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RESEARCH PAPER

An *In-silico* Approach for Identifying Phytochemical Inhibitors Against Nervous Necrosis Virus (NNV) in Asian Sea Bass by Targeting Capsid Protein

Sk Injamamaul Islam[1,*], Moslema Jahan Mou[2], Saloa Sanjida[3], Sarower Mahfuj[1]

[1]Chulalongkorn University, Faculty of Veterinary Science and Technology, Department of Veterinary Microbiology, Bangkok-10330, Thailand.
[2]University of Rajshahi, Faculty of Life and Earth Science, Department of Genetic Engineering & Biotechnology, Rajshahi-6205, Bangladesh.
[3]Jashore University of Science and Technology, Faculty of Applied Science and Technology, Department of Environmental Science and Technology, Jashore-7408, Bangladesh.

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Corresponding Author
Tel.: +0646904801
E-mail: 6378506331@student.chula.ac.th

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Abstract

Nervous necrosis virus (NNV) is a devastating infectious pathogen for fish species with 100% mortality. To date, no specific drugs or vaccines have been developed that can prevent infections in aquaculture caused by NNV. It has been found that the NNV utilizes capsid protein to enter into the host cell in Asian sea bass and cause disease. In this study, we evaluated the inhibitory potential of *Allium sativum* compounds that have been reported to show antiviral activity against various pathogens. The capsid protein was modeled and the binding affinity of all the compounds was calculated with the docking approach and top 2 (PubChem CID: 122130381 and CID 12303662) inhibitory compounds were selected for further ADMET properties and DFT analysis. Both the geometry optimization and redocking of the two inhibitory compounds (PubChem CID: 122130381 and CID 12303662) showed a strong binding affinity of -8.2 and -8.0 kcal/mol, respectively with the capsid protein. The molecular dynamic simulation approach further validated the capsid protein – CID: 122130381 and capsid protein- CID 12303662 complex stability. In conclusion, this study deduces that these *Allium sativum* phytochemicals might act as significant inhibitors of the NNV in sea bass, which can be further validated experimentally.

Introduction

Viral Nervous Necrosis (VNN) disease, also called viral encephalopathy and retinopathy (VER), viral vacuolating, encephalopathy, and retinopathy or piscine neuropathy. Nervous Necrosis Virus (NNV) of the genus Betanodavirus (25-30 nm) is the causative agent of VNN and it consists of 4 genotypes: among them, the red-spotted grouper nervous necrosis virus (RGNNV) genotype shows a comprehensive host range (Pakingking et al., 2018). All growth stages of fish are affected heavily by NNV but mass mortalities were reported in marine finfishes, especially among larvae less than 20 days old (Pakingking et al., 2018). The virus can be waterborne – transmitted from diseased to healthy fish and can infect fish from at least 5 orders, a total number of 16 families of fish species (Huang et al., 2017). When the target organ of NNV was examined under light microscopy, it mainly indicated damage of the central nervous system of the infected fish (spinal cord) and marked vacuolations in the eye retina and brain of fish (Ziarati et al., 2020).

During viral infection, NNV capsid protein binds to nucleolar phosphoprotein B23 and accumulates in the nucleus. Nuclear phosphoprotein Nucleophosmin (B23) plays multiple roles in cellular activities by being

phosphorylated, acetylated, ubiquitylated, and SUMOylated with its functional domains (Okuwaki et al., 2002). During the infection, however, B23 is reallocated from the nucleoli to the nucleoplasm. B23-Capsid protein complex reduction by siRNA decreased viral propagation and cytopathic impact. As a result, B23 directs capsid protein to the nucleus, making NNV replication easier (Mai et al., 2017). Our main goal was to inhibit this capsid protein through antiviral phytochemicals to weaken the role of the B23-Capsid protein complex in viral replication.

Moreover, medicinal plants can play a critical role in the treatment of a variety of ailments, particularly in areas where resources are scarce. Traditional remedies are mostly advocated given the abundance of these plants all over the world (Jalil et al., 2013). Its importance has risen steadily in recent years around the world. Well over 100 effective chemical compounds have been reported and extracted from various components of this plant, which include leaves, flowers, seeds, roots, fruits, and bark have been used traditionally as a treatment for a variety of diseases, as shown in research. Garlic (*Allium sativum*) is one of the oldest cultivated plants on the planet. For over 4000 years, it has been used as a spice, food, and traditional medicine, and it is the most extensively investigated medicinal plant.

Garlic (*Allium sativum L*.) is a fragrant herbaceous plant that is used as a food and a traditional cure for a variety of ailments across the world (G. El-Saber Batiha et al., 2020; Sharma et al., 2021; Tesfaye, 2021). Its extracts and isolated chemicals have been tested for antibacterial, antiviral, antifungal, antiprotozoal, antioxidant, anti-inflammatory, and anticancer properties, among other biological activities (Gaber El-Saber Batiha et al., 2020; Tesfaye, 2021).

To introduce effective medicines in a conventional or standard manner can take a long time, be expensive, and require a significant amount of effort (Lim et al., 2015). For example, high-throughput screening (HTS) is a technique that integrates multiple-well microplate with automated processing to improve drug development by assaying a large number of putative drug-like molecules (Hughes et al., 2011). Additionally, HTS should have abundant resources, as processing a particular HTS program is expensive and involves the use of robotic devices (Szymański et al., 2012). On the contrary, computer-aided drug design, also known as in silico drug design, is a relatively new technology for screening a large database of compounds using a high-throughput approach (Liang et al., 2006). The in silico virtual screening approach aids in the discovery of novel medicines by generating hits for lead compounds in a shorter period and at a cheaper cost (Wichapong et al., 2013). As a result, improved in silico drug design reduces the time required to develop, design, and optimize a novel drug. The virtual screening approach has been used for decades to find the best lead compounds with various structural properties for use with a given biological target (J. Wu et al., 2020). Furthermore, computer-aided drug design has been used to find a wide variety of interesting drug applications and hits utilizing virtual screening, molecular docking, and dynamics simulation techniques (C. Wu et al., 2020). In light of the above-mentioned *Allium sativum* drugs, the goal of this study is to use molecular docking, geometry optimization of highest docked compounds, redocking, and molecular dynamics simulation to screen active compounds of *Allium sativum* against the capsid protein and investigate their interaction pattern. As a result, the goal of this work was to combine virtual screening, molecular docking, and ADMET (absorption, distribution, metabolism, excretion, and toxicity) features strategies to screen potential natural anti-fish drugs.

Method and Materials

Retrieving the Sequence

The NCBI database was used to retrieve the amino acid (aa) sequence of the capsid protein (Accession No. G0Z249) found in Nervous necrosis virus (NNV) and downloaded in FASTA format.

Assessment of Secondary Structure

The secondary structural elements of the toll receptor protein were predicted through the SOPMA tool (Combet et al., 2000; Islam et al., 2022) using the default parameters (window width of 17, number of states of 4, and similarity threshold of 8).

Prediction, Refinement, Validation of 3D Structures

The three-dimensional structure of the target protein was predicted using the Raptorx server (http://raptorx.uchicago.edu/) (Islam & Jahan, 2022b; Xu et al., 2021). The protein 3D structure was refined by the GalaxyWeb server. The structure validity is a crucial stage in homology modeling, which is based on the experimentally validated structure of 3D proteins. The proposed capsid protein model was uploaded to ProSA-web for basic confirmation (Wiederstein & Sippl, 2007). The server foresaw the overall character of the model, which is represented by the z-score. If the expected models' z-scores are outside the scale of the property for local proteins, it indicates that the structure is erroneous (Wiederstein & Sippl, 2007). To determine the overall quality of the suggested drug, a Ramachandran plot analysis was performed using the PROCHECK Server (Morris et al., 1992).

Protein Preparetion

The 3D structure of the protein was modeled and developed using the following criteria: water, metal ions, and cofactors were removed, polar hydrogen

atoms were introduced, nonpolar hydrogen was combined, and gasteiger charges were calculated using AutoDockTools (Islam & Jahan, 2022a).

Retrieval and Preparation of Compounds

Natural phytochemicals from medicinal plants cover a wide range of chemical spaces that can be used in the discovery and development of new drugs. IMPPAT (Indian Medicinal Plants, Phytochemistry, and Therapeutics) is a manually curated database of over 1742 Indian medicinal plants and over 9500 phytochemical compounds that uses cheminformatic methodologies to improve natural product-based drug discovery (Islam & Jahan, 2022a; Mohanraj et al., 2018). Because of virtual screening, the phytochemical of *Allium sativum* has been discovered and obtained from the database. The compounds found in the database were created using accurate AutoDock 4 atom types, merging nonpolar hydrogens, detecting aromatic carbons, and creating a 'torsion tree'. It has been discovered that the AD4 atom type is the same as the elements of the compound for the majority of atoms.

Molecular Docking and Receptor Grid Generation

PyRx is an open-source virtual screening application that can screen libraries of compounds against a given therapeutic target and is primarily used in CADD techniques (Dallakyan & Olson, 2015). PyRx integrates AutoDock 4 and AutoDock Vina as docking wizards with an intuitive user interface, making it a more trustworthy CADD tool. This experiment used PyRx's AutoDock Vina wizard for molecular docking to find the optimum protein and ligand binding poses. For docking objectives, the default configuration parameters of the PyRx virtual screening tools were utilized, and the highest binding energy (kcal/mol) with the negative sign was chosen for further investigation. Subsequently, using the BIOVIA Discovery Studio Visualizer v19.1.0.18287, the binding interaction of the protein–ligands complex was seen.

Predictive Pharmacology

Absorption, Distribution, Metabolism, Excretion (ADME)

In the ADME of a material, the physicochemical, pharmacokinetics, metabolism, and excretion properties of molecules into feces and urine are all recorded. The Swiss-ADME server (http://www.swissadme.ch/) was used to forecast the various pharmacokinetic and pharmacodynamic parameters for the experiments (Daina et al., 2017).

Toxicity Test

In the area of drug discovery and development, initial analysis of a compound's toxicity is critical. Toxicology profiles of drug candidates provide information about the hazards to human health and the environment, as well as the safety and toxicity of chemical constituents. Chemical toxicity is now assessed using computer-assisted in-silico testing without the need for animal experiments. As a result, the ProTox-II (http://tox. charite.de/protox II) website was used to assess the early-stage toxicity of the chosen medication candidates (Banerjee et al., 2018). With ProTox-II, you can identify compounds that are acutely toxic, hepatotoxic, cytotoxic, carcinogenic, mutagenic, and immunotoxic. Using Quantitative Structure-Activity Relationships (QSARs) techniques, the software estimates the toxicity of specified compounds.

Quantum Mechanics (QM)-Based Calculation

An important element of identifying possible active conformation, binding affinity, and strain discipline related to the binding process is the conformation study of a ligand to the binding site of a protein. The computation of lowest energy conformation and structural optimization, which is based on the solution phase and related gas-phase energy, can be used to accomplish this sort of binding. Because metal ions are present in a ligand-protein complex system, conventional molecular mechanics (MM) cannot adequately describe the process (Friesner & Guallar, 2005). As a result, the DFT methods-based QM calculations of two substances were done in this work. After optimizing bond lengths, bond angles, and dihedral angles for possible compounds, the DFT of the compounds was computed using the ORCA quantum chemistry software package (Version 4.1.1) (Hanwell et al., 2012; Maity et al., 2021). Becke's three parameters were combined with Lee-Yang-Parr functionals (B3LYP) and a dispersion correction energy term D3 to calculate DFT (B3LYP-D3). The usual combination of functionalities B3LYP-D3 was chosen for this investigation because it does not directly affect the wavefunction or any other molecular characteristic, and 6-31G**, also known as 6-31G (d, p), was chosen as a basis set to describe all the molecules electronic wave function.

HOMO/LUMO Calculation

In nature, HOMO is primarily an electron donor (nucleophilic), whereas LUMO is primarily an electron acceptor (electrophilic), and the interaction between the electron donor and electron acceptor pair can govern a molecule's other chemical reactivity (Li & Evans, 1995). The HOMO-LUMO gap is the energy difference between two molecular orbitals, and it illustrates the photochemistry as well as the strength and balance of transition metal complexes in organic compounds. To comprehend the sensitivity of atoms against electrophilic and nucleophilic interactions, the HOMO and LUMO energy were computed by using the

Avogadro Software and visualized by Avogadro and Chemcraft software (Hanwell et al., 2012), and the following equation was used to determine the energy difference between two molecular orbital HOMO-LUMO gaps. (1).

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

here, E_{HOMO} is the highest energy occupied molecular orbital energy, E_{LUMO} is the lowest energy unoccupied molecular orbital energy, and E_{LUMO} is the lowest energy unoccupied molecular orbital energy.

Molecular Dynamics Simulation

Molecular dynamics is a computer method for describing the behavior of molecules and determining the stability of protein-protein complexes (Pandey et al., 2016). The iMODS server (http://imods.chaconlab.org/), which performs Normal Mode Analysis, was used to examine the protein and ligand complex's binding stability and flexibility. (NMA) in internal (dihedral) coordinates using an elastic network model (ENM) (López-Blanco et al., 2014). By measuring the deformability, eigenvalues, B-factors, and covariance of four major factors, this tool estimates the direction and range of basic motions of the protein-ligand complex. In general, high eigenvalues make deformation harder (López-Blanco et al., 2014).

Results

Sequence Retrieval and Secondary Structure Inquiry

The amino acid (aa) sequence of the capsid protein (Accession No. G0Z249). was obtained from the NCBI database. There are 338 amino acids in the protein. The α-helix, extended strand (Ee), β-turn (Tt), and random coil (Cc) of the protein (A0A096VJY) were predicted by the SOPMA software to be 55 (16.27%), 83 (24.56%), 12 (3.55%), and 188 (55.62%), respectively (Figure 1). Most proteins contain the α-helix, which is a fundamental structural element. A-helices are formed by hydrogen bonds between the carbonyl oxygen of one peptide bond and the amino acid located three amino acids away. β-strands are also important structural elements of proteins. The protein chains are predominantly linear when β-strands are present. Furthermore, some portions of the protein chain do not form a regular secondary structure or have a consistent hydrogen-bonding pattern. These regions are known as random coils and are found in two locations in proteins (a) Terminal arms and (b) Loops.

SOPMA:

Alpha helix (Hh)	:	55 is 16.27%
310 helix (Gg)	:	0 is 0.00%
Pi helix (Ii)	:	0 is 0.00%
Beta bridge (Bb)	:	0 is 0.00%
Extended strand (Ee)	:	83 is 24.56%
Beta turn (Tt)	:	12 is 3.55%
Bend region (Ss)	:	0 is 0.00%
Random coil (Cc)	:	188 is 55.62%
Ambiguous states (?)	:	0 is 0.00%
Other states	:	0 is 0.00%

Figure 1. Secondary structural elements predicted by SOPMA server.

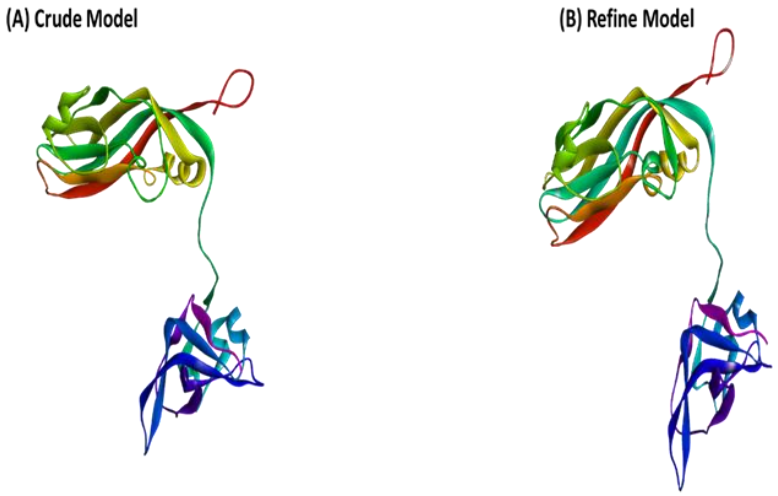


Figure 2. (A) 3D structure of the crude model and (B) 3D structure of refining model.

3D Structure Prediction, Refinement, and Validation

The Galaxy Refine server was used to refine the protein's projected tertiary structure, yielding five refined models and increasing the number of amino acid residues in the favored location. Crude model and refine model 1 with RMSD value .409 were chosen and visualized in Pymol (Figure 2). PROCHECK Server and ProSA-Web online server were used to validate the before and after refined capsid protein model. Ramachandran plot analysis of the before refined structure revealed that 88.3% of the structure was in the favorable zone. However, after refining, the server produced a better result, with 95.2% of residues in the most favored regions (Table 1). The validation quality and potential faults in a basic tertiary structure model are assessed using the ProSA-web server. Validation of the final protein model reveals a Z-score of -7.57 (Table 1).

Retrieval and Preparation of Phytochemicals

The IMPPAT database, an Indian natural, and the medicinal phytochemical compound library were used to find the accessible compounds of the required plant. The phytochemical components found in *Allium sativum* were extracted and recorded in a 2D (SDF) file format. During the ligand preparation procedures, the compounds were produced and optimized, then converted to pdbqt file format for further assessment.

Molecular Docking Analysis

A molecular docking study was first conducted to screen and identify the optimal intermolecular interaction among the desired protein and phytochemical substances. PyRx tools AutoDock Vina wizard were used to perform molecular docking between 48 phytochemical compounds and the protein of choice. The binding affinities discovered during molecular docking of the phytochemical molecule ranged from -3.1 kcal/mole to -7.7 kcal/mol. Based on the binding affinity top 2 of 48 phytochemical compounds have been chosen (Table 2). The docking methods predict PubChem CID: 12303662 and 122130381 inhibitory compounds that bind strongly with the capsid protein with a binding affinity of -7.7 and -7.2 kcal/mol, respectively (Table 2).

Predictive Pharmacology

The SwissADME online tool was used to conduct an early-stage evaluation of Absorption, distribution, metabolism, and excretion (ADME) analysis characteristics for these two compounds. Focusing on hydrophilic nature, solubility, pharmacokinetics, medicinal chemistry, and drug-likeness characteristics, the server assessed the ADME qualities of two compounds (PubChem CID: 122130381 and 12303662). All the compounds have maintained an optimum pharmacokinetics property (Table 3). The study used the ProTox-II webserver to compute the toxicity of the chemical since it is quick, inexpensive, and does not need any ethical concerns. The two compounds PubChem CID: 12303662 and 122130381 were selected previously through different screening processes have been submitted in the ProTox-II web server that determines the oral toxicity, hepatotoxicity, cytotoxicity, carcinogenicity, and mutagenicity of the compounds listed in Table 4. All the compounds have shown no oral toxicity or organ toxicity effect.

Theoretical Calculation

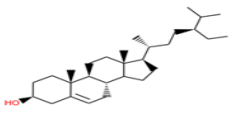
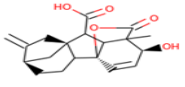
Geometry Optimization

By using the default basis set 6-31G (d,p) in Avogadro, the molecular geometry with the lowest energy value has been selected as the best-optimized

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

Parameters		Initial Model	Refine Model	Remarks
Ramachandran	Most favored region	88.3%	95.2%	Significant
	Additional allowed region	10.9%	4.8%	Significant
	Disallowed region	0.0%	0.0%	Significant
ProSA Web	Z-Score	-7.3	-7.57	Significant

Table 2. The top 3 compounds molecular docking score and ligand structure

Ligand	Molecule Name	Formula	Binding Affinity (kcal/mol)	Structure
CID 12303662	Phytosterols	$C_{29}H_{50}O$	-7.7	
CID 122130381	Gibberellin A7	$C_{19}H_{22}O_5$	-7.2	

one for selected two compounds. The 2D structures and 3D optimized geometries of the compounds PubChem CID: 12303662 and 122130381 have been plotted in Table 5.

Frontier Molecular Orbital HOMO/LUMO Calculation

Gap energy was derived from Equation (1) and illustrated in Figure 3 to evaluate the chemical reactivity and kinetic stability of the selected two compounds, the HOMO, LUMO, and HOMO-LUMO. The calculated FMO energy band gap values found for the compounds CID 12303662 and CID 122130381 were 2.332 eV and 2.8821 eV, respectively, which was considerably higher, indicating kinetic stability and low chemical reactivity of the molecules.

Re-Docking and Interaction

Redocking Score

Re-docking has been performed using the proteins previously acquired binding sites to determine viable docking poses in a confined area. The geometry optimized structure has been docked and the score found for the selected three compounds CID 12303662 and CID 122130381 were −8.0 kcal/mol and −8.2 kcal/mol, which was better than the previously obtained binding score (Table 2). As a result, it can be assumed that the QM-based optimization of the compounds has been effective for the three compounds chosen.

Protein–Ligands Interaction Interpretation

With the desired capsid protein model, the compound CID:122130381 produced three Alkyl interactions with TYR:6 (4.29), VAL:217 (5.46), and ALA:257(4.74), where two pi-Alkyl bonds were discovered to form at the positions ALA: 216 (4.13) and PRO: 215 (5.41) in Figure 4A and Table 6.

With the target protein, compound CID:123036662 has been found to create a conventional hydrogen bon at the positions of ALA:216 (2.49), ALA:216 (4.42), ALA:216 (4.41), and ALA:216 (4.27), where Alkyl bonds have been noted at the positions of LEU:36 (5.03), ILE:7 (5.0) and TYR:76 (4.25) Figure 4B and Table 6. Pi-Alkyl bonds were observed to form exclusively at the VAL:180 (3.77) and PRO:180 (5.07) position of the molecule CID:12303662 as shown in Figure 4B and Table 6.

Molecular Dynamic Simulation

Molecular dynamic simulation results showed stable interactions between capsid protein-CID: 122130381 complex and capsid protein - CID:123036662 complex. The motion stiffness is represented by the eigenvalue associated with each normal mode. Its value is proportional to the amount of energy required to distort the structure. The easier the deformation, the lower the eigenvalue, while the B-factor column provides an averaged RMS (Root mean square) value. On the other hand, the eigenvalue is inversely related to the variance associated with each normal mode. The

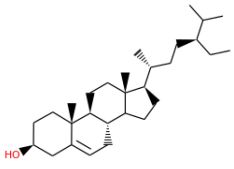
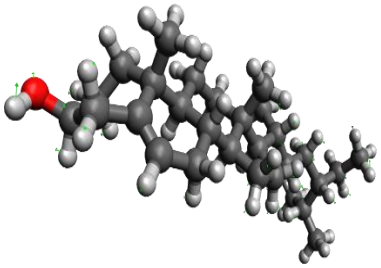
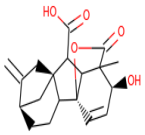
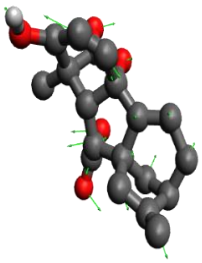
Table 3. List of absorption, distribution, metabolism, and excretion (ADME) and toxicity of compounds

Properties		CID12303662	CID 122130381
Physiochemical properties	MW (g/mol)	414.72	330.38
	Heavy atoms	31	29
	Aro. atoms	11	14
	Rotable bonds	6	5
	H-bond acceptors	1	4
	H-bond donors	1	2
	TPSA (Å^2)	75.99	20.23
Lipophilicity	Log Po/w (Cons)	5.11	6.88
Water solubility	Log S (ESOL)	Soluble	Soluble
Pharmacokinetics	GI absorption	High	Low
	BBB permeant	No	No
	P-GP substrate	No	No
Drug likeness	Lipinski violations	1	0
Medi. chemistry	Synth. accessibility	Very Easy	Easy

Table 4. The toxicity endpoints of chosen two compounds

Classification	Target	CID 12303662	CID 122130381
Oral toxicity	LD50 (mg/kg)	7641.20	841.50
	Toxicity Class	3	1
Organ toxicity	Hepatotoxicity	Inactive	Inactive
Toxicity endpoints	Carcinogenicity	Inactive	Inactive
	Mutagenicity	Inactive	Inactive
	Cytotoxicity	Inactive	Inactive

Table 5. Geometry optimization of compounds

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

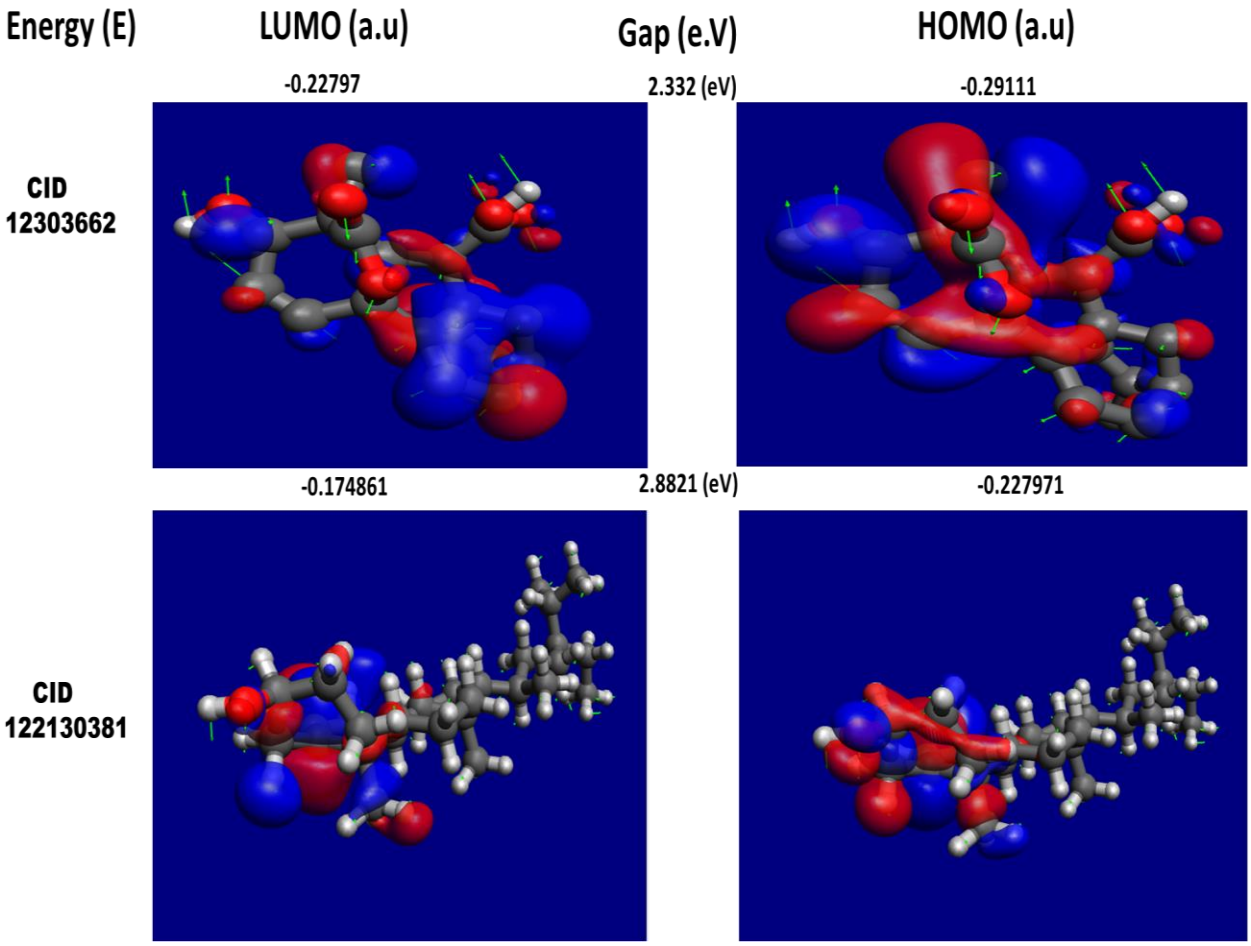


Figure 3. The molecular frontier orbital wave function is shown with negative and positive phases for selected two *Allium sativum* compounds, representing asymmetric HOMO, LUMO, and HOMO-LUMO gaps.

main-chain deformability of a molecule is a measure of its ability to deform at each of its residues. The elastic network model specifies which atom pairs are linked together by springs. The covariance matrix reveals whether two residues are coupled, i.e. whether they move in a correlated, uncorrelated, or anti-correlated manner. The correlation matrix is computed using the Cα Cartesian coordinates.

Discussion

Nervous necrosis virus (NNV) is a highly important fish virus that has caused severe economic losses to the aquaculture industry around the world. The viral polymerase and capsid protein (CP) are encoded by two single-stranded RNA segments in the NNV genome (Buonocore et al., 2019). CP is the only structural protein on the NNV surface, and it plays a role in viral invasion and encapsidation. According to previous studies, CP determines the host specificity and pathogenicity of NNV (Iwamoto et al., 2004; Moreno et al., 2019). Moreover, capsid protein along with heat shock protein (HSP) is the main cause of disease in sea bass as well as other marine fish species. According to several studies, *Allium sativum* is one of the most beneficial traditional medicinal plants on the planet (G. El-Saber Batiha et al., 2020; Tesfaye, 2021). It is now regarded as a valuable source of unique natural compounds for the creation of immunostimulants to treat a variety of illnesses (Aly & Mohamed, 2010).

Because it contains a variety of advanced features and approaches, Computer-Aided Drug Design (CADD) is one of the most promising tools for the selection of new compounds against a given protein (Sastry et al., 2013). The CADD approach has minimized the required time and costs involved in the entire drug discovery process that makes the virtual screening process includes molecular docking, molecular dynamic simulation, ADMET, etc. as integral parts of drug designing (S. Bharadwaj et al., 2021).

In this study, a 3D structure prediction of capsid protein was performed (the lowest energy score was

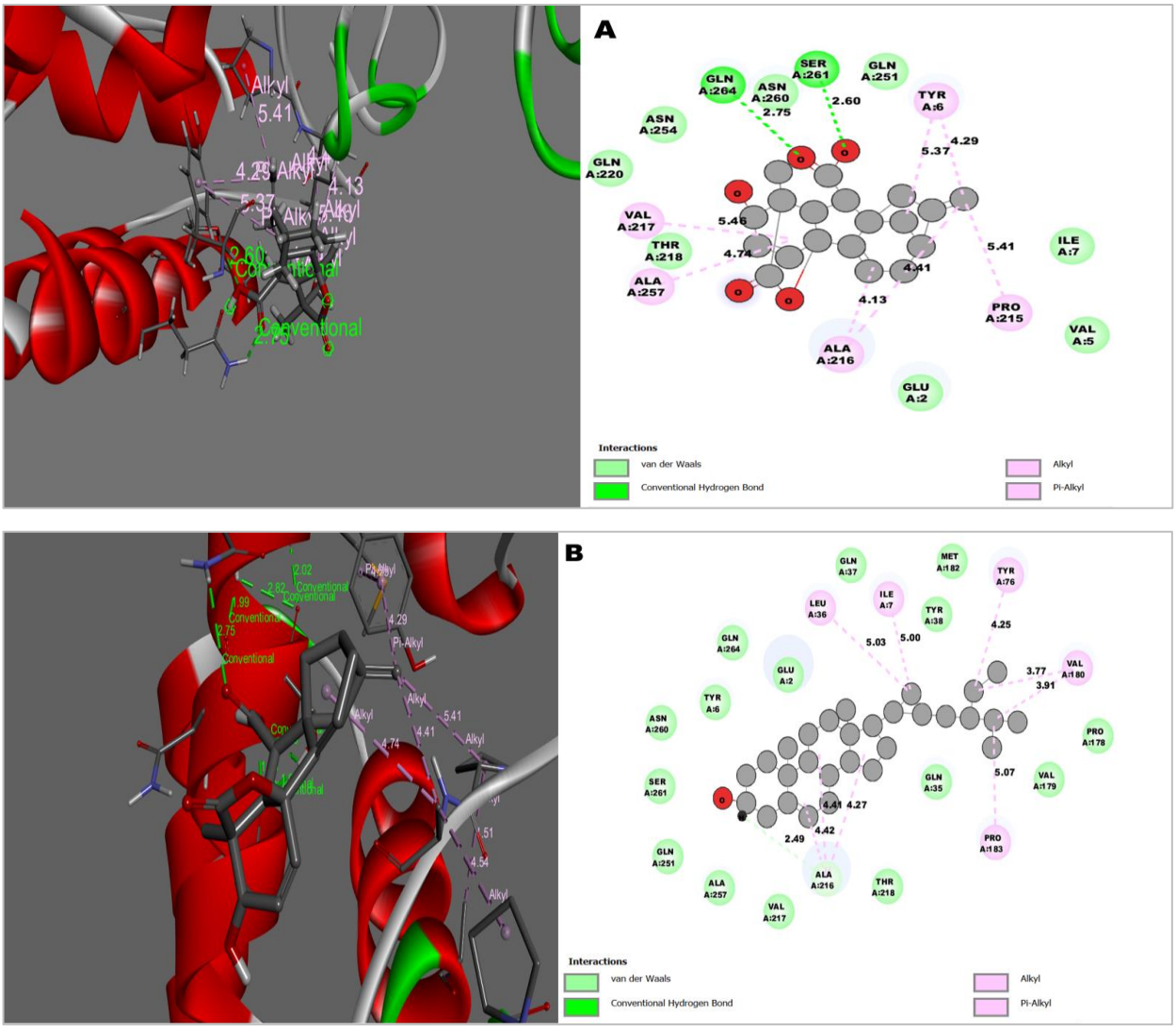


Figure 4. (A): The interaction between the capsid protein and CID 122130381 compound. The 3D interaction has represented the left side of the figure, whereas 2D interaction has depicted on the right side of figure (B): Capsid protein and CID 12303662 compound interaction. The left side of the figure represents 3D interaction, while the right side shows 2D interaction.

used to choose the best model) and the result was the achievement of the best model among the discovered models. After refinement from the Galaxy Refine server, the model quality was improved and the final refined model showed 95.2% in the most favored region in the Ramachandran plot and 0.0% in the disallowed region which indicates good model quality (Rani & Pooja, 2018). While, before refinement, the Z-scores of the protein model was -7.2 and after refinement, this score increased to -7.57. For CADD analysis, we identified potential drugs like compounds from *A. sativum* by molecular docking and *in-silico* process. Initially, the molecular docking process has used to screen the 48 compounds of *A. sativum* from the IMPPAT database, where the top 2 compounds PubChem CID: 12303662 and 122130381 have been selected initially with the highest binding affinities of -7.7 to -7.2 kcal/mol, respectively for further validation. Lipinski's rule of five (RO5) demonstrated the drug-like properties of the selected compounds (Lipinski, 2004; Pollastri, 2010). All two compounds were found to follow the five of Lipinski's rules of drug-likeness properties. The toxicity qualities of the chemical with good ADME properties were used to quantify the detrimental impact on humans or animals (Aljahdali et al., 2021). After toxicity testing, we confirmed that the selected two compounds are non-toxic or low-toxic.

Most computational biologists, chemists, academics, and researchers use geometry optimization, a quantum chemical technique, to find the configuration of minimum energy with the most stable form of a chemical structure. It is a technique for getting as close to accurate geometric measurements as feasible by using rough estimates (Shiv Bharadwaj et al., 2021). Because molecules in the lowest energy state spontaneously reduce their energy by emitting, the geometry with the lowest energy is the most stable. The importance of DFT in CADD-aided drug design is recently been studied also (Tandon et al., 2019). The compounds were investigated and optimized by a computational DFT-based QM simulation. We retrieved and re-docked the geometry optimized compounds by DFT with the desired protein, and the docking energy was significantly above >8.00 kcal / molecular. To determine the reactivity of the compounds, the HOMO-LUMO energy gap was calculated using an FMO model. The HOMO-LUMO gap energy found for compounds CID: 122130381 and CID: 12303662 was high >2.0 eV which confirms the low reactivity correspondence to the bioactivity of the compound (Liu et al., 2020; Tandon et al., 2019).

Using the MD simulation approach on the geometry-optimized re-docked complex structure, we have investigated the stability of the compound concerning the binding sites of the protein. Molecular dynamics simulation is used to confirm the stability of a protein in a complex with ligands (Aljahdali et al., 2021; S. Bharadwaj et al., 2021). It may also assess the stability and stiffness of protein-ligand complexes in a controlled context, such as the human body (S. Bharadwaj et al., 2021). In this study, the MD simulation was carried out in the iMODS server, where NMA assessment was applied to the internal coordinates of the complex. The deformability represents the independent distortion of each residue as depicted by the chain hinge approach. The B-factor and main-chain deformability simulation of the selected capsid protein – CID: 122130381 and capsid protein- CID 12303662 complex systems indicate the best stability of the compounds. The eigenvalue determined for both complexes (capsid protein – CID: 122130381 and capsid protein- CID 12303662) was found to be 1.69988e–04 and 1.54408e–04 respectively. Each typical complex's variance was gradually reduced. All of these findings point to stable binding interactions in both complexes with tight structure and negligible variations (López-Blanco et al., 2011; Samad et al., 2022).

Table 6. List of the interaction between the selected two compounds and capsid protein found during the complex structure analysis and generated through the docking simulation

Compound	Residues	Bond Distance (Å)	Category	Bond Types
CID:122130381	TYR:6	5.37	Hydrophobic	Alkyl
	TYR:6	4.29	Hydrophobic	Alkyl
	VAL:217	5.46	Hydrophobic	Alkyl
	ALA:257	2.74	Hydrophobic	Alkyl
	ALA: 216	4.13	Hydrophobic	Pi-Alkyl
	ALA: 216	4.41	Hydrophobic	Pi-Alkyl
	PRO: 215	5.41	Hydrophobic	Pi-Alkyl
	GLN:264	2.75	Hydrogen Bond	Conventional H-B
	SER:216	2.60	Hydrogen Bond	Conventional H-B
CID:12303662	ALA:216	2.49	Hydrogen Bond	Conventional H-B
	ALA:216	4.42	Hydrogen Bond	Conventional H-B
	ALA:216	4.41	Hydrogen Bond	Conventional H-B
	ALA:216	4.27	Hydrogen Bond	Conventional H-B
	LEU:36	5.03	Hydrophobic	Alkyl
	ILE:7	5.0	Hydrophobic	Alkyl
	TYR:76	4.25	Hydrophobic	Alkyl
	VAL:180	3.77	Hydrophobic	Pi-Alkyl
	VAL:180	3.91	Hydrophobic	Pi-Alkyl
	PRO:183	5.07	Hydrophobic	Pi-Alkyl

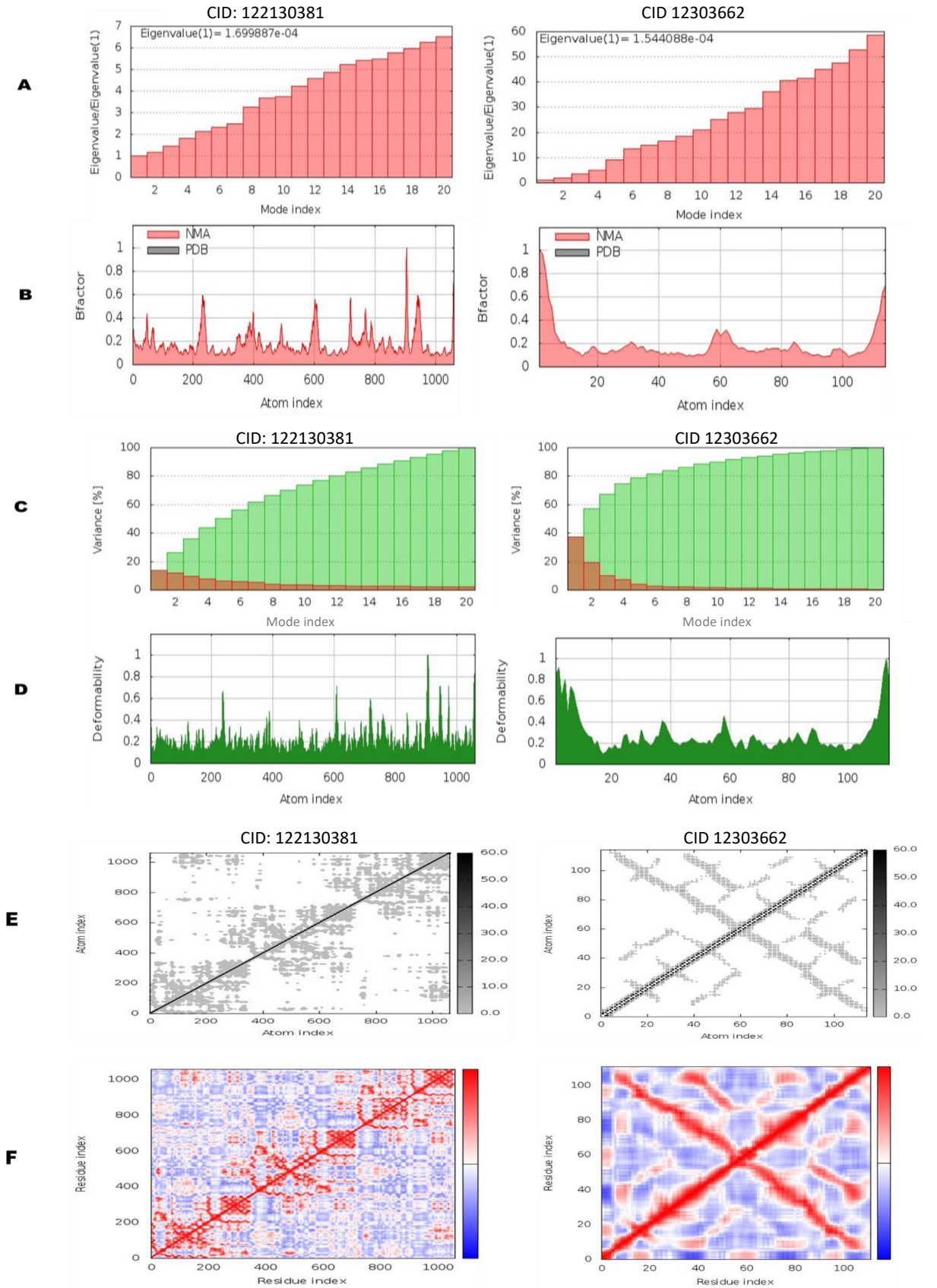


Figure 5. The molecular dynamics simulation of the capsid protein- CID: 122130381 docked complex and capsid protein- CID:12303662 complex (A) The eigenvalue of the docked complex, showing the energy required to deform the structure; (B) Normal mode analysis generates B-factor values, which measure each atoms uncertainty. The experimental B-factor is taken from the corresponding PDB field and the calculated from NMA is obtained by multiplying the NMA mobility by (8pi2).; (C) The variance matrix between complex and residue. Colored bars show the individual (red) and cumulative (green) variances; (D) Deformability simulations on main chains show high deformability in hinges; (E) The Elastic Network model. Each dot in the graph represents one spring between the corresponding pair of atoms. Dots are colored according to their stiffness, the darker grays indicate stiffer springs and vice versa; and (F) The covariance matrix between pairs of residues (red: correlated, white: uncorrelated, blue: anti-correlated).

Conclusion

The study is the first to identify potential antiviral drug candidates targeting capsid protein using compressed in-silico approaches, to the best of our knowledge. An integrative molecular modeling, virtual screening, molecular docking, ADMET, and MD simulation approaches revealed CID:12130381 as potential drug candidates that will help to inhibit the activity of the capsid protein to combat against NNV in Asian sea bass. By determining the activity of the compound through a variety of lab-based experiments, researchers will be able to identify alternative methods for the treatment of NNV infections.

Ethical Statement

Not applicable.

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There is no any funding institution for this study.

Author Contribution

Conceptualization, M.M. and SK; methodology, SK. and M.M.; software, SK.; validation, M.M., and SK.; formal analysis, SK.; investigation, SK.; resources, SK.; data curation, M.M. and SK.; writing— original draft preparation, M.M.; writing—review and editing, SK. All authors have read and agreed to the published version of the manuscript

Conflict of Interest

The author(s) declare that they have no known competing financial or non-financial, professional, or personal conflicts that could have appeared to influence the work reported in this paper.

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