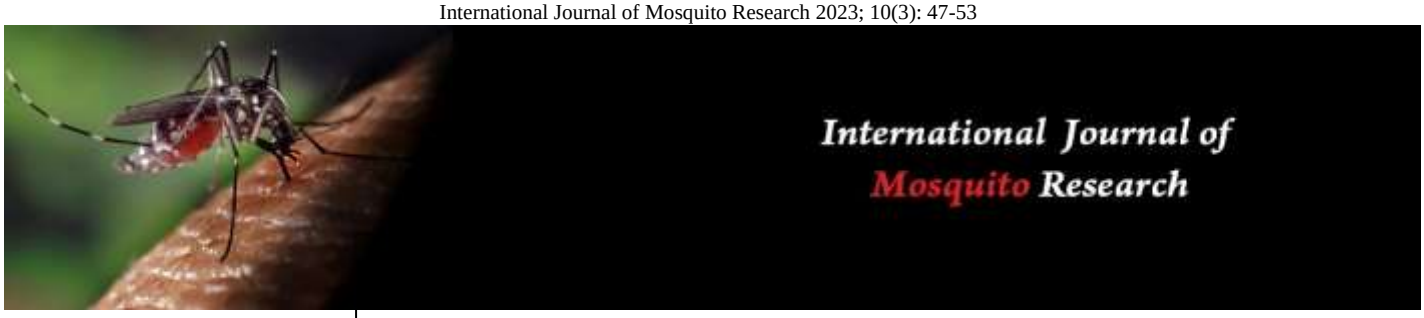

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Identification of the efficiency of Pentane on the bacterial and insecticide proteins of *Aedes aegypti* and *Aeromonas hydrophila* by Insilico methods

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Abstract
Aedes aegypti is known to spread diseases by acting as a vector for Yellow fever virus, *Dengue virus*, *Chikungunya virus*, and *Zika virus*. *Aeromonas hydrophila* is a bacillus with a monotrichous flagellum which is gram-negative, pleomorphic and non-spore-forming. It is a facultative anaerobe that is commonly present in both fresh water and sewage. In this research study, we found out the efficiency of the chemical molecule, Pentane (*Phyllanthus emblica*) on the above two species. From the comparative study, we analyse the anti-microbial and Insecticide properties of Pentane using insilico protocol. The methodology of this study involved protein profiling studies and automated drug docking protocols. All the results were elucidated in 3D form. The complete molecular interaction study clearly elucidated the efficiency of the molecular compound, Pentane with the target proteins, Mucin-5AC and the Outer Membrane Protein Transport Protein of Aedes aegypti and *Aeromonas hydrophila* respectively. It was finally concluded that Pentane had better inhibitive action on *Aeromonas hydrophila* than on *Aedes aegypti*. Hence, Pentane acts as a potential therapeutic agent for diseases associated with *Aeromonas hydrophila*.

Keywords: *Aeromonas hydrophila, Aedes aegypti* and Drug Docking

Introduction
Two Aedes mosquito species, *Aedes aegypti* and *Aedes albopictus*, are responsible for the spread of several important viral illnesses, including yellow fever, dengue, chikungunya, and *Zika virus*. According to the WHO (2016), yellow fever is currently endemic in 47 countries and kills 29,000 to 60,000 people each year. Although dengue-related deaths are much less common, there were an estimated 58.4 million apparent cases of dengue worldwide in 2013 (Stanaway 2016) [1]. From the estimate of 8.3 million in 1990, this is a significant increase (Stanaway 2016) [2]. Dengue caused 1.14 million disability-adjusted life years (DALYs) in total to be lost in 2013 alone (Stanaway 2016) [3].

Chikungunya and *Zika virus* infections typically happen in localised epidemics, unlike the previously two diseases that are both widely spread. They can, however, spread disease across borders as a result of increased international travel (Burt 2017; Sakkas 2016; van Aalst 2017) [4-6]. The most common types of sickness caused by the freshwater, facultatively anaerobic, chemoorganoheterotrophic *Aeromonas hydrophila* bacterium in fish, amphibians, reptiles, birds, and mammals are gastroenteritis, septicemia, and necrotizing fasciitis [7, 8, 9, 10].

Various aquatic and environmental environments, such as silt, estuaries, seaweed, sea grass, used water, drinking water, and food, can harbour Aeromonas species [11, 12]. The *Gamma proteobacteria* genus Aeromonas includes Gram-negative, motile, bacilli or coccobacilli rods that are 1-3.5 m across, non-spore-forming, and have rounded ends. They can convert nitrate to nitrite, are catalase, oxidase, and indol-positive facultative anaerobes, and are typically resistant to the vibriostatic drug O/129.

According to a microbiological examination, *A. hydrophila* is common in the sediment of the Chesapeake Bay and its tributaries, where concentrations range from 4.6 10 2/g to 5 10 3/ml [13]. In shellfish-growing waterways, A. hydrophila cell counts ranged from 3 to 2400 cells/100 ml of water and from 3 to 4600 cells/100 g of oysters.

Material and Methods

Preparation of *Phyllanthus emblica* fruit Extract

The collected *Phyllanthus emblica* fruit 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 *Phyllanthus emblica* fruit 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.

Drug - Protein retrieval system

The amino acid sequence of (YP_003934133.1) the outer membrane transport protein of *Aeromonas hydrophila* and Mucin-5AC (XP_021696657.1) from *Aedes aegypti* were extracted from NCBI GenPept database [14] (https://www.ncbi.nlm.nih.gov/protein/) in FASTA format. Pentane extract present in *Phyllanthus emblica* (CID: 525), was selected from NCBI PubChem Compound database [15] (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 bacterial and mosquito protein sequences were subject to drug docking studies against Pentane using H-Dock server [16] (http://hdock.phys.hust.edu.cn/).

Results and Discussion

Aedes aegypti contains mucin-5AC, the nucleotide length of which is 2043 bp (XM_021840965). Its corresponding amino acid sequence length is 608 aa (XP_021696657) Similarly, *Aeromonas hydrophila* contains an Outer membrane protein which has an amino acid sequence length of 432 aa (WP_011706004.1) (Fig 1 and Fig 2). In this study, we choose the mucin-5AC which plays a vital role in the research on dengue. The most common vector-borne viral disease, dengue, which is caused by the *Dengue virus* (DENV), poses a major health risk to 2.5 billion people globally. Since the mosquito vector of DENV, *Aedes aegypti*, is the main means by which humans are exposed to it, it is essential to identify a novel *Dengue virus* receptor in mosquitoes in order to create new ant-mosquito strategies. During the course of the current investigation, it was identified that peptides may interact with the surface of virion particles and promote viral infection and transportation during the mosquito vector's life cycle.

To find these candidate proteins, we used phage-display library screening against the envelope protein's domain III (EDIII), which is crucial for viral entry and plays a part in host cell receptor binding. The mucin protein was cloned, expressed, and purified for *in vitro* interaction investigations since it had a sequence resemblance to the peptide found during the screening. The effective interactions of mucin was validated with pure EDIII and entire virion particles using *in vitro* pulldown and virus overlay protein-binding assay (VOPBA). In the end, anti-mucin antibodies that block the mucin protein partially lowered the DENV titers in infected mosquitos. Additionally, mucin protein was identified to be localised in *Ae. Aegypti* 's midgut [17].

Although A. hydrophila's strong pathogenic potential has been demonstrated, its survival and multiplication methods against the immune system of the host remain little understood. It has been demonstrated that extracellular traps (ETs), which have a DNA backbone stabilised by antimicrobial compounds, histones and proteases, may be released by mast cells, neutrophils, macrophages, eosinophils and granulocytes. The primary role of ETs is to stop and eliminate the pathogen at the site of infection, thereby preventing it from spreading to other parts of the diseased organism [18].

The compound was extracted from *Phyllanthus emblica* using methanol extract. An identical procedure was used to synthesize 5, 5'-Dimethoxy-2, 2'-[(Pentane-1, 5-diyldioxy) bis (Nitrilo methylidyne)] diphenol (Dong *et al.*, 2009). An ethanol solution (6 ml) of 1, 5-bis (aminooxy) Pentane (134.2 mg, 1.00 mmol) was added to an ethanol solution (10 ml) of 4-methoxy-2-hydroxybenzaldehyde (304.3 mg, 2.00 mmol). At 328 K, the reaction mixture was agitated for 5 hours. After being separated by filtration, the precipitate was repeatedly washed with ethanol and ethanol-hexane (1:4). The substance was vacuum-dried to 204.2 mg of the title compound. Yield, 51.8%. mp. 349–350 K. Anal. Calc. for $C_{21}H_{26}N_2O_6$: C, 62.67; H, 6.51; N, 9.96. Found: C, 62.79; H, 6.68; N, 6.83 [19]. The identified compound, Pentane, was converted from 2D to 3D structure using Insilico protocols. The 2 selected target proteins from *Aedes aegypti* and *Aeromonas hydrophila* were introduced using automated drug docking protocols. The results clearly revealed that the efficiency of the identified chemical compound, Pentane inhibits the proteins.

Pentane's 2D structure is seen in Fig. 2 with coloured atom designations. The NCBI PubChem substance database was used to obtain the image. With coloured atom labels, Fig. 3 shows the 3D structure of Pentane as seen in the Ball and Stick model. Discovery Studio, a cutting-edge molecular visualisation programme, was used to visualise the 3D structure. The target protein of mucin-5AC from *Aedes aegypti* binds to the chemical structure based on the internal Vander Waals force.

Homologous complexes with a sequence identity of less than 30% with the test cases were disregarded throughout the HDOCK server evaluation. The unbound structural sequences were used as input for receptor and ligand as well as the protein-protein docking process. It was successfully determined throughout the evaluation that the projected binding mode had an allowable precision in accordance with the CAPRI criteria. Our findings are consistent with earlier research that has been established [20, 21, 22, 23, 24, 25]. Pentane with mucin-5AC (*Aedes aegypti*) has a larger negative value of -38.68 *kcal/mol* in our drug ratings compared to other drugs (Table 1). Similar to this, the drug scores between Pentane and *Aeromonas hydrophila's* outer membrane protein transport protein indicate a larger negative value of -57.00 *kcal/mol* (Table 1).

Theoretical Cheminformatics guidelines state that a larger negative value denotes a favourable binding relationship between the drug and the receptor. The binding relationship between mucin-5AC and Pentane is depicted in figures 4, 5, 6, 7, 8. Similar binding interactions between Pentane drug and Outer membrane protein transport protein are seen in Figures:

9, 10, 11, 12, and 13 at hydrogen bonds.

Interestingly, we found out that the total length of the protein, mucin-5AC of *Aedes aegypti* was 680 aa. Mucin-5AC protein sequence contains the functional domain, namely, RNA recognition motif domain (IPR000504) [26] which lies within the amino acid range of 347-423 aa. The post-docking results clearly elucidated that the H-bond interaction amino acid involved inside these limits are as follows: SER:365, LEU: 363, ASP: 362, ARG:364.

Similarly, we found out that the total length of the outer membrane protein transport protein of *Aeromonas hydrophila* was 432 aa. The protein sequence contains the functional domain, namely, OMPP1/FadL/TodX (IPR005017) [27] which lies within the amino acid range of 5-432 aa. The post-docking results clearly elucidated that the H-bond interaction amino acid involved inside these limits are as follows: SER:55, SER:52, ARG:56, VAL:53, ILE:156, ASN:155, ARG:239, ASN:221. All the above results were discussed. [28, 29]

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

```
MTTAFFKKSLIAAAVTLASTQTFAAAFQLNEHSASGLGRAYAGEAAVADNASVLSRNPAAMTQFDKMAFS
VSGTYIKPDVDVNGDIYAGSAKMAGASESGIAPSAFVPATYFIQPLNDQWAWGIGLFSNYGLATEYTENF
NGGSIAGNTELLTFNINPNIAYRVNQHFSVGAGLNLVYAKAELNRRAGVLAAVPPLSSVPGIGKDTIVSH
LKGDDWGYGWNVGTMYEVNENNRFALTYRSQVDLKFKGDYQGTSSGFKTVGGELPLDLPAQAEFAGYHRL
NQQFAVHYSVNWTDWSAFQELKATSGQCNSTDGAGVCLYKPEKFKDSTRYSLGGTWYVNPSWEARIGFAY
DNTPIEPEYRSLSIPDSDRVWYSAGATYHIDKDMSVDFGMAYLDGKKVDVNEKLVESNEALRWKGTSHGN
AFLASAQFNMKF
```

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

>XP_021696657.1 mucin-5AC [Aedes aegypti]

```
MALPPLLPYLGINQLRNYTVNLELNLEWVKLNREIWHMVQGMGPFPGAAGFPPTAAQAGLGAEGLSVQVV
KGGGAATGGGGVAGGGGGLAMAVADHQLTSLKQHQEQLNQQHQQHQQQQAVAAAAAAQLANSQTLRNTVS
QYSSQSVLGVPPGGAPPPTSLSSLSAVSSAVPSSGVTSSSSTTSAAVSAITSVPAVGPAGAPPGIAGISN
GIEQQTVSIQTEPETQVSSANNTIINQTNISPNGSSISCSSPSDVATAAALIAASSAQQLVSSGGSSSSS
PGGGLGVGSCIMTTGSSTITTTAGTGTVPLSTAQQQLSVVQSQQQQQNVAAAVAAATALAESKAQPKRLH
VSNIPFRFRDPDLRSMFGQFGTILDVEIIFNERGSKGFGFVTFANSSDAERARERLHGTVVEGRKIEVNN
ATARVQSKKVPAVSSVFLTKPGTVTAPPLAAVCVPWPAEGYRLSMSAWPWLGASAATVGATGAQSAAALA
AAAAAGAAPTSAAAAAAAAAAASSNQAAAAAAAAAANQPMNAAAAAAAAASPLLIAQAAQRAAVAAQRRS
VYFDPYLAAAAASADQQYRLQAAKPVTEVSAQPMIQAAAPLLKTPLSQAQQAYNAATYTAVAARAAYGAA
AAAQPTVAGYATVAGYGREYADPYLGHGIGPVPGYGATMYRGTYNRFAPY
```

Fig 2: FASTA sequence of Mucin-5AC of *Aedes aegypti*

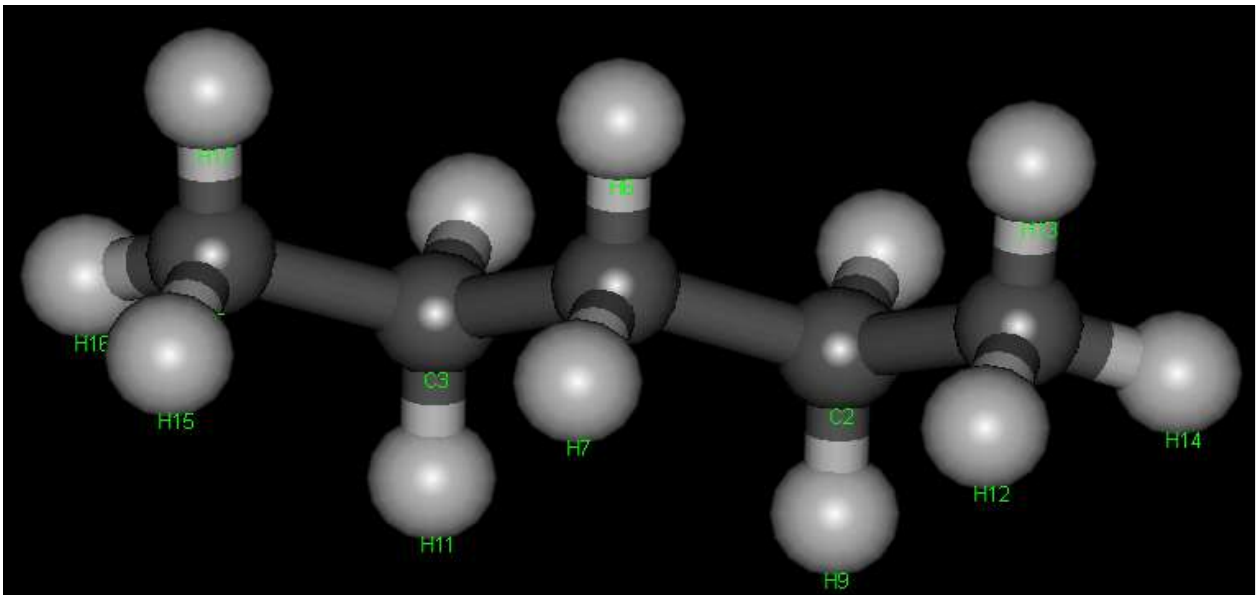

Fig 3: 3D structure of Pentane with respective coloured atoms viewed using Discovery studio software

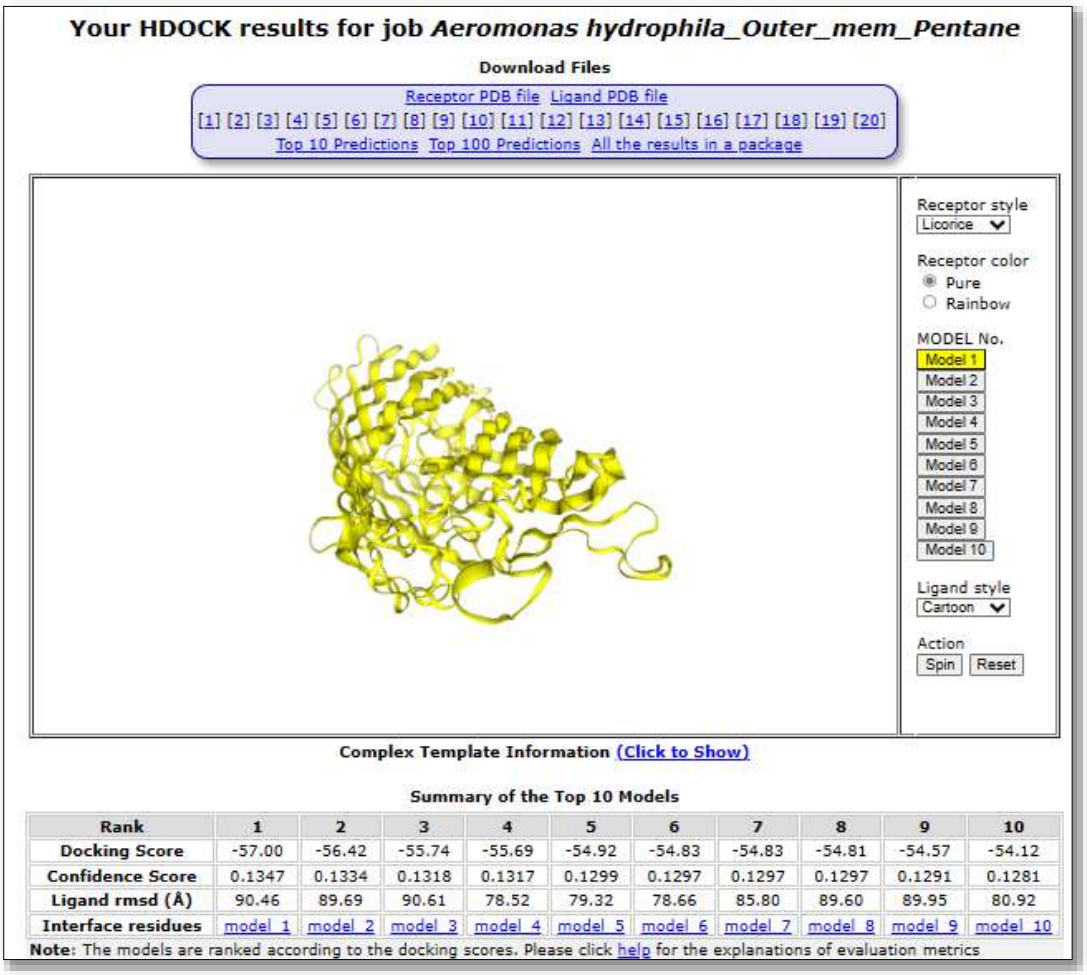

Fig 4: Molecular docking studies on Pentane with *Aeromonas hydrophila* carried out using H-Dock server showing the respective binding scores

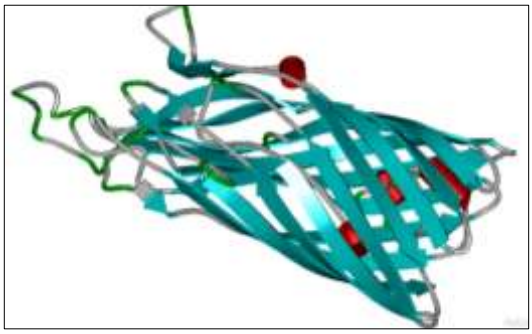

Fig 5: 3D structure of the Outer membrane protein transport protein of *Aeromonas hydrophila* viewed using Discovery Studio software

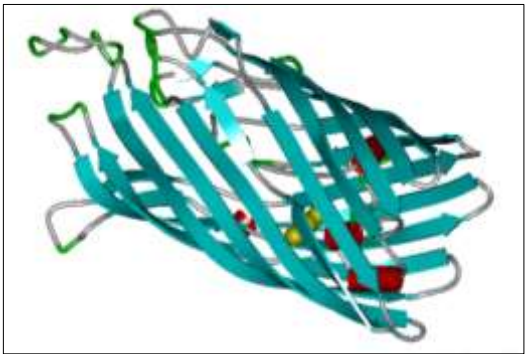

Fig 6: Complex 3D structure of the Outer membrane protein transport protein of *Aeromonas hydrophila* with Pentane viewed using Discovery Studio software

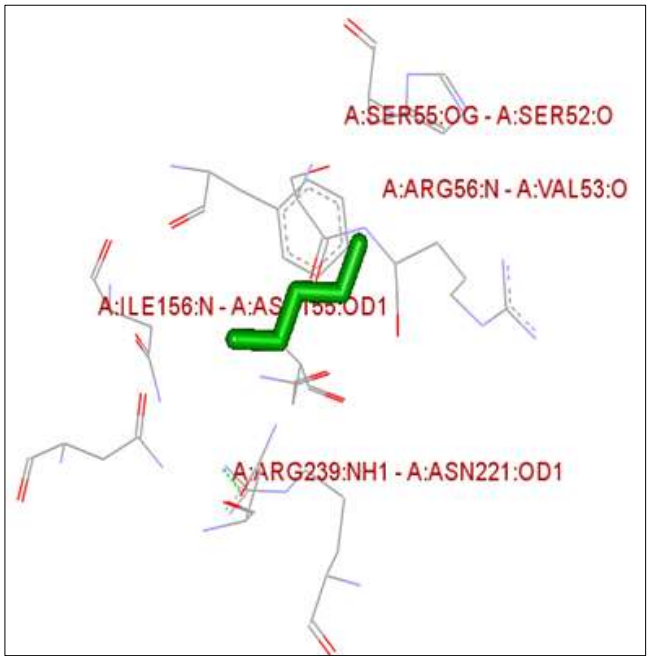

Fig 7: H-Bond interaction between the Outer membrane protein transport protein of *Aeromonas hydrophila* with Pentane showing the respective amino acids viewed using Discovery Studio software

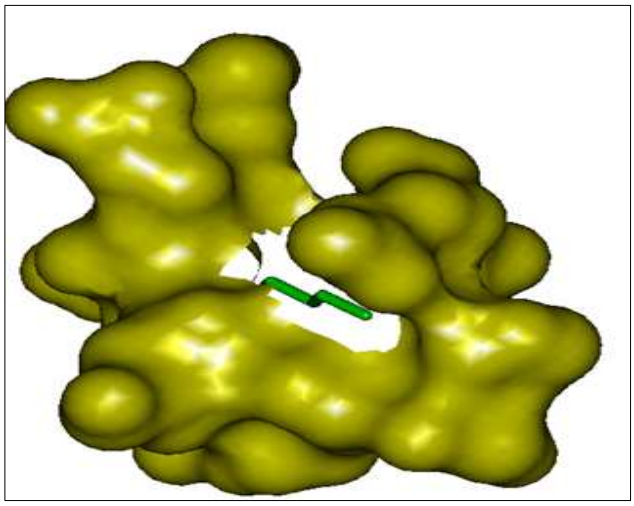

Fig 8: H-Bond interaction between the Outer membrane protein transport protein of *Aeromonas hydrophila* with Pentane in surface model view using Discovery Studio software

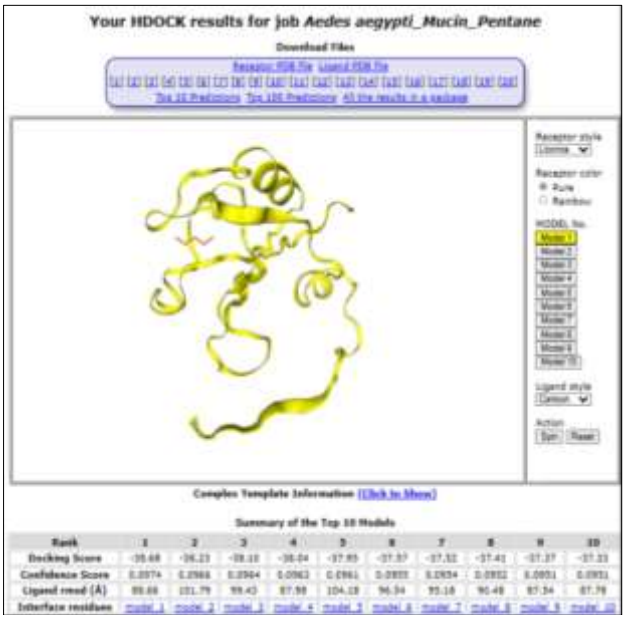

Fig 9: Molecular docking studies on Pentane with *Aedes aegypti* carried out using H-Dock server showing the respective binding scores

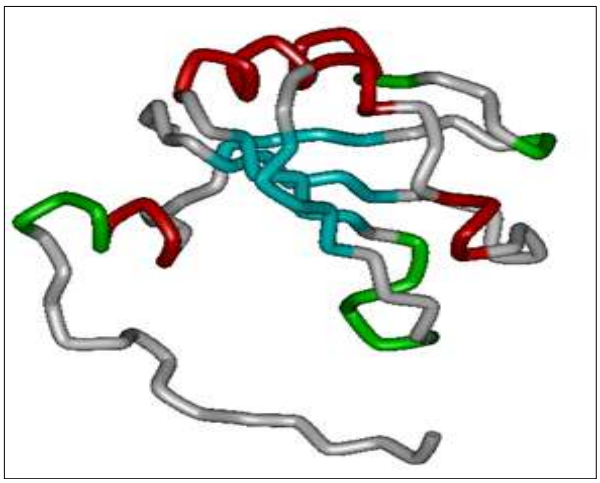

Fig 10: 3D structure of the Mucin 5AC protein of *Aedes aegypti* viewed using Discovery Studio software

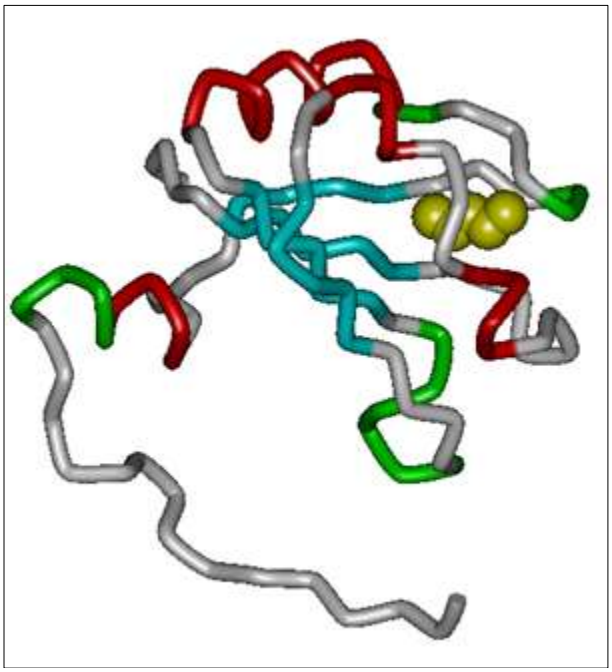

Fig 11: Complex 3D structure of the Mucin 5AC protein of *Aedes aegypti* with Pentane viewed using Discovery Studio software

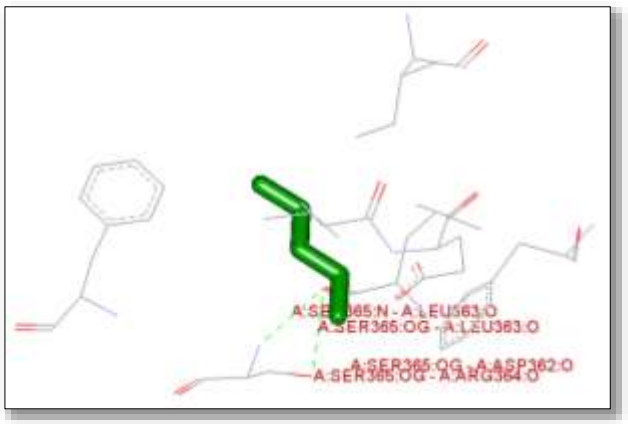

Fig 12: H-Bond interaction between the Mucin 5AC protein of *Aeromonas hydrophila* with Pentane showing the respective amino acids viewed using Discovery Studio software

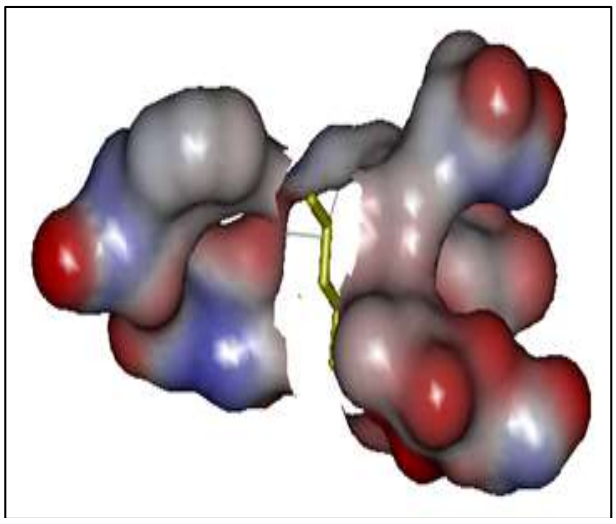

Fig 13: H-Bond interaction between the Mucin 5AC protein of *Aeromonas hydrophila* with Pentane in surface model view using Discovery Studio softwar

Table 1: Drug docking summary

	Target 1	Target 2
Compound 1 Extract (*Phyllanthus emblica*)	*Aeromonas hydrophila* (WP_011706004.1)	*Aedes aegypti mucin*-5AC (XP_021696657.1)
Pentane (CID: 8003)	-57.00 kcal/mol	-38.68 kcal/mol

Conclusion

In this research investigation, it was found that the extract of (*Phyllanthus emblica*) contained Pentane. Pentane was validated to find out its efficiency using Insilico protocols. The protein targets of the outer membrane protein transport protein of *Aeromonas hydrophila* and mucin-5AC of *Aedes aegypti* were docked with Pentane. The overall results clearly elucidated that the molecular interactions of Pentane with the outer membrane protein transport protein of *Aeromonas hydrophila* is better when compared to mucin-5AC of *Aedes aegypti* with Pentane. In conclusion, it can be deduced from our study that Pentane has anti-microbial and insecticide properties. Hence Pentane plays a vital role in the research related to *Aeromonas hydrophila* and *Aedes aegypti*.

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