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In silico Analysis on Docking Studies of Haemolysin Protein in *Vibrio paraheamolyticus*

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ABSTRACT

The natural plants and fruit extract contain antibacterial properties to inhibit the action of disease caused by protein (Haemolysin) present in *Vibrio* species. The drug effectiveness and inhibit the action of disease caused by protein by natural compounds is done by ligand-receptor interactions,pharmacophore and ADMET studies. The ADMET studies were done for 10 natural compounds and 6 were satisfied. The common feature pharmacophore studies were done for 10 compounds (Poses A, B,C,D). The ligand -receptor interactionswere analysed for 60 ligands with common receptor (E,F,G,H,I,J,K,L,M,N). Molecular dynamics and simulation analysed for two natural compounds(Cyanidin) and (Bergapten).

Keywords: *Vibrio paraheamolyticus,* Molecular dynamics, simulation, pharmacophore, Haemolysin.

INTRODUCTION

Vibriosis is a disease caused by Vibrio parahemolyticus that belongs to Genus - Vibrio. These organism are gram-negative, curved shaped bacterium and a important disease causing agent in marine organism like shrimps, fishes, etc. There are reports stating these organisms also causes diseases in human by food-borne infections that associate with eating undercooked seafoods[1,2,3]. Vibriosis have been reported mostly in coastal area and among14 species, Vibrio parahemolyticus is responsible for major outbreaks. Vibrio parahaemolytics causes wound, black lesion and sudden death in fishes like tuna, marakkal, sardines, that causes loss to many seafood companies and hatcheries[4,5,6,7].

A protein secreted by *Vibrio parahaemolyticus*, is a type of hemolysin that is most virulent and acknowledged in past decades as the important pathogenic factor. It is differentiated into thermostable related haemolysin (trh) and thermoreliable direct haemolysin (tdh). Although originally studied for its hemolytic property, TDH has been long suspected to be an enterotoxin involved in most cases of *Vibrio parahaemolyticus*[8,9,10].

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Haemolysin is responsible of lysis of red blood cells and also causes hemolytic cancer in the human. The three types of heamolysin are α, β and γ. α–Haemolysin is mainly responsible for oligomerization of water-filled channels and toxin monomer in cell depolarization, osmotic phenomena and loss of vital molecules leading to its demise. α-Haemolysis has a toxic mechanism to hydrolyze specific membrane lipid causes cell lysis. α-hemolysis has a higher affinity for phosphocholines[11,12].

The lytic process, most commonly seen in leucocytes, is caused by pore formation induced by an oligomerized octamer that organizes in a ring structure. Many studies show that naturally available compounds suppressor or inhibits the bacterial diseases (Vibriosis) caused by hemolysin[13,14,15,16]. The main objective is to test the drug effectiveness to inhibit the action of the haemolysin (which is responsible for causing the disease) obtained from plant extracts, caffeine, curry leaves, flavonoids, grape seed, sweet basil, phenolic, papaya leaf extract compounds.

MATERIALS AND METHODS

Molecular modeling of Hemolysin Protein

To identify the function analysis of the protein hemolysin the sequences of α, β and γ hemolysin was retrieved from the Uniprot database. The homology structure was retrieved from structural database for the protein sequence the structure was predicted using a modeller[9.17]. The modelled structure was analyzed using SAVS (Structure analysis and verification server) and MolProbity (Structure - Validation server) that provides evaluation of mode quality in global and local arrangement of amino acids in Ramachandran plot for the modeled protein. For the predicted structure Energy minimization, Molecular dynamics simulation was performedusing Accelrys Discovery Studio (ADS) 2.0 for 1 nanosecond and stabilized structure model was used.

Identification of drug for Hemolysin Protein from natural plant compounds

The natural drug compounds for hemolysin was identified in various research articles. The identified compound was collected from natural compounds like plant extracts, caffeine, curry leaves, flavonoids, grape seed, sweet basil, phenolic, papaya leaf extract compounds.10 naturally bioactive compounds were identified for hemolysin. Structural analogs for active pharmacologically compounds were retrieved from Pubchem database. Absorption, distribution, metabolism, elimination and toxicity (ADMET) were analyzed by Pharmacokinetic properties. Using Discovery Studio Receptor-ligand docking was done and the docked molecules are viewed for hydrogen bond interactions between the ligand atoms and the amino acid residues of the receptor molecule. The distance between the bonds is calculated and estimated for studying the favourable interactions between the ligand and the receptor molecule. Various interactions such as the alkyl bonds, pi bond, and the respective distance are calculated for the receptor distances are calculated the receptor-ligand complex.

Molecular docking and Molecular dynamics studies on simulation on natural plant compounds

From the modeled hemolysin protein the selected drug compounds was docked by autodock by analyze using drug-likliness and ADMET properties. In ADMET the scores was measured through a multiple scoring functions like piecewise linear potential (PLP1), Ligand Score 1 and 2, and PLP2, Jain, Ludi, Dock score, potentials of mean force, which results 6 compounds satisfy the ADMET Properties based on binding energy the docked compound was selected for the molecular dynamics and simulation studies.

RESULT AND DISCUSSION

Heamolysin

The non-structured target sequence were taken from uniprotKB .The similarity were checked in BLAST and the template structure 3A57A was retrieved from PDB. The sequences were modeled in MODELLER 9.17 server. In script file five DOPE score were generated among that lowest DOPE score was taken as protein structure and results will be executed in modeller window.

The target protein Haemolysin is modelled in modeller 9.17. Thecrystalline structure is viewed in Biovia 4.1.Modelled Protein Haemolysin (Fig 2).Molprobity result for target protein(haemolysin) (Fig 3)

ADMET result analyses and interpretation

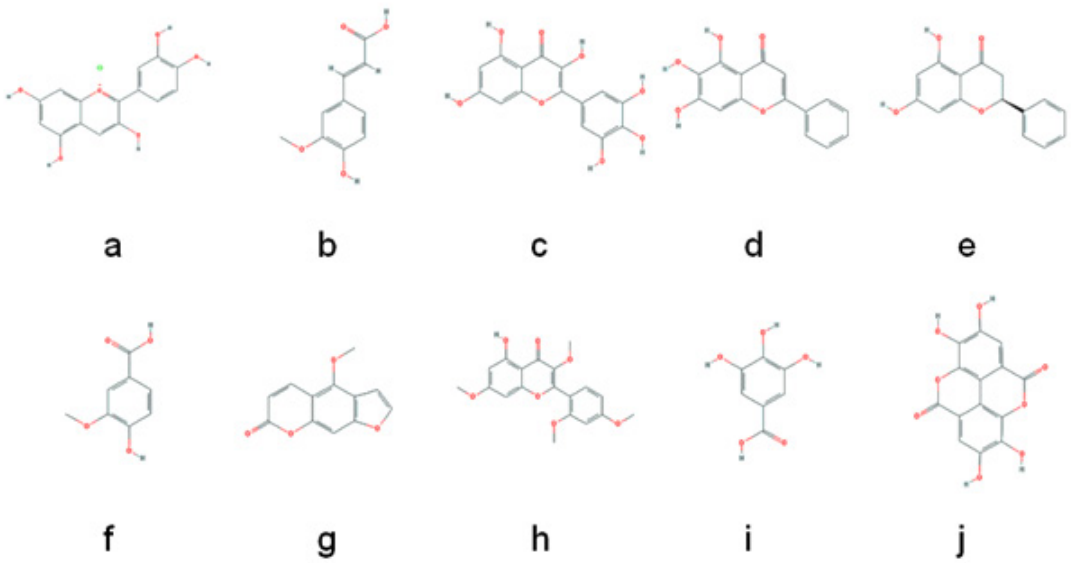
The seven natural compounds of Flavonoids, caffeine ,papaya leaf and grape seed are Subjected to ADMET (Adsorption Distubution, Metabolism, Toxicity studies) studies which gave 6 ligands satisfying the ADMET rules out of 10 compounds. The ADMET results for all the 6 compounds was shown in the given Table -1. (Fig.4). The plot highlights for BBB penetration levels Blue: Very high penetrant; High penetrant;cyan: Medium penetrant; Orange: Low penetrate.An optimal drug should not penetrate the BBB level as it can cause side effects in the CNS. Thus, drug compounds with BBB values 2 and 3 are considered optimal for a drug to be administered.

Pharmacophore result interpretation

Common feature pharmacophore generation is studied for the ligand molecules

Table 1: ADMET Results Analyses

S. NO	PubChem ID	BBB Level	Absorption Level	Solubility Level	Hepatoma Toxicity	CYP_2D	PPB Level	PSA_2D
1	68247	4	0	3	1	1	1	116.631
2	445858	3	0	4	1	0	1	67.861
3	5281672	4	3	3	1	1	1	151.123
4	5281605	3	0	3	1	1	2	83.677
5	68071	2	0	4	1	0	2	67.861
6	8468	3	0	3	1	0	2	67.861
7	2355	2	0	3	1	0	1	47.715
8	70686876	3	0	4	1	1	2	82.766
9	370	3	0	3	1	0	1	100.562
10	5281855	4	1	3	1	0	1	135.723



(a) Cyanidin, (b) Ferulic Acid, (c) Vanillic Acid, (d) Gallic Acid, (e) Myricetin, (f) Baicalein, (g) Pinocembrin, (h) Morin, (i)Ellagic acid, (j) Bergapten.

Fig. 1 : 2D structures of drug for Hemolysin Protein from natural plant compounds

obtained from headsetresult. It generates common feature pharmacopeia models from a set of ligands. It uses Hip-hop hypothesis to generate common feature pharmacophore among a set of active ligands. The algorithm can also optionally use information from inactive to place excluded volume features.The colours of the pharmacopeia are represented in given green, blue and magenta. The green denotes the hydrogen bond acceptors, blue represents the hydrophobic centres and Magenta represents hydrogen bond donors Figure -4.

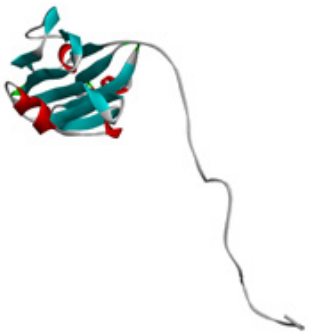


Fig. 2: Haemolysin structure viewed in Biovia 4.1

Absolute energy

Absolute free energy calculations based on the thermodynamic cycle of a set of diverse inhibitors binding to a protein and demonstrating the mean absolute error of the protein or drug.

Conft Number

Conft Nunber or Converted Number is used to convert the structures into their corresponding 3D structure in Discovery Studio and convert interactions energy into pharmacophorepoints location and constraints.

Fit Value

Fit Value verses the negative logarithm of the activity while generating the 10 pharmacophoremodels were generated with corresponding statistical parameters such as cost values, RMSD and Fit Value.

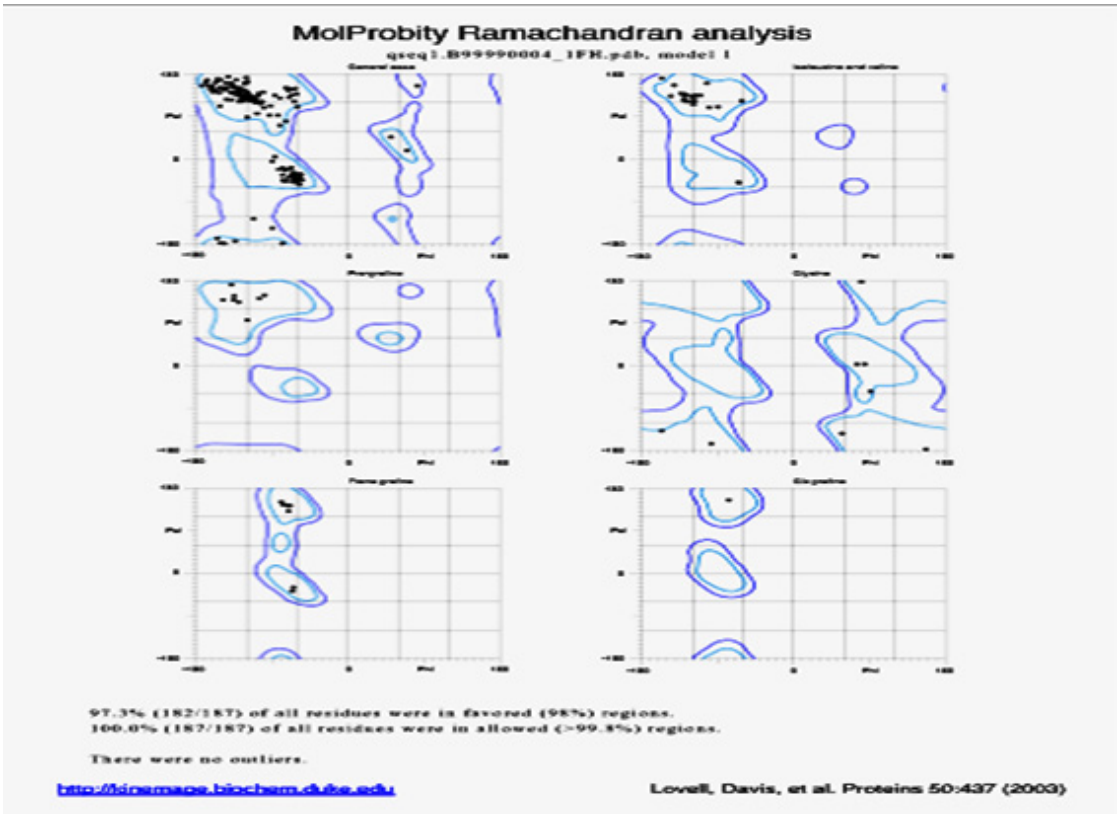


Fig. 3: Ramachandran plot of haemolysin

Study of receptor ligand interactions

The receptor ligand interactions are studied for Ten natural compounds (Figure -5)

Above results for docking done by giving 10 and 4 poses for the ligand-receptor interactions as input. Out of the 61 compounds selected for docking 10 compounds generated successful results for receptor-ligand interactions with 84 poses. The Ligscore,PMF,PLP,Jain Score,Dock Score are the five values used to analyse the docking results. A higher score in all the above values shows a highly stable molecular interactions, Polar attractive interactions,Polar repulsive interactions, solvation of the protein and ligand and an entropy term for the ligand.

Solvation analysis of target protein

The variation of solvation entropy as a function of solute charge has been used to investigate hydrophobic and hydrophilic ordering and the structure-making and structure breaking effects of ion.The solvation energy, free energy, entropy, etc. are defined as the difference in these quantities for the box containing the water molecules and the solute molecule and two separate isolated boxes of volume V, one with the water molecules and one with the solute molecule.

Solvation result of target protein

Study of molecular dynamics and simulation

The dynamics of the receptor are studied using standard cascade dynamics. Molecular dynamics and simulation analyses for cyanidin and bergapten Figure - 6.

Simulation result of kinetic energy graph for cyaniding

Molecular dynamics(MD) is a computer based method for the study of physical movements of atoms and molecules. The atoms and molecules can interact for a fixed period. The trajectories of atoms

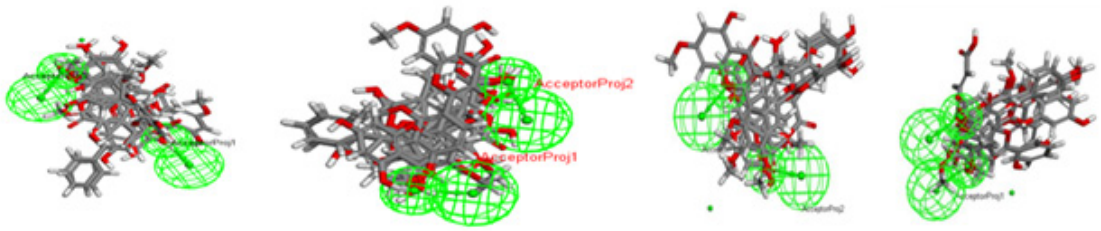


Fig. 4: Common Feature Pharmacophore studies: (A, B, C, D) POSE 1,2,3,4 represents he different positions of ligands binding with the receptor

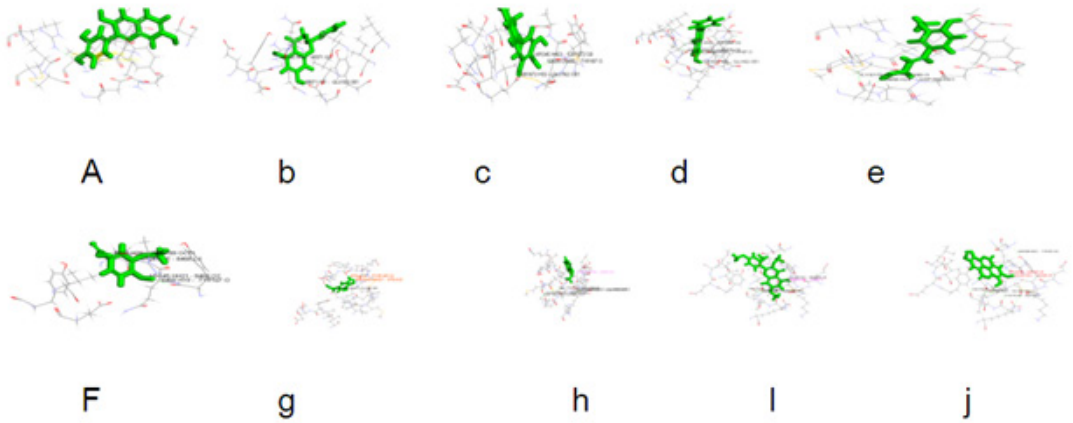


Fig. 5: Ligand-Receptor intreactions represents the receptor -ligands interactions of (a) Cyanidin ,(b) Ferulic acid, (c) Pinocembrin, (d) Myricetin, (e) Baicalein, (f) Vanilic Acid, (g) Galic Acid,(h) Bergapten,.(h) Morin, (j) Ellagic Acid

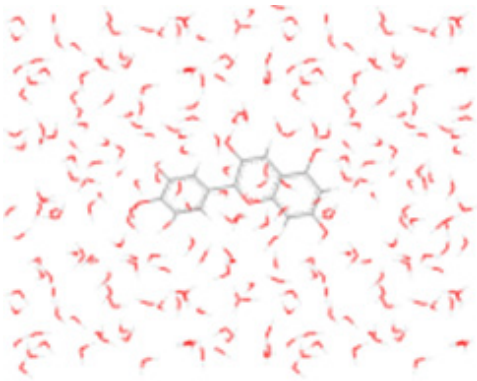


Fig. 6: Solvation of ligand and receptor in solvent and its movement

and molecules are determined by numerically solving Newton's equation of motion. The PDB structure of Docked receptor-ligand is applied for Force field with CHARmm. Using Standard Dynamics method (SD) saves the current location of the coordinates from iteration to iteration. This method is useful for small changes, such as the removal of unfavourable steric contacts .Maximum steps: 2000,RMS:0.0001. Heating- A minimized structure represents the molecule at a temperature close to absolute zero. The equilibrium procedure is continued until various statistical properties of the system become independent of time. Steps:2000,time step:0.001. Save Frequent results-1000.

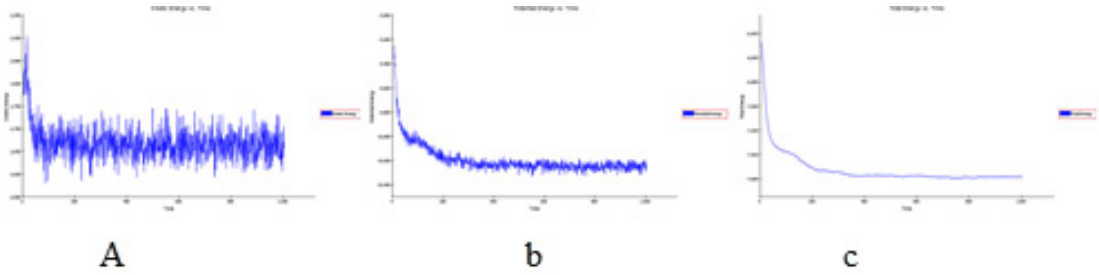


Fig. 7: In simulation Graph shows (a)Kinetic energy vs time (b)Potential energy vs time (c)Total energy vs time mass required to do one work from rest to motion for cyanidin

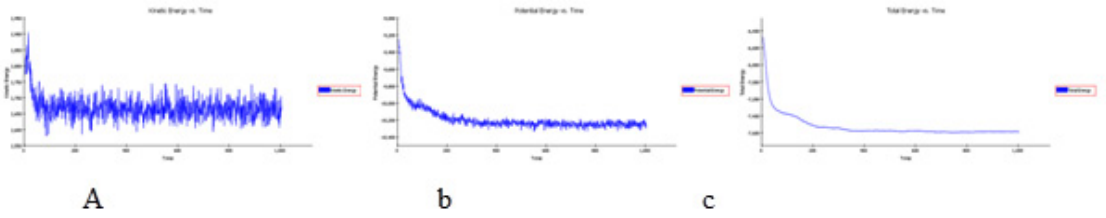


Fig. 8: In simulation Graph shows (a)Kinetic energy vs time (b)Potential energy vs time (c)Total energy vs time mass required to do one work from rest to motion for bergapten

For the final molecular dynamics simulation, Charm takes the equilibrated structure as its starting point. In simulation, the trajectory traces the motions of the molecule through a period of at least several hundred picoseconds. Just as with energy minimization, provision is made to update the list of nonbonded interactions periodically. Additional options for handling nonbonded interactions and the environment are available, which makes the dynamics facility quite flexible.

Max step:1000000.Save frequent result -:true.

Simulation Graph shows (a)Kinetic energy vs time (b)Potential energy vs time (c)Total energy vs time mass required to do one work from rest to motion forbergapten (Figure -7)

Simulation result of kinetic energy graph for Bergapten

In simulation, the kinetic energy the mass required to do one work from rest to motion was calculated (Figure -8).

CONCLUSION

The natural plants and fruit extract contain antibacterial properties to inhibit the action of disease caused by protein(Haemolysin) present in Vibrio species. The drug effectiveness and inhibit the action of disease caused by protein by natural compounds is done by ligand-receptor interactions, pharmacophore and ADMET studies.The ADMET studies were done for 10 natural compounds and 6 were satisfied. The common feature pharmacophore studies were done for 10 compounds receptor. Molecular dynamics and simulation is analysed for two natural compounds (Cyanidin) and (Bergapten). During the simulation studies, these 2 compounds was shown better result and found that there wasn't much significant variation in the conformation. Hence, this identified compounds will be very much useful for to design novel and potential drug, which are considered to be an alternative compound for Hemolysin Protein.

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