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Research Article

Bioactive compounds of *Hemidesmis indices* inhibit the acyl-homoserine lactone synthase

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Abstract

AHL (acyl homoserine lactone) is a signaling molecule responsible for communication in gram negative bacteria, which is responsible for bacterial virulence as well as biofilm formation. The speedy growing in the number of resistant pathogenic bacteria takes controlled to a decrease in the efficacy of the existing antimicrobial agents. Acyl-homoserine lactone synthase plays an important role in the key molecules responsible for the formation of antibiotic resistance of gram-negative bacteria. The molecular docking studies performed by using molecular docking server online respectively in which the oral biofilm target namely N-acyl homoserine (ESAI) (PDB id: 1kzf) have a potential interaction with vanillin and Hexadeconoic acid. In this study, the protein N-acyl homoserine (ESAI) was used from its structure perspectives. The primary and secondary structures were calculated using online tools. Its role in oral biofilm was assessed by molecular docking the compounds present in the root extract of Hemidesmis indices assayed by GC-MS analysis. This in-silico study results throw light on how these active components of Hemidesmis indices are effective in oral biofilm.

Keywords: *Hemidesmis indices*, Docking studies.

1. INTRODUCTION

Quorum sensing is a highly regulated and effective means of communication seen in bacterial colonies. This involves the production of certain chemical messengers called auto-inducers which signals other bacteria of the same or adjacent communities. In gram positive bacteria, the signaling molecules are called auto-inducer peptides (AIP) and in gram negative they are called acyl-homoserine lactones (AHL). The formation of biofilms is regulated by quorum sensing molecules secreted by the bacteria. [1] A biofilm is a group of microbial cells, mainly bacteria, close to the tooth surface and coated by an extracellular polymeric substance. This coating keeps cells and permits enhanced growth rates, along with new parallel gene transfer between cells within the coating, which promotes additional problems. [2] Amoxicillin from the penicillin group is often used to treat dentoalveolar abscesses and periodontitis. Amoxicillin is a broad-spectrum antibiotic that works by binding to penicillin binding proteins in Gram-positive and Gram-negative bacteria and inhibiting the transpeptidation process in bacteria. [3] *Hemidesmus indicus* is one of the important medicinal plants, belongs to the family Asclepiadaceae, which is derived from the word "Asklepios" means God of medicine. It is generally named as Indian Sarsaparill, and in Sanskrit, it is termed as "Anantmool," which means endless root. It is a slim, laticiferous semi-errect shrub. Roots of this plant contains phenolic compounds, steroids, flavonoids, saponins, terpenoids, cardiac glycosides, proteins, tannins and cardiac glycosides. [4] 2-hydroxy 4-methoxy benzaldehyde (2H4MB) is an isomer of vanillin; it is one of the major compounds in the volatile oils of Decalepis hamiltonii and *H. indicus* [5]. Nowadays, the use of complementary and alternative medicine and especially the consumption of botanicals have been increasing rapid worldwide, mostly because of the evidently less frequent side-effects when compared to modern medicine. One such plant is *Hemidesmis indices* is a folk medicinal plant. An attempt has been taken to investigate the oral biofilm activity of identified bioactive compounds of Hemidesmis indices root extract on quorum sensing model through molecular docking. The aim of this research is to investigate the oral biofilm constituents present in the root extract of *Hemidesmis indices* using molecular docking prediction. *In-silico* docking procedures have also been carried out to examine the interactions of the plant components with acyl-homoserine lactone synthase targets. The

widespread uses of *Hemidesmis indices* in traditional medicine have resulted in significant qualitative analysis of the plant and its active principles.

2. EXPERIMENTAL METHODS

2.1 Molecular Docking Analysis

A computational tool offers the advantage of delivering new drug candidates more quickly and at a lower cost. The present work by computational approach used for the following software manipulation of drugs using molecular docking server online web service for calculation of drug likeness. The identified compounds from Hemidesmis indices was used to interact with acyl homoserine lactone synthase retrieved from PDB.

2.2 Protein Sequence

The acyl homoserine lactone protein was retrieved from online database of SWISSPROT. [6] It was obtained through the entry keyword of ESAI protein and searched the entire database. The sequence of acyl-homoserine lactone synthase were shown. The ESAI protein was retrieved in FASTA format and it was used for the further computational analysis.

2.3 Primary Structure Prediction

For physiochemical characterization, theoretical isoelectric point (PI), molecular weight, total number of positive and negative residues, extinction coefficient, [7] half-life, [8 11] instability index,[12] aliphatic index and grand average of hydropathy (GRAVY) [13] were computed using the Expasy protparm server.

2.4 Secondary Structure Prediction

Secondary structure of the protein was determined by using the FASTA sequences of protease and predicted using SOPMA and SOPM .[14]

2.5 Transmembrane Region Identification

The transmembrane region of ESAI protein was examined by SOSUI server. [15] The evaluated transmembrane region was analysed and visualized by pep wheel, [16] using EMBOSS 2.7 suit.

2.6 Homology Modeling and Validation

The protein sequence was subjected for comparative homology modeling via Swiss model [17] and evaluate by Rampage online server.[18] The protein was confirmed by using online server procheck [19] and WHAT IF.[20] The Swiss model executes the sequence alignments and looks for the assumed template protein in the 3D model.

2.7 Protein Preparation for Docking

Docking calculations were carried out on protein models involved in acyl homoserine lactone synthase. The crystalline structure were downloaded from Protein Data Bank website (http://www.rcsb.org/pdb/home/home.do) and saved in pdb format. In the protein id the essential hydrogen atoms, Kollman united atom type charges, and salvation parameters were added with the aid of Auto Dock Tool. Affinity (grid) maps of 20x20x20 A° spacing were generated using Autogrid program. Auto dock parameter set and distance-dependent dielectric functions were used in the calculation of the van der waals and the electrostatic terms respectively.[21]

2.8 GC-MS Compounds

Through the GC-MS analysis of *Hemidesmis indices* performed by [22] identified *Hemidesmis indices*, vanillin and hexadeconoic acid compounds and included in this study.

2.9 Ligand Retrieved

The screened compounds were retrieved from the PubChem compound (http://www.ncbi.nlm.nih.gov/pccompound) and used for the further studies.

2.10 Receptor retrived

The receptor of acyl-homoserine lactone synthase protein was downloaded from the PDB (http://www.rcsb.org/pdb/home/home.do) and the PDB ID: 1KZF.

2.11 Docking studies

Docking calculations were carried out using Docking server.[23] Gasteiger partial charges were additional to the ligand atoms. Non-polar hydrogen atoms were merged, and rotatable bonds were strong. Essential hydrogen atoms, Kollman united atom type charges, and solvation parameters were added with the aid of AutoDock tools. Affinity (grid) maps of xx Å grid points and 0.375 Å spacing were generated using the Auto grid program.[24] Auto Dock parameter set and space dependent dielectric functions were used in the calculation of the van der Waals and the electrostatic terms, individually. Docking simulations were performed using the Lamarckian genetic algorithm (LGA) and the Solis & Wets local search method.[25] Initial position, orientation, and torsions of the ligand molecules were set randomly. All rotatable torsions were released during docking. Each docking experiment was derived from 10 different runs that were set to terminate after a maximum of 250000 energy evaluations. The population size was set to 150. During the search, a translation step of 0.2 Å, and quaternion and torsion steps of 5 were applied.

3. RESULTS AND DISCUSSION

3.1 Primary structure analysis

The acyl homoserine lactone protein (P54656) had gene name as ESAI was retrieved from SWISSPROT as the form of FASTA sequence.

3.2. Sequence Subjected for Modeling

MLELFDVSYEELQTTRSEELYKLRKKTFSDRLGWEVICSQG
MESDEFDGPGTRYILGICEGQLVCSVRFTSLDRPNMITHTF
QHCFSDVTLPAYGTESSRFFVDKARARALLGEHYPISQVLF
LAMVNWAQNNAYGNIYTIVSRAMLKILTRSGWQIKVIKEA
FLTEKERIYLLTLPAGQDDKQQLGGDVVSRTGCPPVAVTT
WPLTLPV

The primary physiochemical parameter was performed and amino acid composition was identified (Table 1 and 2). The results show that the ESAI protein was composed of 22 amino acids with different ratios. Among that

leucine content was more (11.0%) that indicate the hydrophobic nature of protein because it has an aliphatic isobutyl side chain and also essential amino acid. This indicates the sequence length is 210, and the molecular weight of protein was found to be 23844.39, the protein has 5.96 isoelectric points that represent the protein is acidic in nature and it will help to purify the protein molecule. The number of negative charged residues (Asp + Glu) is 24 and number of positive charged residues (Arg + Lys) is 22. The extinction coefficient was 34170 at 280 nm; it may be probable to avoid interference of other substances. The evaluated value used to determine the quantification of protein-protein or protein-ligand interactions. The quantitative measurement of dynamic equilibrium based on the half-life time. The ESAI protein has 30 hours in mammalian reticulocytes; in yeast have 20 hours and 10 hours in *E.coli*. The stability of protein was determined by using the instability index (39.46). The aliphatic index characterize that the volume of protein occupied by aliphatic chains (Alanine, valine, isoleucine and leucine), ESAI protein have 89.10 that denoted unstable in high thermal conditions. Grand Average Hydropathicity denoted that the hydrophobicity of amino acid residues. Here ESAI protein has -0.129 had a reasonable interaction with water molecule. The protein molecule has 4 different atoms such as C,H,N,O, and S, molecular formula was $C_{1072}H_{1675}N_{283}O_{313}S_{10}$.

Table 1: Amino acid composition (%) of acyl homoserine lactone protein computed in protparam:

Amino acids	Numbers	Percentage
Ala (A)	11	5.2 %
Arg (R)	13	6.2 %
Asn (N)	5	2.4 %
Asp (D)	10	4.8 %
Cys (C)	5	2.4 %
Gln (Q)	10	4.8 %
Glu (E)	14	6.7 %
Gly (G)	14	6.7 %
His (H)	3	1.4 %
Ile (I)	11	5.2 %
Leu (L)	23	11.0 %
Lys (K)	9	4.3 %
Met (M)	5	2.4 %
Phe (F)	10	4.8 %
Pro (P)	9	4.3 %
Ser (S)	14	6.7 %
Thr (T)	17	8.1 %
Trp (W)	4	1.9 %
Tyr (Y)	8	3.8 %
Val (V)	15	7.1 %
Pyl (O)	0	0.0 %
Sec (U)	0	0.0 %

Table 2: Parameters computed using Expasy's protparam tool

Name	Accession Number	Sequence length	Mol. Wt	PI	-R	+R	EC	II	AI	GRAVY
ESAI	P54656	210	23844	5.96	24	22	33170	39.46	89.10	-0.129

Mol.wt- Molecular weight; PI- Isoelectric point; -R – number of negatively charged residues; +R – number of positively charged residues; EC – Extinction coefficient at 280 nm; II – Instability Index; AI – Aliphatic Index; GRAVY – Grand average of Hydropathicity.

3.3 Secondary Structure of Protein

The secondary structure of ESAI protein was predicted by using SOPMA and SOPM (Table 3). The protein was α helix with other structures such as extended stand, β turn and random coil. Presents the comparative analysis of SOPMA and SOPM. From which it is clear that random coil is mostly present, when the structure was predicted both by SOPMA and SOPM, followed by extended strand and alpha helix. So this protein is stable in nature.

Table 3: SOPMA and SOPM

Secondary structure	SOPMA	SOPM
Alpha helix (Hh)	78 is 37.14%	59 is 28.10%
3_{10} helix (Gg)	0 is 0.00%	0 is 0.00%
Pi helix (Ii)	0 is 0.00%	0 is 0.00%
Beta bridge (Bb)	0 is 0.00%	0 is 0.00%
Extended strand (Ee)	45 is 21.43%	52 is 24.76%
Beta turn (Tt)	9 is 4.29%	17 is 8.10%
Bend region (Ss)	0 is 0.00%	0 is 0.00%
Random coil (Cc)	78 is 37.14%	82 is 39.05%
Ambigous states (?)	0 is 0.00%	0 is 0.00%
Other states	0 is 0.00%	0 is 0.00%

3.4 Protein Structure Validation

The physiochemical parameters represent the protein primary properties and then the secondary structure was predicted as alpha helical in nature of transmembrane protein in (Fig 1) and then the structure was evaluated by PROTTER and Ramachandran plot represents the protein validation (Fig 2 and 3). The predicted ESAI protein structure was validated by using Ramachandran plot using PROCHECK software that shows the protein molecule contains 198 residues in that 157 amino acid most, 14 amino acids additionally allowed, 0 generally allowed and 0 disallowed regions.

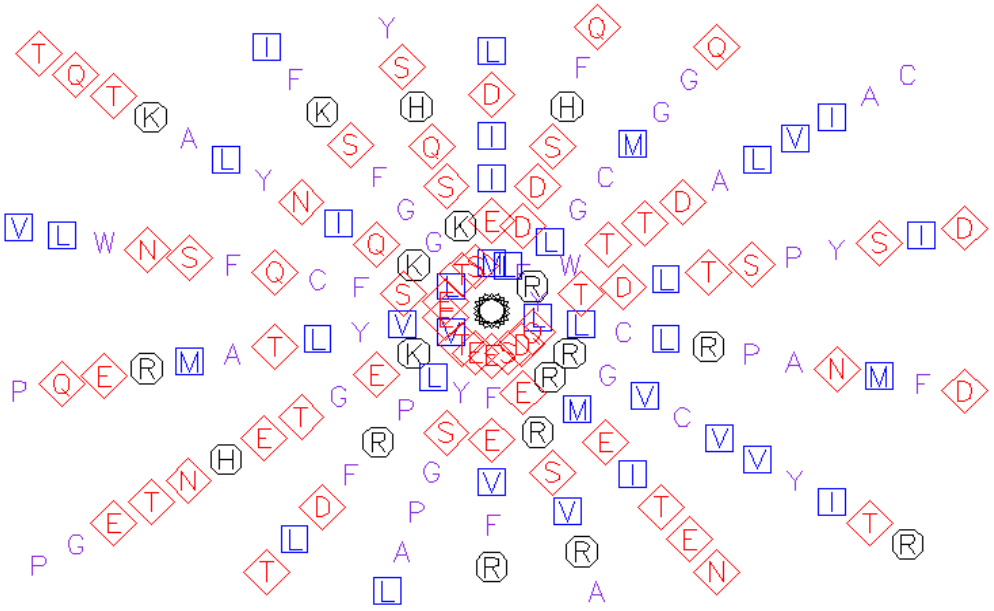


Figure 1: Pepwheel of 1kzf

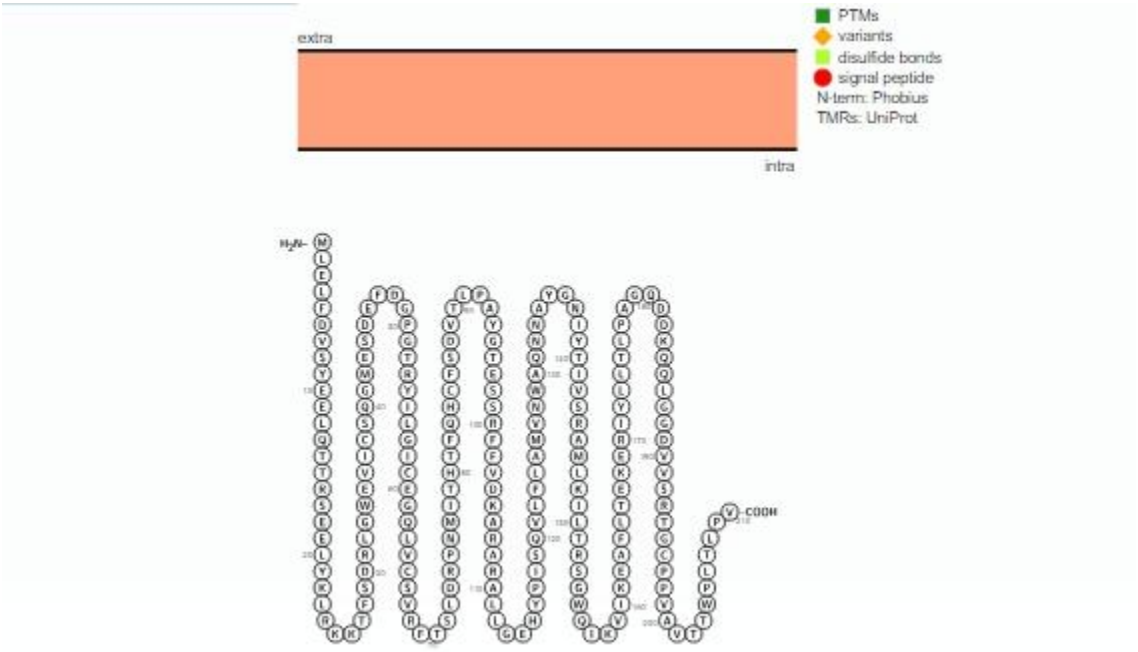


Figure 2: PROTTER Result of Transmembrane region

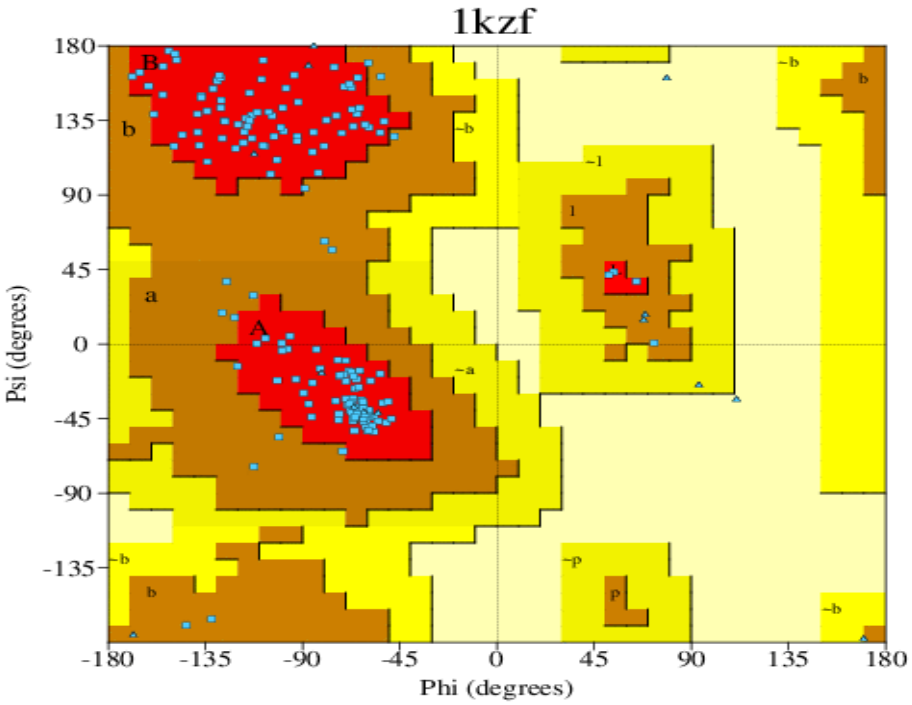


Figure 3: Ramachandran plot

3.5 Molecular Docking

The ESAI protein (Fig 4), the macromolecule and ligands (isolated compounds of *Hemidesmis indices* and Amoxicillin) in (Table 4a & 4b) were subjected to docking studies by using online Auto dock server. The software used to runs 10 docking and were shown in (Table 5). the 3D structure of acyl-homoserine lactone synthase (PDB id: 3kzf) were optimized to achieve minimal potential energy using molecular docking server. The minimization values are summarized. Docking simulation of 10 runs of plant compound vanillin was performed for a set of catalytic active site of acyl-homoserine lactone synthase (Fig 5).

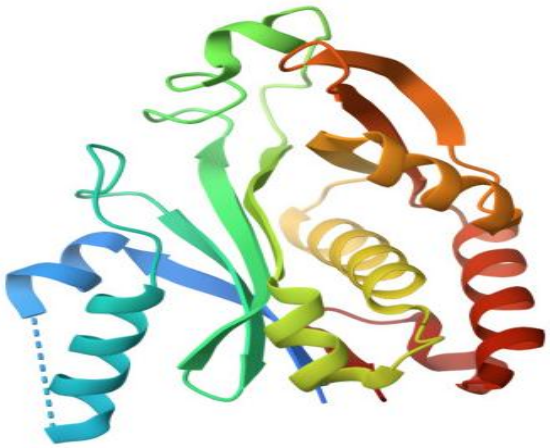


Figure 4: ESAI protein (PDB id: 1kzf)

The best docked conformation was selected based on lowest docking energy and binding free energy. Docking score is a measure of interaction of the ligand to the active site of the target.[26] More negative values indicate more effective stable conformation of the bound ligand target. Our results suggest that the standard drug Amoxicillin with 1kzf (-4.70 kcal/mol) forms hydrogen bonds ILE 141, hydrophobic interactions are MET 42 and VAL 142, other interactions are MET 42, MET 77, SER 99, PHE 101, PHE 102, THR 140, ILE 141, VAL 142, MET 146. Vanillin with 1kzf (-5.15 kcal/mol) forms polar bonds THR 140, hydrophobic interactions are PHE 101, MET 146, VAL 142, MET 126 and other interactions are SER 98, PHE 123, LEU 150. Hexadecanoic acid with 1kzf (-4.03 kcal/mol) forms polar bond ARG 100, hydrophobic interactions are VAL 67, MET 77, PHE 101, PHE 123, MET 126, VAL 142, MET 146, LEU 150, LEU 176, and other interactions are SER 98, ARG 100, THR 140 respectively.

The Synthesis and detection of acyl-homoserine lactones (AHLs) enables many gram negative bacteria to engage in quorum sensing, an intercellular signaling mechanism that activates differentiation to virulent and biofilm lifestyles. The AHL synthases catalyze acylation of S-adenosyl-L-methionine by acyl-acyl carrier protein and lactonization of the methionine moiety to give AHLs. The crystal structure of the AHL synthase, Esal, determined at 1.8 A resolution, reveals a remarkable structure similarity to the N-acetyltransferases and defines a common phosphopantetheine binding fold as the catalytic core. The molecular docking analysis revealed that the active compounds from Hemidesmus indices fits appropriately within the active site of AHLs synthase, exhibiting favorable binding energy. Among that other parameters, confirming the stability and suitability of the interaction.

Table 4a: Standard Drug (Amoxicillin)

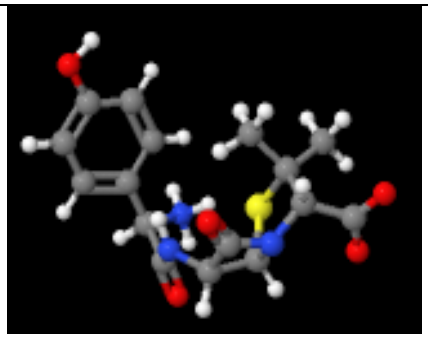
S.No	Name	Mol. Structure
1	Amoxicillin	

Table 4b: Plant compounds identified by GC-MS

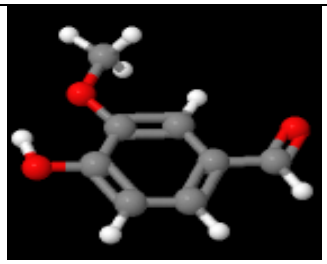
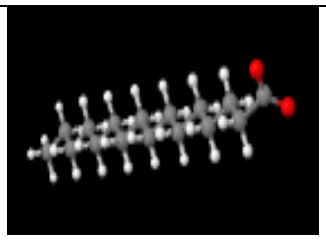
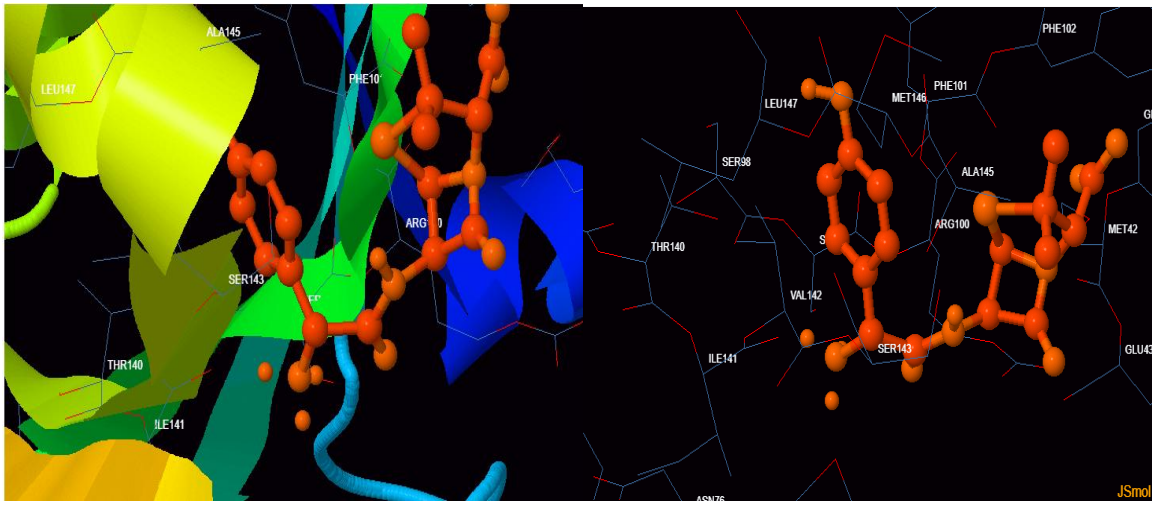
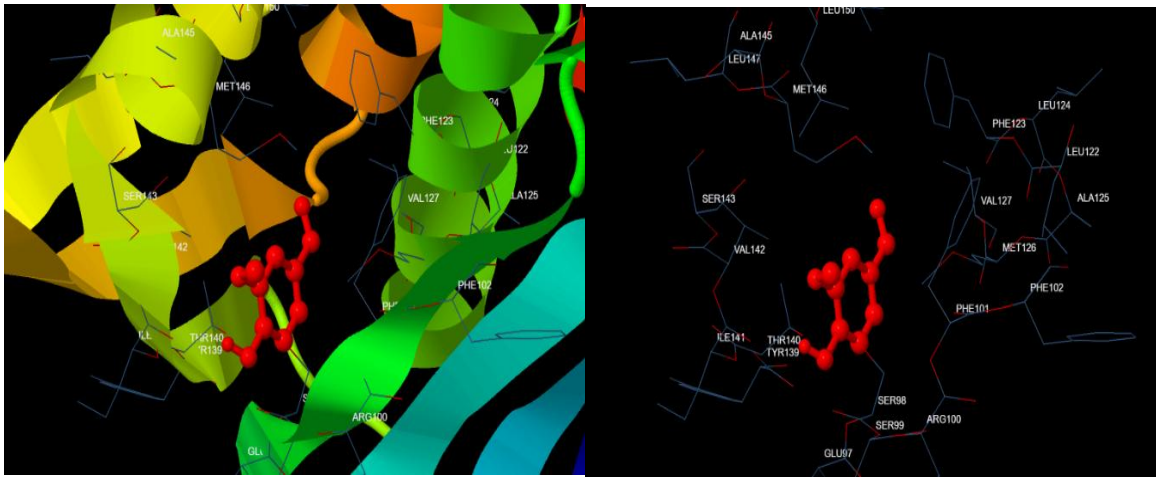
S.No	Name of the compound	Mol. Formula	Mol. Weight	Mol. Structure
1	Vanillin	$C_8H_8O_3$	152	
2	Hexadecanoic acid	$C_{16}H_{32}O_2$	256	

Table 5: Interacting residues responsible for docking

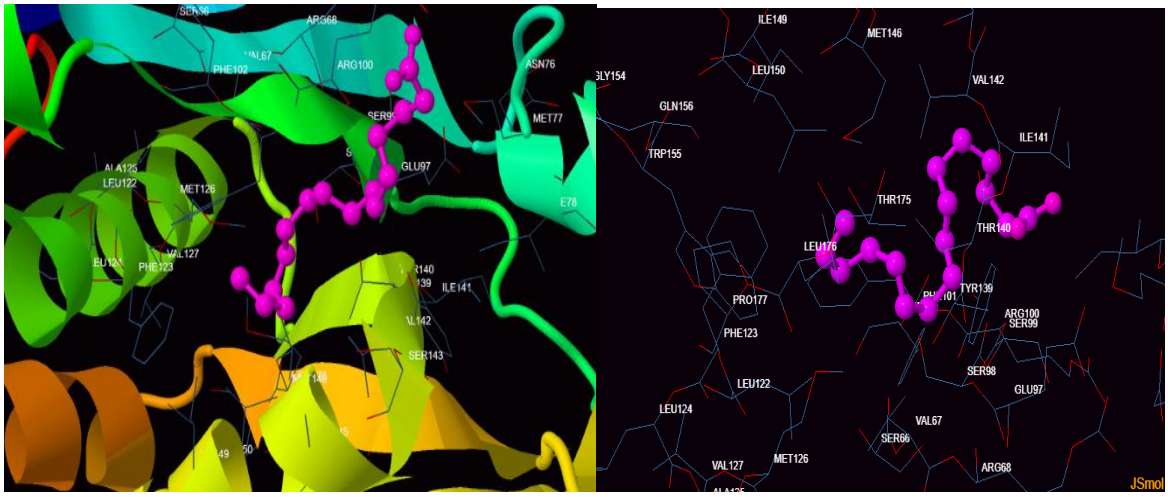
Docking result	Est. free energy of binding	Est. inhibition of constant, Ki, µM	vdW + Hbond + desolv energy	Electrostatic energy	Total intermole energy	Frequency	Interact surface
Amoxicillin (Drug) with 1kzf	-4.70 kcal/mol	357.67	-5.47 kcal/mol	-0.11 kcal/mol	-5.58 kcal/mol	20 %	619.632
Vanillin with 1kzf	-5.15 kcal/mol	167.39	-5.25 kcal/mol	-0.03 kcal/mol	-5.28 kcal/mol	100%	420.442
Hexadecanoic acid with 1kzf	-4.03 kcal/mol	1.10	-7.67 kcal/mol	-0.20 kcal/mol	-7.87 kcal/mol	10 %	642.461



a) Docking interactions of standard drug Amoxicillin with 1kzf



b) Docking interactions of vanillin with 1kzf



c) Docking interactions of Hexadecanoic acid with 1kzf

Figure 5: Docking interactions of a) vanillin, b) Hexadecanoic acid with active sites of 1kzf

4. CONCLUSION

acyl-homoserine lactone synthase plays a vital role in oral biofilm, a detailed study of the physiochemical characteristics helps to understand its role in oral biofilm. The physiochemical parameters also supported the protein properties, and then the protein undergoes with pock finder to elucidate the sites which were ready to docking studies with ligand. In other hand the *Hemidesmis indicus* containing organic compounds were interacting with the acyl-homoserine lactone synthase protein and provide better scoring compare to that of standard drug of amoxicillin by using Auto dock. This study proves that vanillin present in *Hemidesmus indices* enhanced the oral biofilm. This study reveals that vanillin

and hexadecanoic acid, a compound with various therapeutic properties, shows promise as an inhibitor against the AHLs synthase enzyme. The results from the conducted analysis offer significant insights that could assist in the development of novel inhibitors aimed at managing biofilm formation and antibiotic resistance.

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Conflicts of Interest: The authors have given considerable and equal contributions to this research.

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