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Ligand-based Virtual Screening, Quantum Mechanics Calculations, and Normal Mode Analysis of Phytochemical Compounds Targeting Toll-Interacting Protein (Tollip) Against Bacterial Diseases

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Abstract

The *Labeo rohita* (Rohu) Toll interacting protein (Tollip) is ubiquitously expressed in the kidneys, gills, spleen, liver, and blood. Tollip in *L. rohita* has higher eukaryotic structural features and is produced in response to bacterial infections. Several bacterial diseases, such as *Aeromonas hydrophila* and *Vibrio* spp, have been reported in the internal organs of *L. rohita*. The consequences of these bacterial infections can be 100% mortality of fish. There are currently no medicines or vaccines available to prevent or treat infections caused by the involvement of this protein. During bacterial infections, it was discovered that Tollip plays an essential function as a negative regulator of the MyD88-dependent TLR signalling pathway. Therefore, the study aimed to evaluate the inhibitory potentiality of the *Allium sativum* compound against Tollip. *A. sativum* has been reported to show potential antibacterial activity against numerous microbial pathogens. Still, activity against the Tollip-promoted pathogens has not yet been reported. *In silico* virtual screen and molecular docking methods were used in this study to calculate the binding affinity of 48 drug compounds of *A. sativum* against the receptor Tollip. The docking and normal mode analysis methods predict 2 (PubChem CID: 122130381 and CID 12303662) inhibitory compounds that bind strongly with the Tollip with a binding affinity of -9.2 and -8.8 kcal/mol, respectively. The ADMET properties of the compounds also verified the drug resemblance features of the two compounds of *A. sativum*. Furthermore, to evaluate the efficacy of these two potential inhibitors, more *in-vitro* testing is required.

Keywords: *Allium sativum*, ADMET, Bacteria, Docking, Tollip

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1. Introduction

Toll-interacting protein (Tollip) reduces the activation of nuclear factor-kappa through the reduction of autophosphorylation of interleukin-1 receptor-associated kinase and its activity during acute inflammation. In comparison with higher vertebrates, only a limited number of fish species have had the tollip genes in their genomes cloned and characterized. In pathogenic infection of grass carp (*Ctenopharyngodon idella*), Tollip and Interleukin-1 Receptor-Associated Kinase 1 (IRAK-1) were found in the cytoplasm of cytokine-induced killer cells[1]. Each Tollip domain in different fish species has a distinct mix of essential amino acid residues for signal transduction pathway inhibition[1]. Different expression patterns of the Tollip gene have been documented after infection of common carp with *Vibrio anguillarum*[2], sea cucumber with *Vibrio splendidu*[3], and grass carp with *Aeromonas hydrophila*[1]. Furthermore, carp species such as *Cyprinus carpio* and *Labeo rohita* is critical commercial species in South Asian countries, including Bangladesh, India, Pakistan, and Myanmar. Economic loss from diseases will affect the aquaculture industries; thus, preventing bacterial pathogens is pivotal for aquaculture sustainability.

Traditional phytochemicals can be essential in curing different ailments, particularly in areas with limited resources. Given the availability of these plants throughout the world, traditional medicines are often advised[4]. To begin with, traditional pharmaceuticals are less harmful than contemporary drugs; this is one of the critical reasons for the extraction and synthesis of essential chemicals from plants[5]. *Allium sativum* (Garlic) is a common medicinal herb that has grown in popularity in recent years worldwide. It comprises a wide range of physiologically active chemicals with various implementations. Approximately 100 therapeutic chemical substances have been recorded and extracted from multiple elements of this plant, including leaves. Research proves that flowers have traditionally been used to cure various diseases. Additionally, *A. sativum L.* is a fragrant, herbaceous plant that is eaten and used as a traditional treatment for a variety of diseases all over the globe. *A. sativum* extracts and isolated chemicals have been examined for antibacterial, antiviral, antifungal, antiprotozoal, antioxidant, anti-inflammatory, and anticancer properties, among other biological activities[6]. In addition to this, *A. sativum* has a more significant concentration of sulfur compounds, which are the chemical components responsible for its curative properties. Garlic has several benefits for aquaculture, including the promotion of growth, improvement of immunity, stimulation of appetite, and strengthening of control over bacterial and fungal diseases[7].

Presenting targeted therapies in a traditional or standard method can be time-consuming, costly, and labour-intensive[8]. High-Throughput Screening (HTS), for example, is a technology that combines multiple-well microplates with automated processing to enhance drug development by testing a large number of suspected drug-like compounds[9]. Furthermore, HTS should have abundant resources because processing a specific HTS program is costly and requires employing robotic machines[10]. *In-silico* drug design is a relatively new methodology that employs a high-throughput method to screen an extensive database of compounds[11]. The *in-silico* virtual screening method assists in developing innovative medications by producing hits for lead compounds in a shorter time and at a lower cost[12]. The amount of time required to create, design, and optimize a brand-new drug is reduced as a direct consequence of improvements made to *in-silico* drug design[13,14]. For decades, the virtual screening approach has been used to find the best lead compounds with various structural properties for a specific biological target[15]. Additionally, using virtual screening, molecular docking, and dynamics modeling approaches, computer-aided drug design has been utilized to discover a wide range of potential medicinal applications and hits[16–18] Tollip,. This study examines the interactions between the active components of *A. sativum* and the Tollip using molecular docking, geometry optimization of the best-docked compounds, and molecular dynamics modeling. A combination of virtual screening and Molecular Docking was used in this study to screen prospective natural anti-fish medicines based on their ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) properties.

2. Materials and Methods

2.1 Retrieving the Sequence and Assessment of Secondary Structure

The amino acid (aa) sequence of the Toll interacting protein (Tollip) (Accession No.: QGN03866.1) was retrieved from the NCBI database and downloaded in FASTA format. The SOPMA program was used to predict the secondary structural components of the Tollip[19] using the default parameters of the SOPMA server.

2.2 Prediction, Refinement, and Validation of 3D Structure

The three-dimensional structure of the target protein was predicted using the RaptorX server[20,21]. The predicted 3D model of the target protein was then refined by the GalaxyWeb server[22]. Structural validity is a crucial component of homology modeling based on experimentally verified 3D protein models[23]. The proposed toll receptor protein model was submitted for preliminary approval to ProSA-web[24]. The z-score indicates how accurately the server predicted the model's overall quality. If the z-scores of the expected models exceed the scale of the property for local proteins, the structure is incorrect[24]. A Ramachandran plot analysis was performed utilizing the Ramachandran Plot Server to establish the overall quality of the drug molecules[25,26].

2.3 Preparation of Protein

Using AutoDock tools, water, metal ions, and cofactors were eliminated, polar hydrogen atoms were added, nonpolar hydrogen was combined, and gasteiger charges were calculated to model and determine the 3D structure of the protein[27].

2.4 Compound Retrieval and Preparation

Phytochemicals from medicinal plants encompass various chemical spaces employed in medication development and discovery[27]. To promote the development of natural products-based medications, IMPPAT uses cheminformatic approaches to collect 1742 Indian medicinal plants and 9500 phytochemical compounds[28]. The phytochemical of the ginger plant (*Allium sativum*) was found and collected from the database because of virtual screening. AutoDock 4 atom types were assigned accurately to generate the compounds in the database, nonpolar hydrogens were merged, aromatic carbons were recognized, and a 'torsion tree' was constructed. The AD4 atom type has been revealed to be the same as the compound elements for most atoms.

2.5 Molecular Docking and Receptor Grid Generation

The PyRx virtual screening tool AutoDock Vina was used to generate a protein receptor grid[29]. A molecular docking study was conducted using the PyRx virtual screening tool AutoDock Vina to determine the binding mechanism of the necessary protein with selected phytochemicals. PyRx is an open-source virtual screening application commonly used in CADD techniques to screen libraries of compounds against a specific target for the treatment. PyRx combines AutoDock 4 and AutoDock Vina docking wizards with an intuitive user interface, making it a more reliable CADD tool. PyRx's AutoDock Vina molecular docking wizard was used to discover the best protein and ligand binding poses in this experiment. PyRx virtual screening tools were configured for docking objectives using their default settings, and the maximum binding energy (kcal/mol) with a negative sign was chosen. BIOVIA Discovery Studio Visualizer v19.1.0 was then used to visualize the binding interaction between the protein and ligands[27].

2.6 Absorption, Distribution, Metabolism, and Excretion (ADME) and Toxicity test

ADME measures the adverse effects of drugs by monitoring the physical, pharmacokinetic, metabolic, and excretory properties of molecules in urine and feces[30]. The Swiss-ADME system was employed to anticipate the different pharmacokinetic and pharmacodynamic parameters for this study[31]. In drug research and development, accurate recognition of chemical toxicity is critical[32]. The *in-silico* toxicity of the targeted individuals was assessed using the Toxicity Estimation Software Tool (T.E.S.T) version 4.2.1. In addition, Quantitative Structure-Activity Relationships (QSARs) were used to estimate certain chemicals' toxicity.

2.7 Quantum Mechanics (QM)-Based Calculation

The computation of minimal energy conformation may achieve this sort of binding, and structural optimization focused on the solution phase and related gas-phase energy. Standard Molecular Mechanics (MM) cannot adequately describe the process in a complicated ligand-protein system attributed to the presence of metal ions[33]. Density Functional Theory (DFT) is currently used to answer more than 80–90% of all QM-based computations. Therefore, three components' DFT-based QM calculations were performed in this study. Following optimizing bond lengths, bond angles, and dihedral angles for probable compounds, the DFTs were performed with the ORCA

Sequence:
MATTISTQRGQVYIGELPQDFLRITPTQQQQQVQLDAQAARQLQYGGSLGTAGRLSITVV
QAKLAKNYGMTRMDPYCRIRLGYAVYETPTAHNGAKNPRWNKVIQCTVPPGVDSFYLEI
FDERAFSMDDRIAWTHVTIPESLREGTVVDEWYSLSGRQGDDKEGMINLVMSFASIPAGM
MMQPQPVVLMPTVYQQGVGYVPIAGVPGMYNQGVVPMGMPAVPAVTSQSAPCSEEDLK
ALQDMFPNLDKEVIRTVLEAQQGNKDAAINSLLQMAEEL

Figure 1. The amino acid (aa) sequence of the Toll receptor protein (Tollip).

quantum chemistry software tool (Version 4.1.1)[34,35]. For the computation of DFT (B3LYP-D3), Becke's three parameters were combined with Lee-Yang-Parr functionals (B3LYP) and an energy term to correct dispersion. As a basis set, 6-31G**, also known as 6-31G (d, p), and B3LYP-D3 were selected for this study since they have no direct impact on the charge distribution or wavefunction of molecules.

2.8 Frontier Molecular Orbital HOMO/LUMO Calculation

Electrons from the HOMO pass to the LUMO during the electrophilic-nucleophilic process, producing an energy differential between the two molecular orbitals[36]. The HOMO-LUMO gap is the energy differential between two molecular orbitals that illustrates organic transition metals' photochemistry, strength, and stability. To understand the susceptibility of atoms to electrophilic and nucleophilic interactions, the HOMO and LUMO energies were calculated and displayed using the Avogadro and Chemcraft tools[35]. The following equation was used to compute the energy difference between two molecular orbital HOMO-LUMO gaps.

$$\Delta E_{(gap)} = E_{LUMO} - E_{HOMO} \quad (1)$$

2.9 Normal Mode Analysis

Molecular dynamics was used on computers to explain the behavior of molecules and to assess if protein-protein interactions are stable[37]. With the iMODS server, normal mode analysis using an Elastic Network Model (ENM) in internal (dihedral) coordinates was conducted to test the binding stability and flexibility of the protein and ligand complex[38]. Four factors estimate the degree and range of protein-ligand motion: deformability, eigenvalues, B-factors, and covariance. In general, a high Eigenvalue makes deformation more difficult[38].

3. Results

3.1 Sequence Retrieval and Inquiry into Secondary Structure

Toll receptor protein amino acid sequence (Accession No.: QGN03866.1) is available in the NCBI database. There are 276 amino acids in the protein. Figure 1 provides additional information on the protein. The α-helix (Hh), extended strand (Ee), β-turn (Tt), and random coil (Rc) of the protein (A0A096VJY) were predicted by the SOPMA software to be 75 (27.17%), 47 (17.03%), 21 (7.61%), and 133 (48.19%), accordingly Figure 2.

3.2 Three-Dimensional Structure Prediction and Refinement

A five-fold refinement was achieved using the Galaxy Refine server, resulting in more amino acid residues in the favored position. The scores above represent the enhanced model's quality compared to the other models. Pymol was used to display the crude model and refine model 1 (RMSD.409) (Figure 3).

SOPMA :

Alpha helix	(Hh)	:	75 is 27.17%
310 helix	(Gg)	:	0 is 0.00%
Pi helix	(Ii)	:	0 is 0.00%
Beta bridge	(Bb)	:	0 is 0.00%
Extended strand	(Ee)	:	47 is 17.03%
Beta turn	(Tt)	:	21 is 7.61%
Bend region	(Ss)	:	0 is 0.00%
Random coil	(Cc)	:	133 is 48.19%
Ambiguous states	(?)	:	0 is 0.00%
Other states		:	0 is 0.00%

Figure 2. Secondary structural elements feature by SOPMA server.

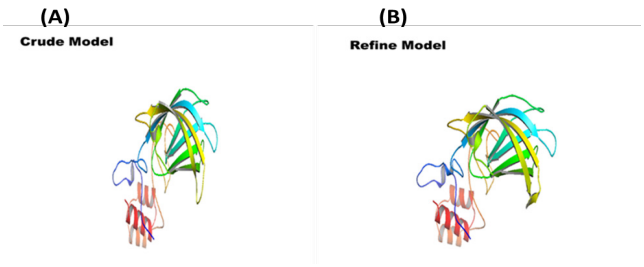


Figure 3. **(A)** 3D structure of the crude model and **(B)** 3D structure of refine the model.

3.3 Validation of a Three-Dimensional Structure

To verify the Tollip protein model before and after it was modified, the Ramachandran Plot Server and the ProSA-Web web server were employed. According to the Ramachandran plot server, the analysis of the previously revised structure indicated that 94.714% of the structure was in the favorable zone. The rampage server delivered a superior result after refinement, with 97.797 % of residues in the desired locations (Table 1). The ProSA-web server was used to assess the level of validation and any defects in a basic tertiary structure model. Following the completion of the validation process, the final protein model was given a Z-score of -8.22 (Table 1).

3.4 Retrieval and Preparation of Phytochemicals

To find the accessible compounds of the required plant, we used the IMPPAT database, a natural Indian plant, and a medicinal phytochemical compound library. *Allium sativum* phytochemical components were isolated and recorded in a 2D (SDF) file format. The compounds were created and optimized during the ligand production methods before being transferred to the pdbqt file format for further evaluation.

3.5 Molecular Docking Analysis

The target protein was first screened for, and the optimal intermolecular interaction between the target protein and phytochemical compounds was determined using molecular docking studies. The PyRx AutoDock Vina wizard was used to execute molecular docking between 48 phytochemical constituents and the protein of choice. According to Supplementary Table 1, the binding affinities determined during the molecular docking of the phytochemical molecule varied from -3.1 kcal/mol to -9.2 kcal/mol. Table 3 shows the top three phytochemical compounds based on binding affinity. The docking algorithm suggests three inhibitory chemicals (PubChem CIDs: 12303662, 15558507, and 12303662) that bind strongly to the Tollip protein with binding affinities of -9.2, -9.0, and -8.8 kcal/mol, respectively (Table 2).

3.6 Absorption, Distribution, Metabolism, and Excretion (ADME) and Toxicity Test

ADMET qualities are one of the most significant features for creating viable drug candidates against a specific target. The Lipinski rule of five, often known as RO5, was used to ascertain the drug-like qualities of certain molecules. The three compounds with the highest binding affinities received the RO5. Table 3 displays the ADMET properties of the top three drug-like, a molecule of *A. sativum*. All the compounds considered had low molecular weight, appropriate H-bond donors and acceptors, no toxicity, no mutagenicity, a reasonable distribution rate, a short excretion time, and an excellent LogP value, according to the SwissADME and T.E.S.T servers (Table 3).

3.7 Theoretical Calculation of the Selected Ligand Compounds

3.7.1 Geometry Optimization

Geometry optimization is a strategy for using approximate guesses to acquire as near-to-exact geometric

Table 1. Validation of selected protein model by Ramachandran and z-score studies

Parameters		Initial Model	Refine Model	Remarks
Ramachandran	Highly Preferred	94.714%	97.797%	Significant
	Preferred	4.405%	1.762%	Significant
	Questionable	0.881%	0.441%	Significant
ProSA Web	Z-Score	-7.04	-8.22	Significant

Table 2. Molecular docking score and ligand structure of the top 3 compounds

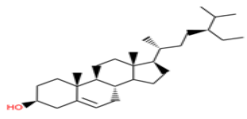
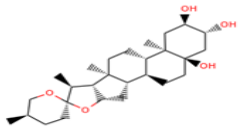
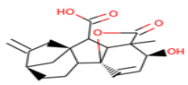
Ligand	Molecule Name	Formula	Binding Affinity (kcal/mol)	Structure
CID 12303662	Phytosterols	$C_{29}H_{50}O$	-8.8	
CID 15558507	Agapanthagenin		-8.7	
CID 122130381	Gibberellin A7		-9.2	

Table 3. List of Absorption, Distribution, Metabolism, Excretion (ADME), and toxicity of compounds.

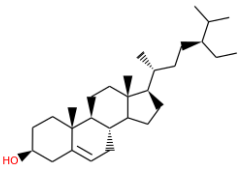
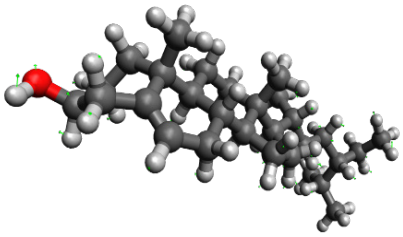
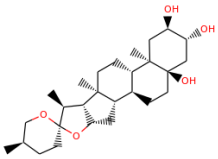
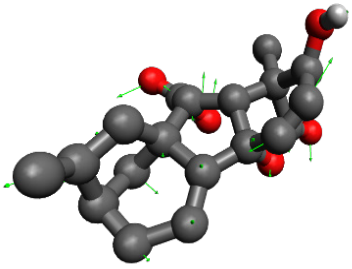
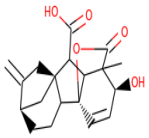
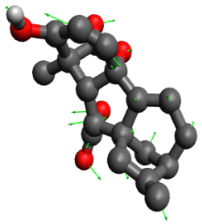
Ligand	Molecular Weight	H Bond Acceptors	H Bond Donors	Log P	Lipinski's rule of 5 violations	LD 50	Skin Sensitization
CID 12303662	414.72 g/mol	1	1	8.02	1	7641.20	Negative
CID 15558507	448.64 g/mol	5	3	3.88	0	1784.98	Negative
CID 122130381	330.38 g/mol	4	2	1.91	0	841.50	Negative

measurements as possible[39]. Because molecules in the lowest energy state naturally reduce their energy by emitting, the geometry with the lowest energy is the most stable. Using Avogadro's default basis set 6-31G (d,p), the molecular shape with the lowest energy value was chosen as the best-optimized one. Table 4 shows the 2D structures and 3D optimized geometries of the compounds CID 12303662, CID 15558507, and CID 122130381.

3.7.2 Frontier Molecular Orbital HOMO/LUMO Calculation

Using the gap between HOMO and LUMO to determine atom sensitivity to nucleophilic and electrophilic attacks, kinetic stability, and chemical hardness or softness of molecules, we can gauge the stability of chemical bonds[39]. In the nucleophilic reaction, the electrons from the HOMO orbital are free, while the electrons from the LUMO orbital are freer to participate. Soft molecules have the lowest kinetic stability and the highest chemical reactivity. Because electron addition to the high-energy LUMO orbital gap is unlikely, compounds containing high frontier (HOMO/LUMO) orbital gaps should exhibit low chem-reactivity or bioactivity and good kinetic stability. The energetic stability of molecules with a large FMO energy gap is due to their low chemical reactivity and strong kinetic stability compared to molecules with a low kinetic energy gap[40]. Because of Equation (1), gap energy was calculated and presented in Figure 4 to assess the chemical reactivity and kinetic stability of the three chemicals chosen: HOMO, LUMO, and HOMO-

Table 4. Geometry optimization of compounds

Compound ID	Crude (2D)	Optimized (3D)
CID 12303662		
CID 15558507		
CID 122130381		

LUMO. The calculated FMO energy band gap values for CID 12303662, CID 15558507, and CID 122130381 were 2.332 eV, 1.6521 eV, and 2.8821 eV, respectively, which was much higher, indicating the compounds' kinetic stability and low chemical reactivity (Figure 4).

3.8 Protein-Ligand Re-Docking and Interaction Analysis

3.8.1 Redocking Score

Re-docking was done utilizing previously obtained protein binding sites to discover potential docking poses in a limited region. For CID 12303662, CID 15558507, and CID 122130381, the optimized geometry structure docked, resulting in binding scores of 9.7 kcal/mol, 9.9 kcal/mol, and 10.1 kcal/mol, respectively, which were better than previous binding scores (Table 3). Consequently, the QM-based compound optimization may be considered adequate for the three compounds selected.

3.8.2 Protein–Ligands Interaction Interpretation

With the desired Tollip protein model, the compound CID:122130381 produced two Alkyl interactions with PHE:1033 (4.67) and ILE:688(4.77), where one pi-Alkyl bond was predicted to form at the positions PHE: 619 (5.17) in Figure 5A. One conventional hydrogen bond and one carbon-hydrogen bond were predicted at the location of LYS:681(6.39) and SER:318(3.47), respectively. The interaction investigation of the compound CID: 15558507 revealed two Alkyl bonds at the location of LYS: 261 (4.32) and HIS: 263 (4.47) and two pi-alkyl bonds at the location of ALA:349(5.13) and PHE:58(5.30) Figure

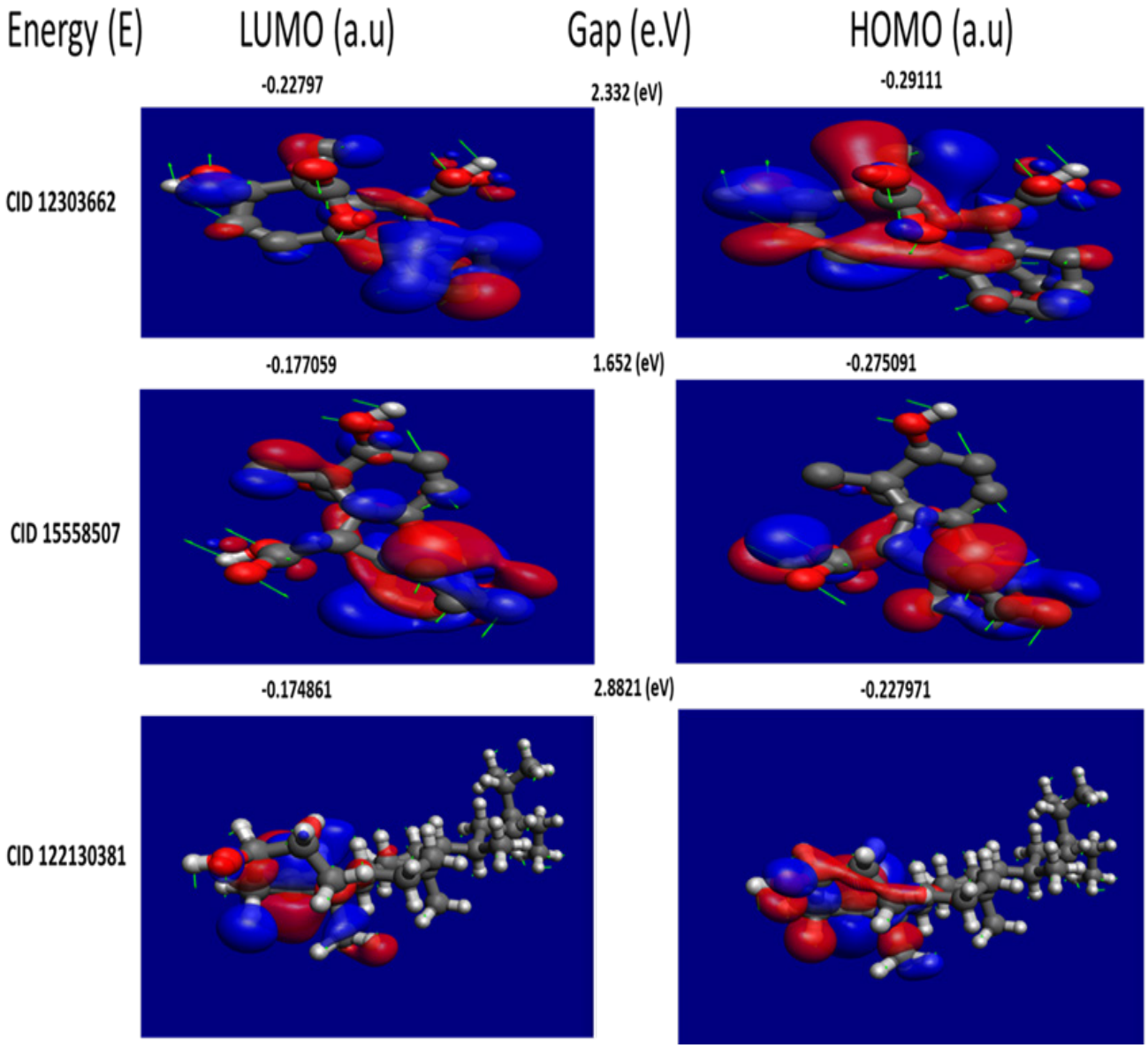


Figure 4. For three *Allium sativum* compounds, the molecular border orbital wave function is illustrated with negative and positive phases, reflecting asymmetric HOMO, LUMO, and HOMO-LUMO gaps.

5B. With the target protein, compound CID:123036662 has been found to create an alkyl bond at the positions of VAL:314 (4.14) and two pi-alkyl bonds at the position of PHE:1030 (4.74) and TYR:1019 (4.60) Figure 5C.

3.9 Normal Mode Analysis (NMA)

Molecular dynamic simulation results showed stable interactions between Tollip protein-CID: 122130381 complexes, Tollip protein- CID 15558507, and Tollip protein - CID:123036662 complex (Figure 6). Each normal mode is associated with an eigenvalue representing motion stiffness. According to its value, it measures how much energy is needed to distort the structure. The lower the eigenvalue, the simpler the deformation, whereas the B-factor column offers an averaged RMS (Root Mean Square) value. The eigenvalue, on either hand, is inversely proportional to the variance associated with each normal mode. Molecular deformability refers to the ability of a molecule to deform at each of its residues. According to the elastic network model, springs link atom pairs together. Depending on the covariance matrix, there can

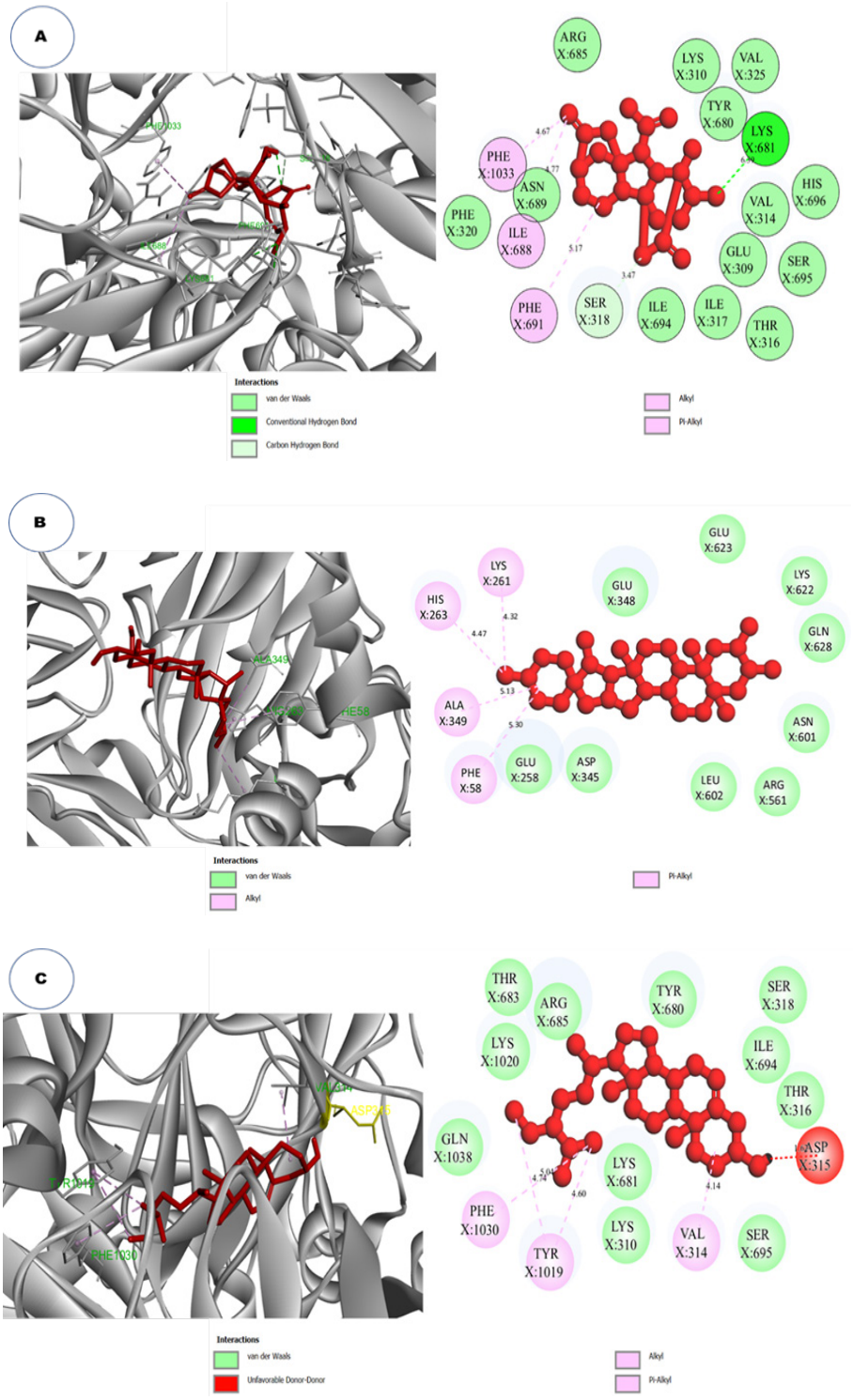


Figure 5. **(A)** The Tollip protein interacts with CID 122130381 chemicals. The 3D interaction is displayed on the left side of the figure, while the 2D interaction is depicted on the right side. **(B)** The Tollip protein interacts with CID 15558507 chemicals. The 3D interaction is displayed on the left side of the figure, while the 2D interaction is on the right side. **(C)** The interaction of the Tollip protein with CID 12303662 chemicals. The left side of the image depicts 3D interaction, whereas the right side depicts 2D interaction.

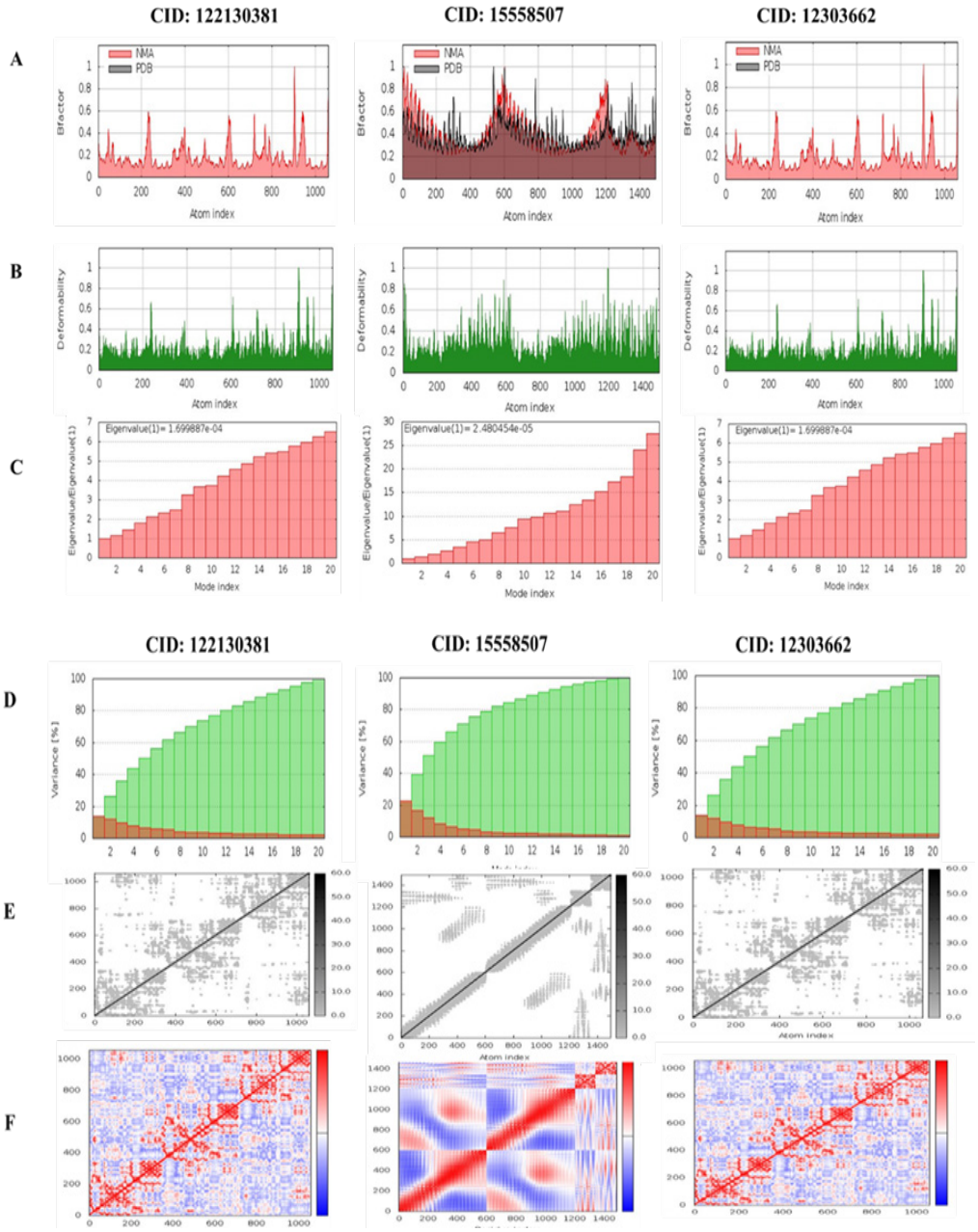


Figure 6. The molecular dynamics simulation of the Tollip protein- CID: 122130381, Tollip protein- CID 15558507 and Tollip protein - CID:123036662 docked complex **(A)** Normal mode analysis yields B-factor values, which quantify the instability of each atom **(B)** Deformability simulations on major chains reveal that hinges have high deformability **(C)** The docked complex eigenvalue represents the energy necessary to distort the structure **(D)** The covariance matrix between residue pairs (red: correlated, white: uncorrelated, blue: anti-correlated). **(E)** According to the elastic network hypothesis, atoms and springs are connected. The hue of stiff springs is a deeper grey **(F)**, The variance matrix between complex and residue.

be correlated, uncorrelated, or anti-correlated motions between residues.

4. Discussion

Bacterial infection in animals can be carried on by an overabundance of the toll receptor protein in their blood and other internal organs[41]. Tollip serves as a negative regulator during bacterial infections by inhibiting the TLR signaling pathway mediated by MyD88[42]. The differential expression patterns of the Tollip gene have been reported in numerous bacterial infections in carp species[43]. When TLRs are activated, cytokines are released, allowing for effective innate and adaptive immune responses. However, the involvement of individual Tollips varies depending on the infection location and pathogen[43]. The essential amino acids exert the critical functions of Tollip, such as phospholipids recognition and ubiquitination in fish species such as *Labeo rohita*[44]. *L. rohita* Tollip gene expression is elevated in multiple organs in response to TLR and NOD-like Receptor (NLR) ligand stimulation and *Aeromonas hydrophila, Edwardsiella tarda,* and *Bacillus subtilis* infections, with noteworthy differences in their kinetics[44]. Bacterial infectious diseases are a main dangerous problem in Aquaculture farming. It causes multiple diseases in fish and has considerable virulence in many carp species[45]. Freshwater fish toll-interacting protein has higher eukaryotic structural motifs and is found in pathogen-associated molecular pattern stimulations and bacterial infections[46]. Therefore, finding a solution to improve the functionality against the bacterial inclusion in different body organs of fish targeting the Tollip have some advantages for aquaculture sustainability.

Almost all components of the *Allium sativum* plant have therapeutic properties and have been used as traditional medicine or remedies for numerous diseases since antiquity[7]. It is a rich source of unique natural ingredients for developing pharmaceuticals to treat various diseases. Furthermore, phytochemicals found in plants may boost the innate immune system, have antibacterial properties, and are redox-active molecules with antioxidant properties, all of which may aid in improving the fish species' overall physiological state. Many researchers have investigated the benefits of phytochemicals in disease prevention[47]. Garlic has a variety of bioactive chemicals that have biological characteristics for animal health. Ajoene, alliin, and allicin are among them, and they have biological activities such as growth stimulation, antibacterial, antiviral, antioxidant, and antiparasitic[48]. Garlic has been used in the aquaculture of different species, such as rainbow trout (*Oncorhynchus mykiss*), spotted grouper (*Epinephelus coioides*), catfish (*Clarias gariepinus*), tilapia (*Oreochromis niloticus*), guppy fish (*Poecilia reticulata*), goldfish (*Carassius auratus*), and barramundi (*Lates calcarifer*)[48]. As a result, its use may be an effective way to battle infections, enhance organism health by utilizing natural resources, and as an alternative to antibiotics[48].

Computer-aided Drug Design (CADD) is one of the most promising technologies for selecting novel compounds against a specific protein since it integrates multiple complex characteristics and methodologies[49]. The CADD technique has reduced the time and expenses associated with the drug discovery process, making the virtual screening process, which includes molecular docking, molecular dynamic simulation, ADMET, and others, an integrated aspect of drug design[39]. A 3D structure prediction was made (the model with the lowest energy score was chosen), and the best model among the revealed models was achieved. The 3D structure of the protein model showed acceptable Z-scores (-8.22) and preferable localization of the amino acid residues in the Ramachandran plot. This result is similar to the previous studies which have experimented on fish species through the *in-silico* application[17,50,51].

In this study, we applied molecular docking and normal mode analysis to predict the best interacting *A. sativum* compounds with Tollip protein by following the previous *in-silico* drug design work experimented on the nervous necrosis virus in fish[17]. The molecular docking procedure was initially employed to screen the phytochemical compounds of *A. sativum* from the database. The top three compounds with the most significant binding affinities of -9.2 to -8.7 kcal/mol were chosen for further confirmation. Like prior studies, the RO5 exhibited drug-like characteristics of the chosen compounds[52,53]. All three substances followed five of Lipinski's rules of drug-likeness qualities. To measure the negative impact on animals, the toxicity attributes of the chemical with good ADME features were applied[54]. We confirmed that the three chemicals chosen are non-toxic or low-toxic after toxicity testing. A computational DFT-based QM simulation was used to evaluate and optimize the chemicals. The docking energy was much higher than >9.00 kcal/molecule when we recovered and re-docked the shape optimized by DFT with the required protein[17].

The HOMO-LUMO energy gap was computed using an FMO model to assess the reactivity of the compounds. The HOMO-LUMO gap energy found for compounds CID: 122130381 and CID:123036662 was high >2.0 eV, confirming the low reactivity correspondence to the compound's bioactivity[13,18]. We evaluated the stability of the chemical about the protein binding sites using the normal mode analysis technique on the geometry-optimized re-docked complex structure[39,54]. It may also be used to determine the stability and flexibility of protein-ligand complexes in a given experimental situation, such as in humans[39]. The B-factor, main-chain deformability simulation, and eigenvalue of the selected protein - CID: 122130381 and protein - CID: 123036662 complex systems showed that the compounds are the most stable[13].

5. Conclusion

To the best of our knowledge, this is the first investigation to uncover prospective antibacterial medication candidates targeting the Tollip protein utilizing compressed *in-silico* techniques. An integrated molecular modeling, virtual screening, molecular docking, ADMET, and MD simulation technique identified CID:12130381 as a promising therapeutic candidate that will aid in inhibiting the toll receptor protein's function. Researchers will be able to explore alternative strategies for treating bacterial infections by measuring the functionality of the substance through a range of lab-based studies.

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7. References

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