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In silico Identification of Hederagenin from *Syzygium aromaticum* as a Potential Therapeutic Agent against *Aeromonas hydrophila* Infection in *Labeo rohita*

Shishir Goswami[1,2], Arti Goel[2], Ruby Singh[1*] and Prachi Bhargava[3]

[1]*Gevanam Genomics, Govardhan enclave, Lucknow – 226301, Uttar Pradesh, India; rubys922@gmail.com, contact@gevanamgenomics.com*

[2]*Amity Institute of Microbial Technology, Amity University, Noida – 201303, Uttar Pradesh, India*

[3]*Shri Ramswaroop Memorial University, Lucknow – 226301, Uttar Pradesh, India*

Abstract

Aeromonas hydrophila is a Gram-negative bacterium that poses a significant threat to aquaculture, leading to high mortality rates in *L. rohita* and substantial economic losses. A key virulence factor of this pathogen is the Outer Membrane Protein-A (OmpA), which plays a crucial role in bacterial pathogenicity. This study focuses on the characterisation and structural prediction of porin OmpA using *in silico* approaches. The amino acid sequence of OmpA was retrieved in FASTA format from the Protein Data Bank (PDB), followed by primary structure analysis, including GRAVY index, amino acid composition, instability index, phosphorylation sites, and signal peptide cleavage site prediction. Secondary structure prediction using the SOPMA server revealed a heterogeneous structural composition. Further, homology modelling techniques were employed to estimate the 3D structure of the functional site of OmpA. The modelled 3D structure of OmpA was utilised for *in silico* screening of bioactive compounds from two medicinal plants, *Acanthus ilicifolius* (45 compounds) and *Syzygium aromaticum* (Clove) (171 compounds). Molecular docking analysis identified Hederagenin ($C_{30}H_{48}O_4$), a compound from *Syzygium aromaticum*, as a potential natural prophylactic and therapeutic agent against *A. hydrophila*.

Major Findings: This study highlights the significance of Hederagenin as a promising candidate for drug development, offering a natural approach to controlling bacterial infections in aquaculture.

Keywords: *Aeromonas hydrophila*, Bacterial Infections, Molecular Docking Simulation, Membrane Protein, Phosphorylation

1. Introduction

Rohu, also called *L. rohita*, is a species of freshwater fish of the Cyprinidae family. It is indigenous to freshwater environments such as rivers in South Asia, especially those found in Bangladesh, India, Nepal, Pakistan, and Myanmar[1]. The fast growth rate, high market demand, and capacity to adapt to a wide range of environmental circumstances make rohu one of the most economically significant species in aquaculture. According to recent studies (2022), passive data collection suggests a high mortality rate (around 60%) for *L. rohita* in aquaculture.

**Author for correspondence*

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Bacterial infections are a significant contributor to this mortality, bringing the survival rate down to approximately 56.67%. These infections cause severe lethargy, bleeding, ulcers, discolouration, and redness in the fish[2,3].

Although infections with *A. hydrophila* are more frequently linked to fish and other aquatic animals, they can sometimes infect immunocompromised people, especially if the fish is undercooked or contaminated. *A. hydrophila* carrying contaminated fish, like Rohu, can cause food-borne illness when consumed by humans[4]. Several factors can influence the impact on human health, such as eating contaminated fish may cause symptoms like nausea, vomiting, diarrhoea, abdominal pain, and fever in certain situations. Usually, these signs appear many hours to several days after eating tainted food[5]. The full genome sequence of *A. hydrophila* strain ATCC 7966T is now available, enabling in silico investigation of the genome for a variety of biological purposes. In silico analysis of the *A. hydrophila* genome was conducted in order to identify possible targets for vaccines and drugs, taking into consideration the potential risks that *A. hydrophila* may represent to people[6]. In the year 2021, *A. hydrophila*'s outer membrane proteins (OMPs) were found to be an interesting candidate for vaccine development since they play a range of functional roles in virulence and disease. The goal was to create an *in silico* model of the beta-barrel OMP found in the 22 genuine A. hydrophila pangenomes[7]. Thus, further in this research paper, *in silico* techniques- PDB, Blast-p, Discovery Studio4.2, SwissModeler, Protparam, SOPMA and Signalp6.0 will be used.

The primary goal of this study is to predict the structure of the porin OmpA protein, which is not available in the Protein Data Bank, and directs us to use the FASTA sequence available to model the structure of the protein and check its stability using primary and secondary structure analysis *in silico* applications. Molecular models of this protein of *A. hydrophila* as documented in this study may provide a valuable aid for designing an inhibitor or better ligand against bacterial gastrointestinal tract disease and wounds, which could play a significant role in prevention or cure for such fatal infections in fish as well as in humans[8,9]. This could protect aquatic life from microbiological infections and hence contribute to the acceleration of national economies[10,11].

After having access to a molecular model of our target protein, the secondary goal of our study takes place where we approach with herbal and plant compounds for molecular docking with virtual screening, as these compounds tend to be more promising to cure diseases and safer than synthetic compounds, both to humans and the environment[12]. Further, we need to categorise a list of compounds which would inhibit the effect of bacterial protein by the *in silico* method of virtual screening. This method aims to achieve two key objectives: minimising the complexity of potential molecules by focusing on essential chemical features and efficiently identifying those that interact with the target protein[13]. After categorising a list of compounds, we prepare both protein and ligand for molecular docking to obtain the top 10 compounds with the least binding energy.

Developing successful treatments for *A. hydrophila* requires an understanding of how well plant-based chemicals work against the pathogen. With differing degrees of inhibition, different Essential Oils (EOs) have shown antibacterial action against *Aeromonas* species, conducted in February 2021. With surprisingly low MIC values ranging from 0.015 to 0.031 µg/mL, *Syzygium aromaticum* EO demonstrated notable inhibitory effects on *A. hydrophila* (ATCC 49140) and other *Aeromonas* species. This demonstrates *S. aromaticum's* potential as a natural antibacterial. The exact mode of action and the particular bioactive substances in charge of this inhibition are still unknown, though. To maximise their therapeutic use, guarantee their effectiveness, and maybe create focused treatments for bacterial infections in aquaculture, these chemicals must be identified and characterized[14].

Bacterial infections in aquaculture, particularly those caused by *A. hydrophila*, pose a significant challenge, often leading to high mortality rates and economic losses. While synthetic antibiotics are widely used, their overuse contributes to antimicrobial resistance, highlighting the need for sustainable alternatives. Essential oils, particularly from medicinal plants like *Syzygium aromaticum*, have shown promising antibacterial properties. However, the specific bioactive compounds responsible for these effects and their molecular mechanisms remain largely unexplored.

This study further hypothesises that bioactive compounds from *Syzygium aromaticum*, particularly Hederagenin, can effectively inhibit *A. hydrophila* by targeting the Outer Membrane Protein-A (OmpA), a key virulence factor. To test this, we employ an *in silico* approach, including structural characterisation of OmpA and molecular docking analysis, to identify potential inhibitory interactions. By bridging computational and natural product-based approaches, this study aims to provide new insights into plant-derived antimicrobial strategies for aquaculture disease management.

1.1 Objective

This study aims to characterise and predict the structural properties of the Outer Membrane Protein-A (OmpA) of *A. hydrophila* using *in silico* approaches and to evaluate the antimicrobial potential of bioactive compounds from *Syzygium aromaticum* against this pathogen. Given the demonstrated inhibitory effects of *S. aromaticum* essential oil on *A. hydrophila* and other *Aeromonas* species, this research focuses on identifying and characterising specific bioactive compounds responsible for antibacterial activity. Through molecular docking and computational screening, the study seeks to determine the efficacy of these natural compounds, particularly Hederagenin ($C_{30}H_{48}O_4$), in targeting OmpA, thereby contributing to the development of plant-derived therapeutic strategies for bacterial infections in aquaculture.

The objectives of this research also include:

(i) Characterisation and Structural Prediction of OmpA: Retrieve the amino acid sequence of OmpA from the Protein Data Bank (PDB) and perform primary structure analysis, including assessments of GRAVY index, amino acid composition, instability index, phosphorylation sites, and signal peptide cleavage sites.

(ii) Secondary Structure Prediction: Utilise the SOPMA server to analyse the secondary structure of OmpA, providing insights into its heterogeneous composition.

(iii) Homology Modelling: Employ homology modelling techniques to construct a 3D model of the functional site of OmpA, essential for subsequent docking studies.

(iv) *In silico* Screening of Bioactive Compounds: Screen bioactive compounds from *Acanthus ilicifolius* and *Syzygium aromaticum* (clove) against the modelled OmpA structure to identify potential natural inhibitors.

(v) Molecular Docking Analysis: Conduct molecular docking studies to evaluate the binding affinity of identified compounds, focusing on Hederagenin ($C_{30}H_{48}O_4$) from *Syzygium aromaticum* as a promising candidate.

2. Materials and Methods

2.1 Protein Characterisation

2.1.1 Retrieval of Porin OmpA Sequence

Protein sequence of porin OmpA was retrieved in FASTA format from the Protein Data Bank of NCBI using Reference Sequence: WP_341671190.1 and then further used for *in silico* characterisation and secondary structure prediction[15]. Reference/template proteins were gathered from the Swiss-Prot protein knowledge-base and the National Centre for Biotechnology Information (NCBI) database. Both databases contain freely available protein sequences and related data.

2.1.2 In silico Characterisation and Secondary Structure Prediction

The program ProtParam from ExPasy (Expert protein analysis system) was used to determine the amino acid content of the matching protein sequence[16]. Using the data from the primary structural analysis, the percentage of hydrophobic and hydrophilic residues was manually determined based on their respective characteristics. The protein's stability in a test tube can be estimated using the physico-chemical parameters, such as its theoretical isoelectric point (pl), molecular weight, total number of positive and negative residues, extinction coefficient, half-life, and II (Instability Index). The AI (Aliphatic Index) of a protein indicates the proportion of its volume that is occupied by aliphatic side chains. These side chains come from the amino acids alanine, valine, isoleucine, and leucine. A protein's thermostability can be determined using the AI, and a peptide or protein's GRAVY (Grand Average of Hydropathy) value is determined by dividing the total hydropathy value of all of its amino acids by the total number of residues in the sequence. Thus, further describe if a protein is hydrophilic or hydrophobic using the Expasy's ProtParam prediction server[17]. Secondary structure prediction was done by using the SOPMA server[18,19] as in Figure 1. The NetPhos-3.1 server was used for studying potential phosphorylation sites of protein[20] as in Figure 2. Further Signal P-6.0 server, an *in silico* application which is used to predict the presence of signal peptides and the location of their cleavage sites in proteins from Archaea, Gram-positive Bacteria, Gram-negative Bacteria and Eukarya in given sequences[21] as in Figure 3.

2.1.3 3D Structure Prediction of Porin OmpA Protein

Homology modelling is a computer method used in the field of protein structure prediction that makes use of templates, or known protein structures, to predict the three-dimensional structure of a target protein whose structure is unknown. The technique is based on sequence alignment, in which target sequence

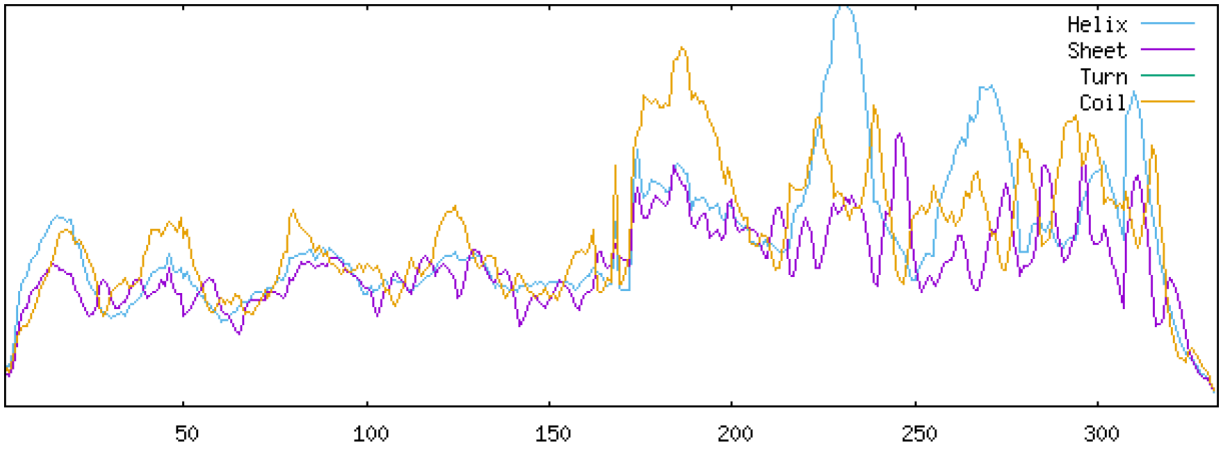


Figure 1. Result of SOPMA.

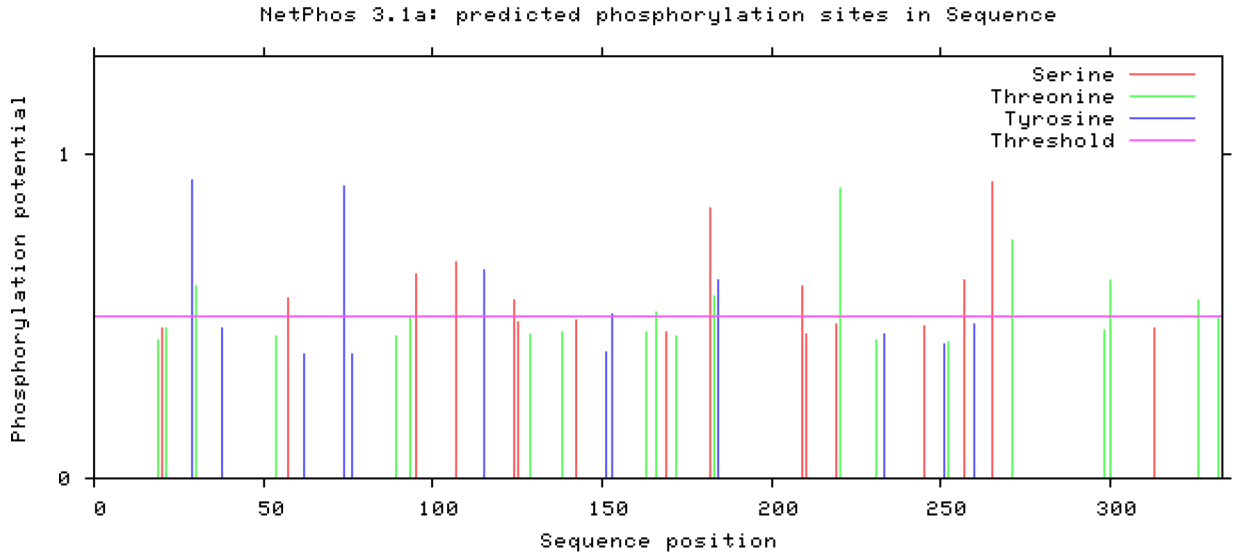
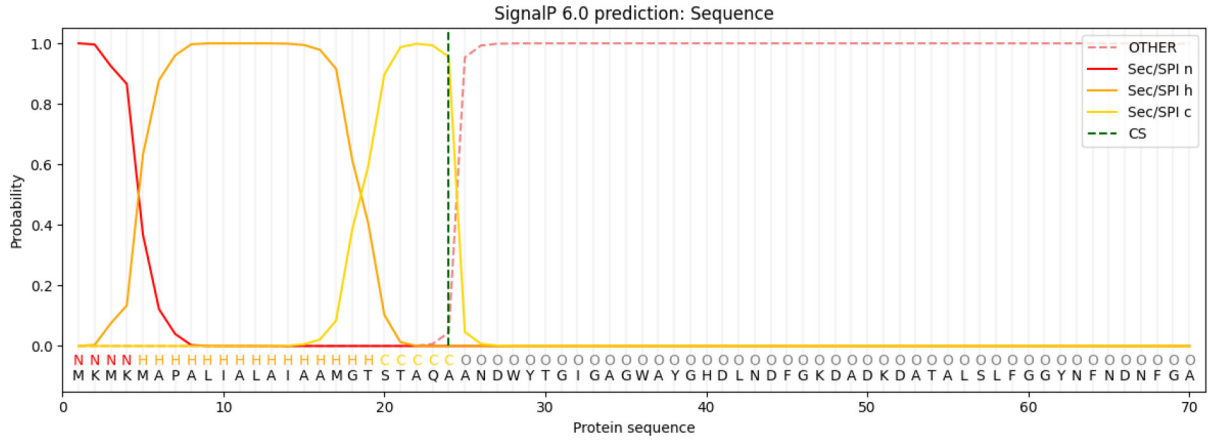


Figure 2. Result of NetPhos analysis.


SignalP 6.0 prediction: Sequence
OTHER
Sec/SPI n
Sec/SPI h
Sec/SPI c
CS
Probability
1.0
0.8
0.6
0.4
0.2
0.0
N N N N H H H H H H H H H H H H H H C C C C C O O O

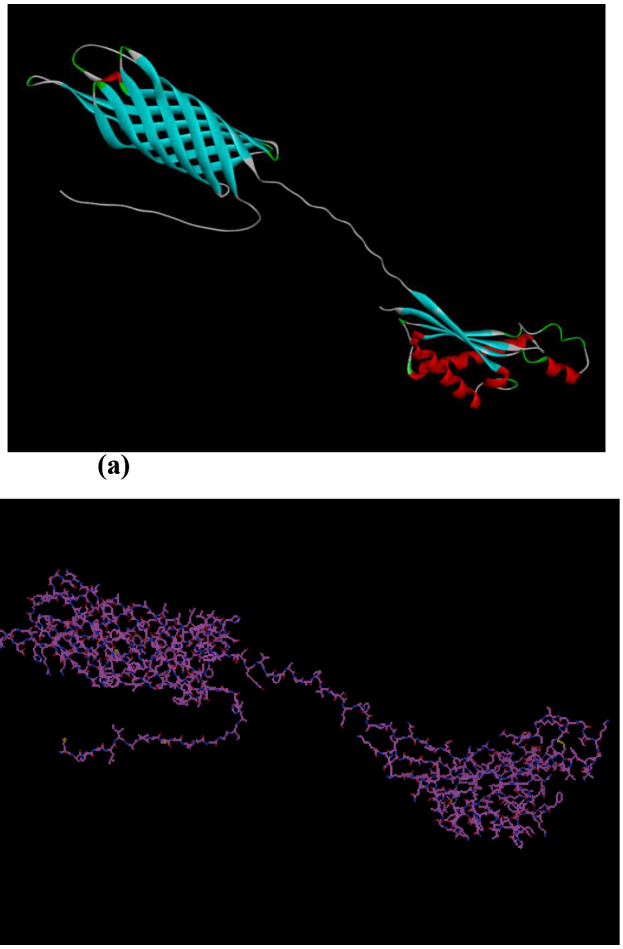


Figure 4. 3D structure of porin OmpA from Swissmodeler. **(a)** Discovery studio visualiser. **(b)** AutodockVina tool.

amino acid residues are mapped to matching template sequence residues[22-25]. The accuracy of the predicted structure hinges on the sequence similarity between the target and template proteins, which can be assessed using online bioinformatics tools like BLASTp[15,26]. As a protein's function is primarily determined by its three-dimensional conformation, knowledge of the 3D structure is crucial for various proteomics studies. This research focuses on predicting the 3D structure of porin OmpA protein from *A. hydrophila* using two *in silico* tools, the first being SwissModeler which uses FASTA sequence of available protein and develops a homologous model as in Figure 4(a), where the second tool being Modeller10.5 which uses FASTA sequence as well as six selected proteins in .pdb structure which had sequence identity more than 20.00% further developing a protein structure with the highest similarity to understand its potential interaction with infection occurrence in *L. rohita*[27] as in Figure 4(b).

2.2 Molecular Interaction and Binding Affinity Analysis

2.2.1 Virtual Screening

The most important computer-based technology (*in silico*) used in the pharmaceutical industry is virtual screening[28]. This research used a combination of reviewing scientific literature and searching a public database (Lotus and PubChem) to identify 171 molecules of *Syzygium aromaticum* (Clove) and 45 compounds of *Acanthus ilicifolius* with potential antibacterial properties against fish diseases. The selection considered published data on these molecules' effectiveness. These compounds are further screened on established guidelines (Lipinski's rule of five) to identify the most promising candidates for further studies using molecular docking[29].

2.2.2 Molecular Docking

In silico techniques like docking are crucial for predicting how molecules interact. Docking allows us to virtually simulate how one molecule binds to another in the most stable way[30]. In this study, docking is used to identify the ideal ligand (binding molecule) for our protein, Porin OmpA. This ligand would ideally inactivate the expression of *A. hydrophila's* protein in *L. rohita*. Docking helps researchers find the best possible complex between the protein and potential inhibitors[31]. We used a computational tool called AutoDock1.5.7 to dock both natural ligands (the protein's usual binding partners) and promising molecules identified through literature searches into a modelled 3D structure of the porin OmpA protein[32]. All these steps were progressed under the Autodock protocol with the help of Autodock Grid box set at X=59.501, Y= -8.219, Z=1.071 and dimensions at X=58, Y=58, Z=50.

2.3 Protein Characterisation

The majority of the amino acids in the target protein are negatively charged, polar, and hydrophilic, according to a primary structural analysis. Tables 1 and 2 make clear that there are a total of 33 negatively charged residues (Asp + Glu) and a total of 28 positively charged residues (Arg + Lys)[32].

Table 1. Amino acid composition of *the* Porin OmpA protein of *A. hydrophila*

Ala (A) - Alanine	44.0	13.2%
Arg (R) - Arginine	11.0	3.3%
Asn (N) - Asparagine	17.0	5.1%
Asp (D) - Aspartic Acid	21.0	6.3%
Cys (C) - Cysteine	2.0	0.6%
Gln (Q) - Glutamine	12.0	3.6%
Glu (E) - Glutamic Acid	12.0	3.6%
Gly (G) - Glycine	39.0	11.7%
His (H) - Histidine	3.0	0.9%
Ile (I) - Isoleucine	10.0	3.0%
Leu (L) - Leucine	30.0	9.0%
Lys (K) - Lysine	17.0	5.1%
Met (M) - Methionine	7.0	2.1%
Phe (F) - Phenylalanine	12.0	3.6%
Pro (P) - Proline	15.0	4.5%
Ser (S) - Serine	16.0	4.8%
Thr (T) - Threonine	20.0	6.0%
Trp (W) - Tryptophan	4.0	1.2%
Tyr (Y) - Tyrosine	12.0	3.6%
Val (V) - Valine	29.0	8.7%
Pyl (O) - Pyrrolysine	0.0	0.0%
Sec (U) - Selenocysteine	0.0	0.0%
[B]	0.0	0.0%
[Z]	0.0	0.0%
[X]	0.0	0.0%

Aromatic residues, particularly those containing phenolic or indolic functionalities, are known chromophores, readily absorbing Ultraviolet (UV) radiation; this property allows for the indirect quantification of proteins through UV spectroscopy[33]. In our investigation, the protein displayed an extinction coefficient of 40005 $M^{-1}cm^{-1}$ at 280 nm, which corresponds to a presumed fully oxidised state of all cysteine residues[33]. Interestingly, a theoretical reduction of all cysteines resulted in a predicted extinction coefficient of 39880 $M^{-1}cm^{-1}$. Proving its importance in further docking studies. Porin OmpA's half-life, as determined physiologically, is thirty hours. A protein's half-life is the length of time it takes for its protein pool to be reduced by half. The stability of proteins as a whole and their half-lives are strongly correlated with the presence of the N-terminal amino acid[18].

Since proteins that exceed the threshold of 40.00 are deemed unstable, the target protein is determined to be stable, with an instability index of 20.02. Porin OmpA has a mixed secondary structure, meaning that it primarily consists of random coils, alpha helices, and extended strands. This suggests that it is a better target against bacterial gastrointestinal diseases because the gastrointestinal tract is the target action site of *A. hydrophila* in both humans and fish[34]. Secondary structure analysis was performed using the SOPMA server.

Table 2. Atomic composition

Carbon	C	1557
Hydrogen	H	2438
Nitrogen	N	422
Oxygen	O	477
Sulfur	S	9

3D structure prediction or molecular model of this Porin OmpA protein of *A. hydrophila* in this study may provide a valuable aid against bacterial gastrointestinal disease and fin rot in Rohu fish, wherein it also acts as a major lead in the treatment of any GIT disease in humans due to the raw consumption of fish. Thus, predicting its 3D structure will also help in the identification of drugs that can be used against the expression of this bacterial protein in *L. rohita* by application of the docking approach for the treatment of this infection.

2.4 Retrieval of Protein Sequences

>WP_341671190.1 porin OmpA [*A. hydrophila*]

MKMKMAPALIALAIAAMGTSTAQAANDWYTGIGAGWAYGHDLNDFGKDADKDATALSLFG

GYNFNDNFGAELGYLYAGKGGVDGVDFKTQGATLSGLARLPLGDIFSVFAEGGAYFNHVNG

NGSSDNGTAPLAGLGVTAKLSDLIDLQARYRYMWNLGDEQKTWETNMSVATLELVMHPNRS

TYVAPVAAPAPEPVPEPVVVDKNFALSSDVLFAFGKSTLKPEGVAALNTLYQQIVGVQPKDGS

AVVVGYTDRIGSDAYNLKLSEARARTVADFLVGKGLPAGKVAIEGRGEANPVTGTQCNGIKA

KAQLISCLAPDRRVEVRVTGVQQVTQ

Formula: $C_{1557}H_{2438}N_{422}O_{477}S_9$

Total number of atoms: 4903

The presence of leucine, alanine and valine in a protein is used by the aliphatic index to estimate its thermal stability. Lower thermal stability of protein indicates a more flexible structure, but here it is high enough (AI = 85.32)[35]. The grand average of hydropathicity (GRAVY) value for a protein is calculated as the total

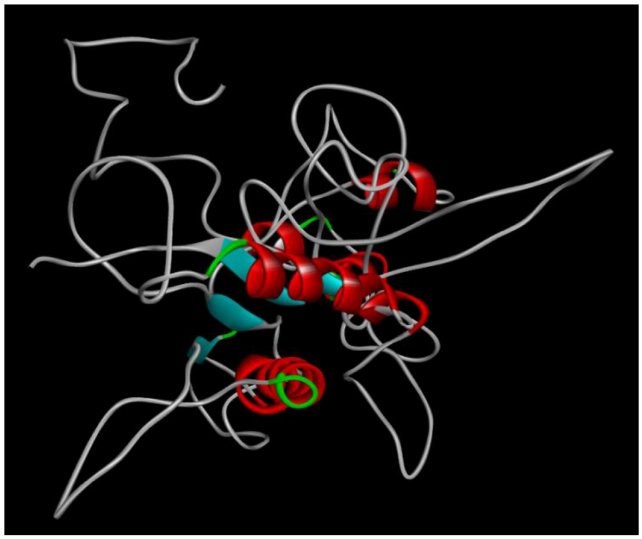
of hydropathy values of all amino acids, divided by the number of residues in the sequence (GRAVY = -0.048). This value of GRAVY indicates that this protein could result in better interaction with water[36]. To further explore the protein's structure, we employed the SOPMA program to predict its secondary elements. The analysis revealed a predominance of random coils and alpha helices, alongside a minor presence of beta sheets. These findings suggest a protein with a partially ordered structure.

Furthermore, phosphorylation, the addition of phosphate groups to proteins, plays a crucial role in regulating their function. Identifying phosphorylation sites is a critical first step in elucidating the protein's regulatory mechanisms. The bioinformatics approach is one of the major approaches in the determination of phosphorylation sites, along with the other two genetic and biochemical approaches[37].

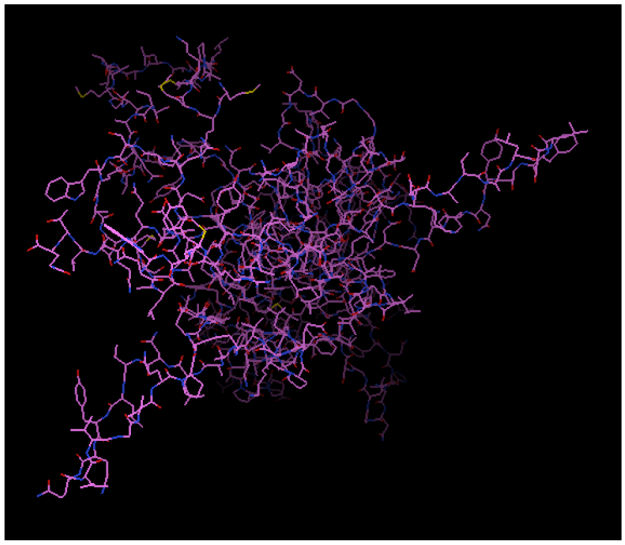
Eukaryotic proteins rely on serine, threonine, and tyrosine residues for phosphorylation, a crucial post-translational modification. In Porin OmpA protein, we obtained number of SERINE=10, THREONINE=9 and TYROSINE=7. Proteases, enzymes that cleave proteins, target specific peptide sequences. These specific sites can be used to cleave off an affinity tag, thereby restoring the natural protein sequence[38-40]. Figure 3 exemplifies the use of Signal P 6.0 software to identify such cleavage sites in the signal peptide of Porin OmpA. Results are illustrated in terms of Signal Peptide (Sec/SPI)- 0.9931, (Sec/SPII)- 0.0009, (Tat/SPI)- 0.0008, (Tat/SPII)- 0.0008 and (Sec/SPIII)- 0.0007. For Porin OmpA protein Cleavage site is between positions 24 and 25. Probability 0.955314, respectively[41].

2.5 Molecular Modelling

SwissModeler from Expasy was used for the prediction of 3D structure of the query protein Porin OmpA (NCBI Reference Sequence: WP_341671190.1), based on the template protein W0A1Y9.1, with sequence identity of 89.79%, with Ramachandran favoured 92.75% [42]. Modeller from salilab was also used to predict the 3D structure of protein Porin OmpA (NCBI Reference Sequence: WP_341671190.1), based on the template protein 6TOP(A); human dynamin, with sequence identity of 35.79%, E value of 0.00[43]. Understanding the three-dimensional (3D) structure of a bacterial protein target is paramount in the development of effective antibiotics. This knowledge allows researchers to design drugs that specifically bind to the target protein, disrupting its function and inhibiting bacterial growth. The model with the DOPE score -22130.12891 is selected as the best model[44]. This predicted structure reveals the detailed 3D structure of the Porin OmpA protein, which was further visualised using Discovery Studio as in Figure 5(a) and AutodockTools in Figure 5(b). This predicted structure reveals the detailed 3D



(a)



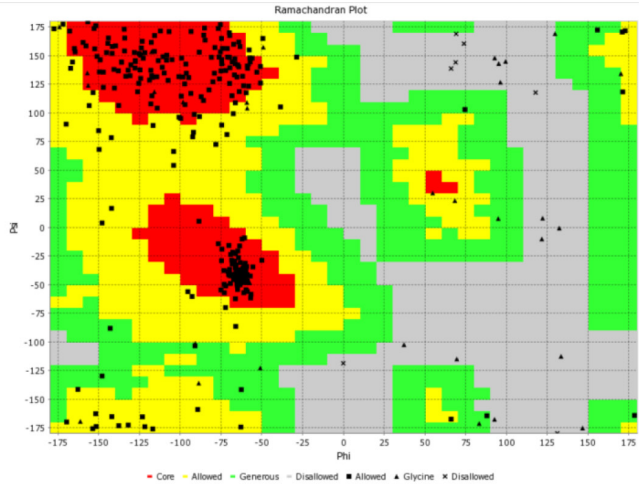
(b)

Figure 5. 3D structure of porin OmpA from Modeller. **(a)** Discovery studio visualiser. **(b)** AutodockVina tool.

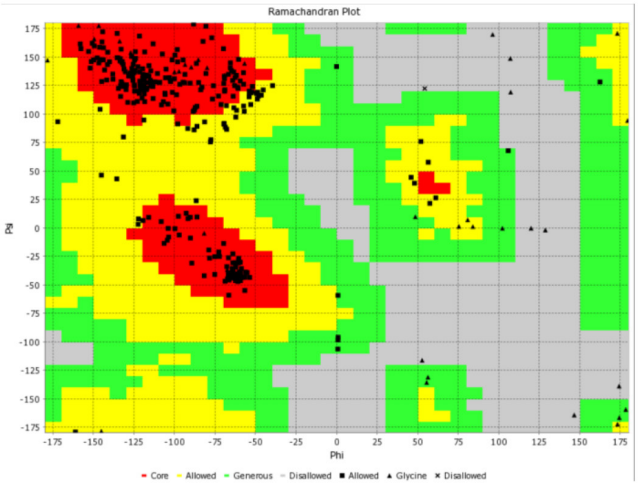
structure of the Porin OmpA protein has ninety-eight alpha helices and fifty-five beta sheets as given by SOPMA[45].

2.6 Virtual Screening by Discovery Studio

Research in the biological sciences and material sciences is the main target market for Discovery Studio, a flexible set of tools created for molecular modelling and simulation by BIOVIA (a Dassault Systèmes brand)[46]. Strong construction and visualisation capabilities are available for a broad variety of molecular structures, ranging from tiny chemical compounds to big macromolecules such as nucleic acids and proteins. Advanced protein modelling features, including protein-protein docking, homology modelling, and the ability to predict protein structures and functions, are all included in the suite[27].



(a)



(b)

Figure 6. Ramachandran plot. **(a)** The modeller generated the porin OmpA protein. **(b)** SwissModeller generated porin OmpA protein.

2.7 Protein Preparation by AutoDockTools-1.5.7

AutoDockTools (ADT) is a user-friendly graphical interface designed to facilitate the use of the AutoDock suite of programs, which are widely used for molecular docking simulations[30,31]. Developed by the Scripps Research Institute, ADT simplifies the process of preparing input files, running docking simulations, and analysing the results, as per Figure 6.

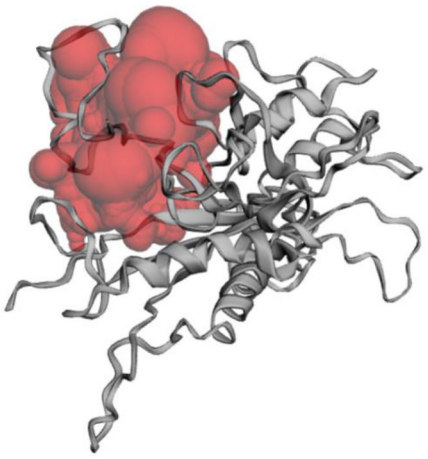
Perl and Open Babel were the programming tools which are used to commend the docking using AutodockVina for compounds to our target protein, porin OmpA.

2.8 Molecular Docking Studies

Porin OmpA was identified as our target protein as per literature reviews and research papers, and so its FASTA sequence was obtained from the Protein Data Bank (PDB), which was used to model its 3d structure using homology modelling (Modeller10.5 & Swiss-Modeller)[27].

These 3d structures were then analysed using Discovery Studio to identify any bond breakage and then opened with AutoDockTools-1.5.7 to prepare proteins for ligation. Polar Hydrogen was added to the protein along with charges on molecules (Gasteiger and Kollman charges), and assigning atoms Ad4 type will merge non-polar hydrogen atoms and charges, assign aromatic carbons, and prompt the user to write a PDBQT file of proteins required for docking. Then, a grid box is assigned to the proteins to specify the active site present on the proteins for compound interactions. The active sites present on proteins were identified using CastP and PyMol, as shown in Figure 7. Then, a grid box is assigned to the proteins to specify the active site present on the proteins for compound interactions, as shown in Figure 8. After grid box preparation, the proteins were docked and ready and saved in PDBQT format in the command directory to run Autodock Vina along with ligands.

2.8.1 *Porin OmpA - Protein's Binding Area Identification*



PocID	Area (SA) Å^2	Volume (SA) Å^3
1	2029.371	2336.407

PocID	Chain	SeqID	AA	Atom
1	A	1	MET	CA
1	A	1	MET	CB
1	A	1	MET	CG
1	A	1	MET	SD

Figure 7. Binding site analysis.

2.8.2 *Porin OmpA- Grid Box Preparation on Active Site of Protein*

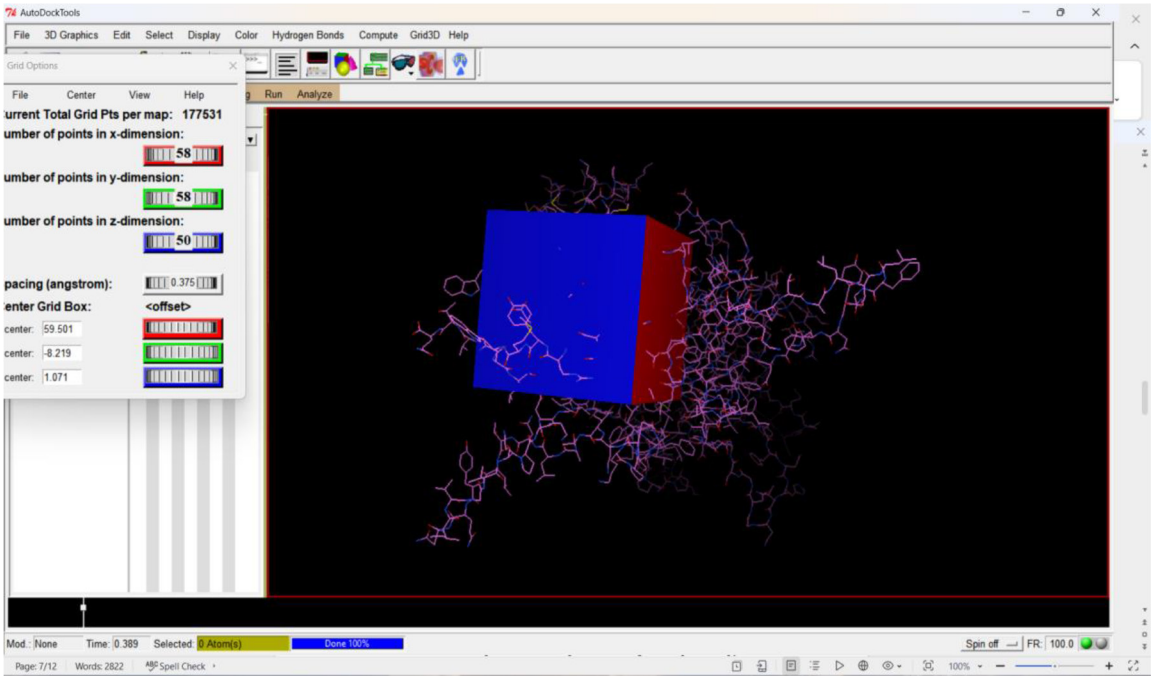


Figure 8. Binding site analysis.

Ligands are specific compounds which are attached to their target proteins to either express or suppress some functions of the protein. As for this case, Porin OmpA is highly expressed by *A. hydrophila* in *L. rohita,* which needs to be suppressed, and for that purpose, our primary candidate was *Acanthus ilicifolius's* 45 compounds, which were identified by *in silico* tools, as it was mentioned in the usage for treatment of *A. hydrophila* infection in *L. rohita* by wet labs, which gives us our lead compound, Amyrin($C_{30}H_{50}O$), with a minimum binding energy reaching -12.7.

Table 3. Binding energy of *Acanthus ilicifolius* compounds

S. No.	Molecule Name	Protein Name	Binding Energy
1	Amyrin	Porin OmpA	-12.7
2	Lupeol	Porin OmpA	-12.0
3	Vicenin 2	Porin OmpA	-11.7

Which was then overtaken by the lead compound of *Syzygium aromaticum* (Clove), which was identified as per novel manual review, as it had better minimum energy than *Acanthus ilicifolius*. *Syzygium aromaticum* (Clove) belongs to the family Myrtaceae and is commonly known as *Syzygium aromaticum* (Clove), native to the Maluku Islands, also known as the Spice Islands, in Indonesia's North Maluku Province. It shows Anti-pathogenicity by analysing wet lab results of its oil extract being used against *A. hydrophila* infection in *L. rohita*, hence composed of compounds most suitable for docking with Porin OmpA.

To access all compounds of *Syzygium aromaticum* (Clove), the compound databank was used to download a total of 171 components in .sdf format and saved in the command directory. Then OpenBabel command line was used to batch convert 171 compounds to .PDBQT format for running AutodockVina. After ligand preparation, a .txt directory was prepared containing all ligands in .pdbqt format only, which is then used to run the Perl command for docking.

After running Autodock on 171 compounds, it showed many compounds with minimum binding energy lowest reaching -18.3.

Casuarictin ($C_{41}H_{28}O_{26}$) is the compound that reached -18.3 in binding energy and binding interaction. Where Casuarictin failed in toxicity administration. As for the compound that was perfect with the highest binding interaction and with the least toxicity was Hederagenin ($C_{30}H_{48}O_4$), with binding energy reaching -15.0. As depicted in Tables 3 and 4.

3. Results

The interaction between the best 10 compounds of *Syzygium aromaticum* (Clove) with Casuarictin, Syzyginin B, Pedunculagin, Pterocarinin A, Hederagenin, Corilagin, Caryophyllin, 6"-O-Acetylisoquercitrin, 1-desgalloyleugeniin, Rugosiin with porin OmpA were represented in Figure 9 (a-j) by AutoDock Visual Tool.

Table 4. Binding energy of *Syzygium aromaticum* (Clove) compounds

S. No.	Molecule Name	Protein Name	Binding Energy
1	Casuarictin	Porin OmpA	-18.3
2	Syzyginin B	Porin OmpA	-17.0
3	Pedunculagin	Porin OmpA	-16.9
4	Pterocarinin A	Porin OmpA	-15.7
5	Hederagenin	Porin OmpA	-15.0
6	Corilagin	Porin OmpA	-14.6
7	Caryophyllin	Porin OmpA	-14.6
8	6"-O-Acetylisoquercitrin	Porin OmpA	-14.5
9	1-desgalloyleugeniin	Porin OmpA	-13.8
10	Rugosiin	Porin OmpA	-12.7

4. Discussion

When *A. hydrophila* enters the human gut, it adheres to intestinal epithelial cells and produces virulence factors that aid in infection. Adhesion factors allow the bacteria to adhere to and colonise the intestinal mucosa. Enterotoxins include heat-labile enterotoxin (LT), which causes watery diarrhoea, heat-stable enterotoxin (ST), which alters electrolyte transport and causes diarrhoea, hemolysins and cytotoxins, which cause cell damage, inflammation, and intestinal injury, and proteases and lipases, which increase bacterial survival by weakening host defences. As such, to prevent this bacterial disease, the target protein was identified using a selection method and conducted virtual screening on more than 20 herbal plants, out of which 45 compounds were selected from *Acanthus ilicifolius* and 171 compounds from *Syzygium aromaticum*. The top 10 compounds were chosen from the compounds of *Acanthus ilicifolius* and *Syzygium aromaticum*, whereas the compound with the minimum binding energy and the least toxicity was found to be Hederagenin of *Syzygium aromaticum* for the current study.

Understanding the bioactivity of *Syzygium aromaticum* is essential for elucidating its antimicrobial properties. The application of *Syzygium aromaticu*m essential oil (SyEO) as a non-bactericidal additive in livestock feed presents a promising strategy for promoting intestinal colonisation while effectively controlling zoonotic pathogens such as

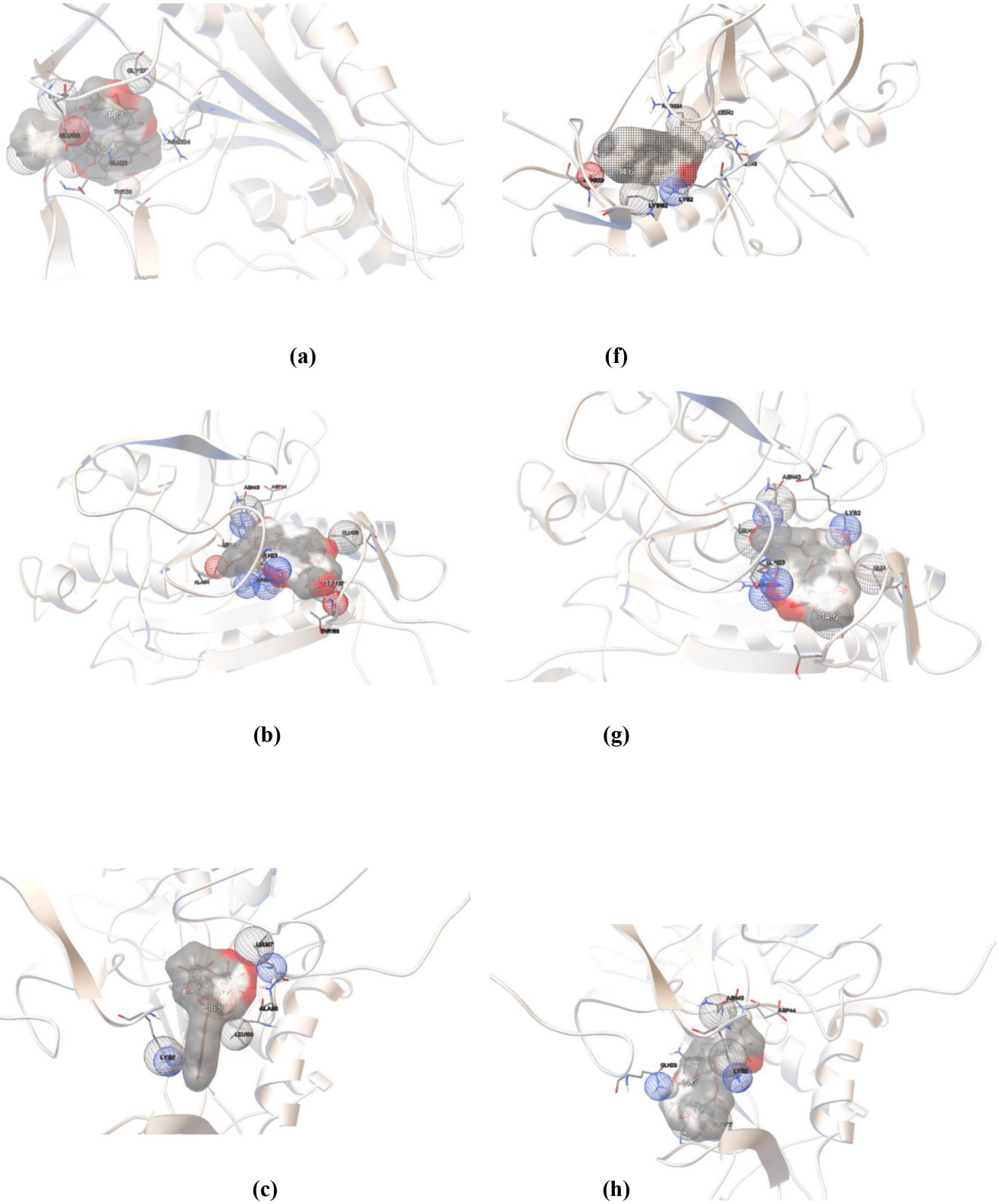


Figure 9. Protein-ligand complex analysis.

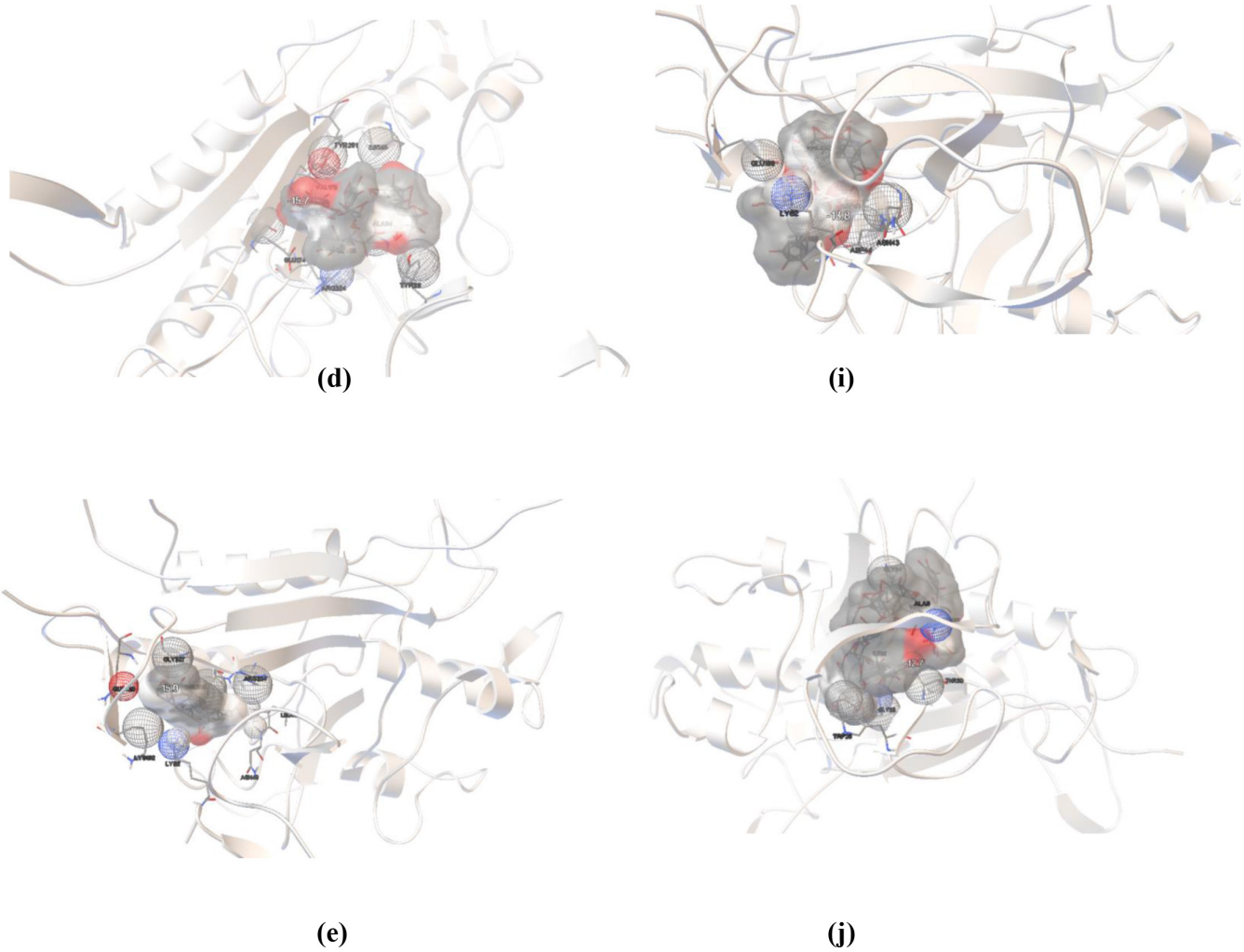


Figure 9. Continued...

Salmonella spp. This study was explored to showcase the potential of *Syzygium aromaticum* Essential Oil (SyEO) as an effective anti-colonisation agent against *Salmonella Typhimurium* using Caenorhabditis elegans as a model organism in recent year 2021. Toxicity evaluation of Essential Oils (EOs) in *C. elegans* revealed that SyEO exhibited the highest safety profile, with an LD50 of 271.6 ± 14.9 µg/mL. Notably, SyEO significantly reduced bacterial colonisation in the nematode's digestive tract by 0.70 log CFU/nematode at a non-toxic concentration of 150 µg/mL. While its Minimal Bactericidal Concentration (MBC) against *S. Typhimurium* was determined to be 625 µg/mL, the reduction in bacterial load suggests an anti-colonisation mechanism independent of direct antibacterial action. Further analysis indicated that SyEO inhibited bacterial swimming motility by 19.56%, potentially limiting pathogen adherence and persistence within the host. These findings highlight the potential of *Syzygium aromaticum* as a natural intervention for controlling pathogenic bacterial colonisation, reinforcing its value in antimicrobial research and sustainable disease management[47].

5. Conclusion

Proteins, as fundamental components of life, play a critical role in biological processes, and the analysis of the amino acid sequence of Porin OmpA provides valuable insights into its functional properties. This study highlights the significant threat posed by *Aeromonas* bacteria, particularly *A. hydrophila*, to both aquatic ecosystems and human health. The molecular models of Porin OmpA documented in this research offer a foundation for designing inhibitors to combat

bacterial infections, including gastrointestinal tract diseases. Additionally, Hederagenin from *Syzygium aromaticum* was identified as a promising compound with minimal binding energy and low toxicity, suggesting its potential for protecting aquatic life from microbial infections. These findings contribute to the development of control measures and quality monitoring systems, ultimately benefiting both the aquatic industry and human populations. Future work could explore the application of Hederagenin and other inhibitors in mitigating bacterial outbreaks.

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