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Identification of potential antiviral drug compound against Erythrocytic necrosis virus by targeting Major capsid protein

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ABSTRACT

A member of the Iridoviridae family has been detected as the erythrocytic necrosis virus (ENV), which causes viral erythrocytic necrosis (VEN) in 20 marine and anadromous fishes. The major capsid protein (MCP) is the main structural protein of iridoviruses and is responsible for causing disease in various fishes. It has been found that the VEN utilizes major capsid protein (MCP) to enter the host cell and blocking the virus entry by targeting the protein can reduce the economic losses caused by the pathogen. The main objective of the study was to evaluate the inhibitory potentiality of 48 compounds *Allium sativum* is one of the medicinal plants which has been reported to show potential antiviral activity against various pathogens, but activity against the capsid protein promoted pathogens has not yet been reported. The MCP was retrieved, modeled, refined, and validated in this experiment. The binding affinity of 48 compounds was calculated against the MCP with the docking, ADMET, and molecular dynamics (MD) simulation approaches which predict PubChem CID 12303662 inhibitory compound that binds strongly with the major capsid protein with a binding affinity of -9.7 after optimization. For theoretical calculation, the HOMO-LUMO gap score was also calculated. The best ADMET compounds were selected for the optimization analysis and re-docking. As a result of the research, it is possible to deduce that these Allium sativum phytochemicals might act as significant inhibitors of the MCP. More in-vitro testing is needed to establish their effectiveness.

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Introduction

Iridoviruses are big cytoplasmic DNA viruses with particular hosts in insects or vertebrates. The major capsid protein (MCP) is the main constituent of non-enveloped icosahedral viral particles that appears to be highly conserved across members of the Iridoviridae,

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Phycodnaviridae, and African swine fever virus families [1]. MCP genes have only been cloned and described in a small number of fish species, in comparison to higher vertebrates. The MCP is a late gene expressed during viral infection, and it forms aggregated in both the nucleus and cytoplasm of infected cells [2]. Each MCP domain in different fish species has a distinct mix of essential amino acid residues for signal transduction pathway inhibition [3]. Following infection of rainbow trout, grouper fish species with Erythrocytic necrosis virus, distinct expression patterns of the MCP gene have been described [4]. Economic loss from this disease will affect the aquaculture industries and thus prevention of any viral pathogens is pivotal for sustainability.

Medicinal plants can play a critical role in the treatment of a variety of ailments, particularly in areas where resources are scarce. Because they have been implicated in treatments for several infectious and non-infectious disorders, they are viewed as a supplemental approach to controlling viral diseases [5]. Traditional remedies are mostly advocated given the abundance of these plants all over the world [5, 6]. For example, an ethnobotanical study of medicinal plants used to treat human and livestock ailments in Hulet Eju Enese Woreda, East Gojjam Zone of Amhara Region, Ethiopia [7]. Before anything, traditional drugs have less detrimental consequences than modern drugs, which is one of the main reasons why essential chemicals are extracted and produced from plants [8]. *Allium sativum* typical medicinal plant whose importance has risen steadily in recent years around the world. It contains a large number of biologically active compounds with a variety of structures [9]. For centuries it was used as a traditional remedy for most health-related disorders [10]. In many nations, garlic and its products are primarily used for culinary and therapeutic purposes [10]. As evidenced by the study, over 100 beneficial chemical compounds have been recorded and isolated from various components of this plant, including leaves, flowers, seeds, roots, fruits, and bark. These components have been utilized traditionally as a treatment for a range of ailments. Garlic is one of the world's oldest cultivated plants [11, 12]. 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 is a fragrant herbaceous plant that is used as a food and a traditional cure for a variety of ailments across the world [12, 13]. It has been reported to possess several biological

properties including anticarcinogenic, antioxidant, antidiabetic, renoprotective, anti-atherosclerotic, antibacterial, antifungal, and antihypertensive activities in traditional medicines. Sulfur-containing phytoconstituents including alliin, allicin, ajoenes, vinyldithiins, and flavonoids like quercetin are abundant in *A. sativum*. *A. sativum* extracts and isolated chemicals have been tested for antibacterial, antiviral, antifungal, antiprotozoal, antioxidant, anti-inflammatory, and anticancer properties, among other biological activities [14].

Introducing effective medicines in a conventional or standard manner can take a long time, be expensive, and require a significant amount of effort [15]. 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 [16]. Additionally, HTS should have abundant resources, as processing a particular HTS program is expensive and involves the use of robotic devices [17]. 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 [18]. 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 [19]. 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 [20]. 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 [21]. 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, and molecular dynamics simulation to screen active compounds of Allium sativum against the Erythrocytic necrosis virus (Iridovirus) 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.

Materials and Methods

Retrieving the sequence

The Uniprot database (https://www.uniprot.org/uniprot/) was used to retrieve the amino acid (aa) sequence of the major capsid protein (Accession No.: A0A4D6QIF4) found in Erythrocytic necrosis virus (Irido Virus) and downloaded in FASTA format.

Assessment of Secondary Structure

The secondary structural elements of the toll receptor protein were predicted through the SOPMA tool [22-24] using the default parameters (window width of 17, number of states of 4, and similarity threshold of 8).

Prediction, Refinement, and Validation of Three-Dimensional Structures

The three-dimensional structure of the target protein was predicted using the RaptorX server (http://raptorx.uchicago.edu/) [25-27]. 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 toll receptor protein model was uploaded to ProSA-web for basic confirmation [28, 29]. 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 [28, 30]. To determine the overall quality of the suggested drug, a Ramachandran plot analysis was performed using the Ramachandran Plot Server (https://zlab.umassmed.edu/bu/rama/) [31].

Preparation of protein

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 [32, 33].

Retrieval and preparation of compounds

Phytochemicals derived from naturally occurring medicinal plants cover a wide range of chemical spaces that can be used in drug development and discovery. IMPPAT stands for Indian Medicinal Plants, Phytochemistry, and Therapeutics, 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 [34]. Because of virtual screening, the phytochemical of the ginger plant (*Allium sativum*) has been discovered and obtained from the database. The compounds found from the database were created by assigning accurate AutoDock 4 atom types, merging nonpolar hydrogens, detecting aromatic carbons, and establishing 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

The PyRx virtual screening tool AutoDock Vina was used to create a protein receptor grid [35]. The molecular docking investigation was carried out using the PyRx virtual screening program AutoDock Vina to find the binding mechanism of the required protein with chosen phytochemicals. Prix 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. PyRx integrates AutoDock 4 and AutoDock Vina as docking wizards with an intuitive user interface, making it a more trustworthy CADD tool [24]. 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.

ADME Analysis

The physicochemical, pharmacokinetics, metabolism, and excretion properties of molecules in urine and feces are all listed in the ADME of a substance [47]. The Swiss-ADME server (http://www.swissadme.ch/) was used to forecast the various pharmacokinetic and pharmacodynamic parameters for the experiments [25].

Toxicity Test

In the area of drug discovery and development, an initial analysis of a compound's toxicity is critical [48]. 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 [11]. With ProTox-II, you can identify compounds that are acutely toxic, hepatotoxic, cytotoxic, carcinogenic, mutagenic, and immunotoxic [49]. 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 confirmation 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 [36]. In the last few years, QM-based calculations have helped to improve the scoring functions that can represent the electronic structure, electronic alterations, and reaction-specific charges in a molecular system. Surprisingly, density functional theory (DFT) is currently used to answer more than 80–90% of all QM-based computations. As a result, the DFT methods-based QM calculations of three 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) [37, 38]. DFT was calculated by combining Becke's three parameters with Lee-Yang-Parr functionals (B3LYP) and a dispersion correction energy term D3 (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.

Frontier Molecular Orbital HOMO/LUMO Calculation

Kenichi Fukui's frontier molecular orbital (FMO) theory or Fukui functions, created in the 1950s, relies on the highest energy occupied (HOMO) and lowest energy unoccupied molecular orbital (LUMO). The FMO of a molecule is the “frontier” of an electron that helps to identify the energy difference between two orbitals HOMO and LUMO. HOMO is predominantly an electron donor (nucleophilic) and LUMO is an electron acceptor

(electrophilic) in nature and the interaction between electron donor and electron acceptor pair can control other chemical reactivity of a molecule [39]. Electrons from the HOMO move to the LUMO during the electrophilic-nucleophilic process, creating an energy difference between two molecular orbitals. The energy difference between two molecular orbitals is termed the HOMO-LUMO gap, which illustrates the photochemistry and the strength and balance of transition metal complexes of 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 [38], and the following equation was used to determine the energy difference between two molecular orbital HOMO-LUMO gaps. (1).

$$\Delta E(gap) = ELUMO - EHOMO \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (1)$$

here, ΔE is the HOMO-LUMO gaps, ELUMO is the lowest energy unoccupied molecular orbital energy, and EHOMO is the highest energy occupied molecular orbital energy.

Molecular dynamics simulation

Molecular dynamics is a computational approach that was applied to describe the molecule's behavior as well as to measure the stability of protein-protein complexes [40]. The binding stability and flexibility of the protein and ligand complex were analyzed using the iMODS server (http://imods.Chaconlab.org/) which performs Normal Mode Analysis (NMA) in internal (dihedral) coordinates using an elastic network model (ENM) [41]. This tool estimates the direction and range of the basic motions of the protein-ligand complex by measuring four main factors: deformability, eigenvalues, B-factors, and covariance. Generally, deformation is much harder when the eigenvalue is high [41].

Result and Discussion

Sequence Retrieval

The amino acid (aa) sequence of the Erythrocytic necrosis virus (Irido Virus) (Accession No.: A0A4D6QIF4) was obtained from the Uniprot database. There are 388 amino acids in the protein. Fig. 1 provides additional information on the protein.

Amino acid sequence:

MSSNNPATYLPTAYVDLATFKNSVLEEDYMYSLSNSMTYFMREVRKCTPLTQIPTIFTIQ
SGQPSFGNNFSVLVSRSTDYLLNCWLRVTIPEVTLLDTNPHGADGRIRWTKNLMHNLIEE
VTLSFADFPTQTIDSYFLDFWAAFTTSASKKSGYDTMIGNVDDLISPHGPNEPLKSKILN
LPIPFFFSRDSGIALPTGALLYTETRISFKLRNWNQLLILENANPLPNTPNGGVPMVGPS
IERAPMLTGVTVWGDAVAADPLERSYMATCERDLLIEQVKQAPAQTYEPVNNPNKSFDVR
FSHAVKALLFGVRNITYPNVWSNWTTASPVIDDRLIMLEPSGAYDPINDVTIKYDSTTRL
QNVGADYFSHVNPYYHAPTIPDSIGYHMYSYALDLMGIDPQGSTNFGRLNNVTIVPNASN
EAKIGYQGTGAVGSGRDHPQRY

Fig 1. The amino acid (aa) sequence of the Erythrocytic necrosis virus (Irido Virus).

Secondary Structure Inquiry

The alpha helix (Hh), extended strand (Ee), beta-turn (Tt), and random coil (Cc) of the protein (A0A4D6QIF4) were predicted by the SOPMA software to be 101 (22.85%), 95 (21.49%), 15 (3.39%), and 231 (52.26%), accordingly Fig. 2

SOPMA :

Alpha helix (Hh)	:	101 is 22.85%
310 helix (Gg)	:	0 is 0.00%
Pi helix (Ii)	:	0 is 0.00%
Beta bridge (Bb)	:	0 is 0.00%
Extended strand (Ee)	:	95 is 21.49%
Beta turn (Tt)	:	15 is 3.39%
Bend region (Ss)	:	0 is 0.00%
Random coil (Cc)	:	231 is 52.26%
Ambiguous states (?)	:	0 is 0.00%
Other states	:	0 is 0.00%

Fig 2. Secondary structural elements

Three-Dimensional Structure Prediction and Refinement

The Galaxy Refine server (RMSD .509) 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. When compared to the other models, the scores listed above indicate the improved model's caliber. Crude model and refine model 2 were chosen and visualized in Pymol Fig. 3.

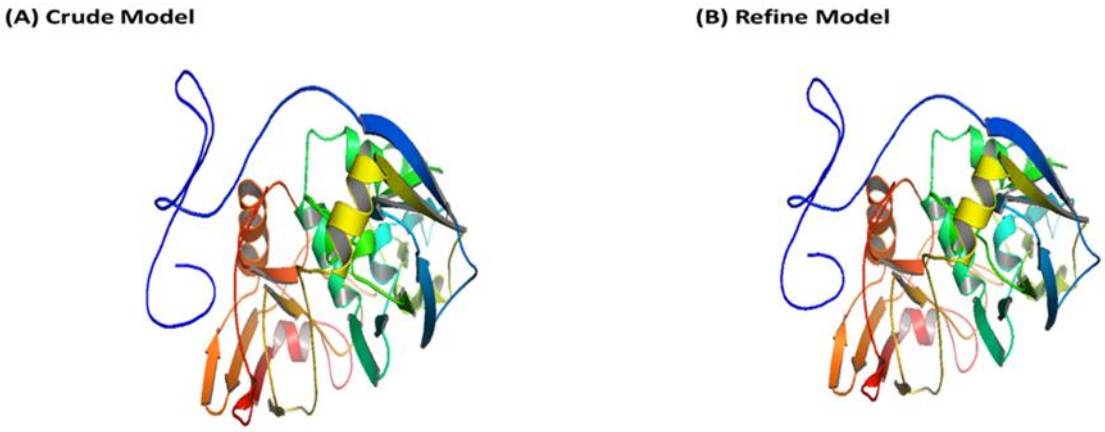


Fig 3. (A) 3D structure of the crude model and (B) 3D structure of refining the model

Validation of a three-dimensional structure

Ramachandran Plot Server and ProSA-Web online server were used to validate the before and after revised major capsid protein model. Ramachandran plot analysis of the before refine structure revealed that 94.474% of the structure was in the favorable zone, as per the Ramachandran plot server. After refining, the rampage server produced a better result, with 96.842% of residues in the preferred 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 -5.59 Table 1.

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

Parameters		**Initial Model**	**Refine Model**	**Remarks**
Ramachandran	Highly Preferred	94.474%	96.842%	Significant
	Preferred	4.211%	3.158%	Significant
	Questionable	1.316%	0.000%	Significant
ProSA Web	Z-Score	-5.24	-5.59	Significant

Retrieval and preparation of phytochemicals

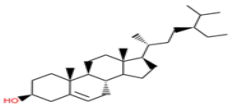
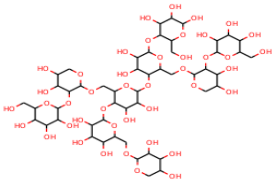
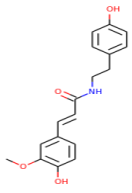
The IMPPAT database, an Indian natural, and the medicinal phytochemical compound library were used to find the accessible compounds of the required plant. The phytochemical components found in *Allium sativum* were extracted and recorded in a 2D (SDF) file format.

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/mol to -9.2 kcal/mol, as reported in Supplementary Table 1. Based on the binding affinity top 3 of 48 phytochemical (total 3) compounds have been chosen in Table 2. The docking methods predict 3 (PubChem CID: 12303662, 44630412, and 5280537) inhibitory compounds that bind strongly with the major capsid protein with a binding affinity of -8.8, -8.7, and -9.2 kcal/mol, respectively Table 2.

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

Ligand	**Molecule Name**	**Formula**	**Binding Affinity (**kcal/mol)	**Structure**
CID 12303662	Phytosterols	$C_{58}H_{98}O_2$	-8.8	
CID 44630412	Monosaccharide Glc4Xyl3Gal2	$C_{51}H_{86}O_{43}$	-8.7	
CID 5280537	Moupinamide	$C_{18}H_{19}NO_4$	-9.2	

Absorption, distribution, metabolism, and excretion (ADME) analysis

The ADME characteristics of chemical compounds are crucial in determining a drug's effectiveness. Pharmacokinetics-related failure in clinical stages can be reduced by optimizing ADME characteristics, which is complex and demanding in the drug design and trial process [25]. It has been discovered that assessing ADME at an early stage in the clinical drug development process can lower attrition rates. As a result, the SwissADME online tool was used to conduct an early-stage evaluation of ADME characteristics for four drugs. Focusing on hydrophilic nature, solubility, pharmacokinetics, medicinal chemistry, and drug-likeness characteristics, the server assessed the ADME qualities of three compounds (PubChem CID: 12303662, 44630412, and 5280537). All the compounds have maintained an optimum pharmacokinetics property Table 3.

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

Properties		**CID 12303662**	**CID 44630412**	**CID 5280537**
Physio-chemical properties	MW (g/mol)	827.40	1387.20	313.35
	Heavy atoms	60	94	23
	Aro. atoms	0	0	12
	Rotatable bonds	15	22	7
	H-bond acceptors	2	43	4
	H-bond donors	2	26	3
	TPSA ($Å^2$)	40.46	20.23	78.79
Lipophilicity	Log Po/w (Cons)	13.48	-14.63	3.25
Water solubility	Log S (ESOL)	Moderately Soluble	Soluble	Moderately Soluble
Pharmacokinetics	GI absorption	Low	Low	High
	BBB permeant	No	No	No
	P-GP substrate	No	Yes	No
Drug likeness	Lipinski violations	0	3	0
Medi. chemistry	Synth. accessibility	Very Easy	Easy	Medium

Toxicity Test

Toxicity testing is an essential and crucial phase in pharmaceutical development that aids in determining the adverse levels of toxic compounds on people, wildlife, plants, and the surroundings. Traditional toxicity testing of chemicals necessitates the use of an in vivo

animal model, which is time-consuming, costly, and fraught with ethical issues [42]. As a result, computer-aided in silico toxicity measurements of chemical compounds might be regarded beneficial in the drug development phase. 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 three compounds (PubChem CID: 12303662, 44630412, and 5280537) selected previously through different screening processes have been submittedton the ProTox-II web server that d+etermines the acute 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.

Table 4. The toxicity endpoints of chosen four chemicals include acute toxicity, hepatotoxicity, cytotoxicity, carcinogenicity, and mutagenicity

Classification	**Target**	**CID 12303662**	**CID 44630412**	**CID 5280537**
Oral toxicity	LD50 (mg/kg)	1190	3140	500
	Toxicity Class	4	4	4
Organ toxicity	Hepatotoxicity	Inactive	Inactive	Active
Toxicity endpoints	Carcinogenicity	Inactive	Inactive	Active
	Mutagenicity	Inactive	Inactive	Inactive
	Cytotoxicity	Inactive	Active	Inactive

Selection of the compound

The best ADMET compound was selected for further analysis. Although ADME qualities of the selected three compounds showed good results as a suitable drug candidate toxicity test found only the CID 12303662 compound of *Allium sativum* with the best qualities (Table-4). The other compounds CID 44630412 and CID 5280537 showed cytotoxicity and hepatotoxicity respectively. As a result, for further calculation, we selected only CID 12303662 for geometry optimization and molecular dynamics simulation analysis.

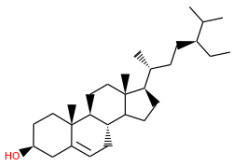
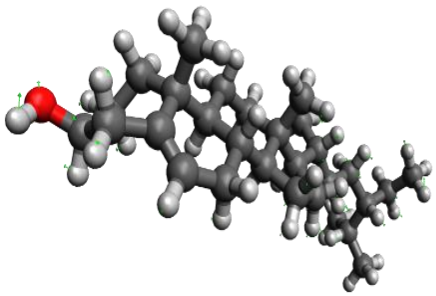
Theoretical Calculation

Geometry Optimization

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 [43]. Because molecules in the lowest energy state spontaneously reduce their energy by emitting, the geometry with the lowest energy is the most stable. 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 one. The 2D structures and 3D optimized geometries of the compound CID 12303662 have been plotted in Table 5.

Table 5. Geometry optimization of compounds

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

Frontier Molecular Orbital HOMO/LUMO Calculation

Molecular structure and reactivity are now explained largely using the FMO in organic chemistry. Through the use of HOMO-LUMO bandgap energy, electronic and optical properties of molecules can be described. In addition to determining atom sensitivity to electrophilic and nucleophilic attacks, chemical kinetic stability, chemical hardness, and softness of molecules, the gap between HOMO and LUMO helps to determine the stability of chemical bonds [43]. In the nucleophilic reaction, the electrons located from the HOMO orbital are freer, while the electrons located from the LUMO orbital are freer to participate. Soft molecules have low HOMO-LUMO gap energies, the lowest kinetic stability, and high chemical reactivity. Because there is a low probability of electron addition to the high-energy LUMO orbital gap, molecules with high frontier (HOMO/LUMO) orbital gaps should have low chem-reactivity or bioactivity and high kinetic stability. Low chemical reactivity and high kinetic stability are responsible for the energetic stability of molecules with a high FMO

energy gap compared to molecules with a low kinetic energy gap [44]. Therefore, to evaluate the chemical reactivity and kinetic stability of the selected three compounds the HOMO, LUMO, and HOMO-LUMO, gap energy was calculated from Equation (1) and shown in Figure 4. The calculated FMO energy band gap value found for the compound CID 12303662 was 3.332 eV, which was considerably higher, indicating kinetic stability and low chemical reactivity of the molecules.

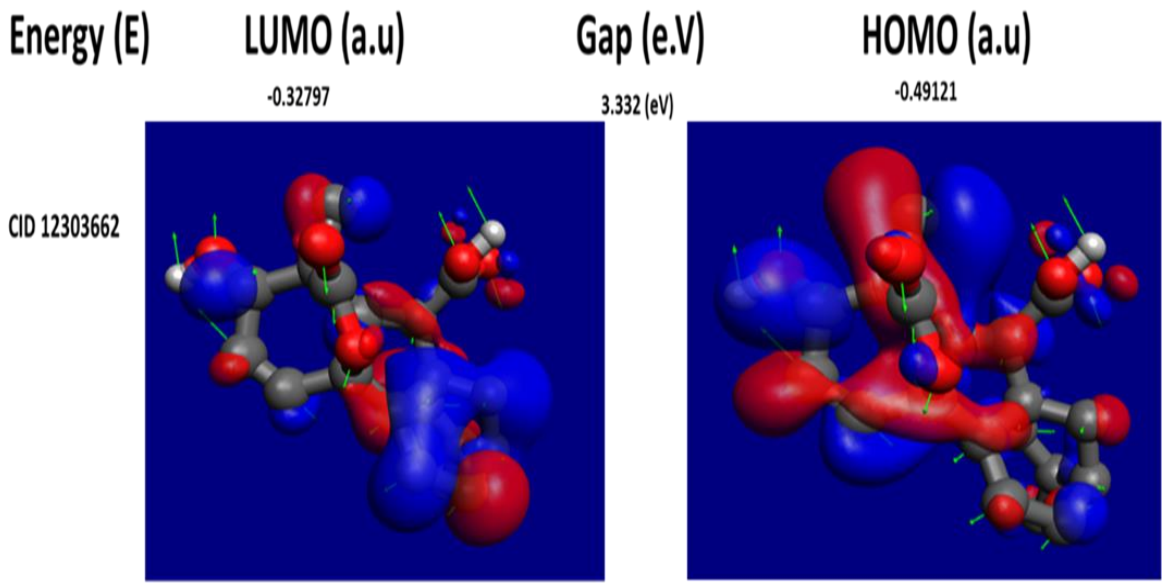


Fig 4. The molecular frontier orbital wave function is shown with negative and positive phases for selected *Allium sativum* compound CID 12303662, representing asymmetric HOMO, LUMO, and HOMO-LUMO gaps

Re-Docking and Interaction

Redocking Score

Utilizing the previously obtained binding sites of the protein, re-docking has been performed to identify possible docking poses in a restricted area. The geometry optimized structure has been docked and the score found for the selected three compounds CID 12303662 was −9.7 kcal/mol, which was better than the previously obtained binding score (Table 3). Therefore, it can be considered that the QM-based optimization of the compounds changed into effective for the chosen three compounds.

Protein–Ligands Interaction Interpretation

With the target protein, compound CID:12303662 has been found to create a conventional hy at the positions of ALA:216 (2.49), ALA:216 (4.42), ALA:216 (4.41) androgen bond 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) Fig. 5C 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 Fig. 5 and Table 6.

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

Compound	Residues	Bond Distance (Å)	Category	Bond Types
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

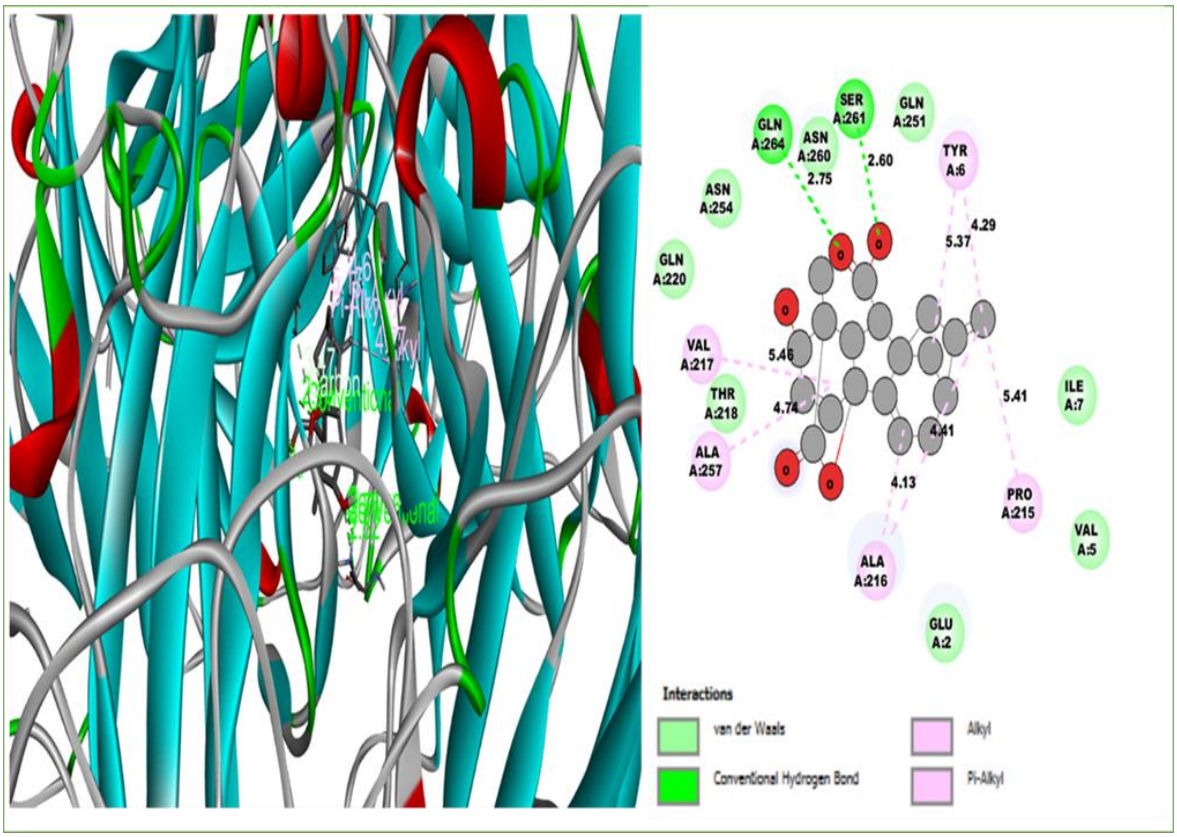


Fig 5. The interaction between the major capsid protein and CID 12303662 compound. The 3D interaction has represented on the left side of the figure, whereas the 2D interaction has been depicted on the right side of the figure accordingly.

Molecular Dynamic Simulation

Molecular dynamic simulation results showed that major capsid protein binds with CID 12303662. Here highest binding affinity complex is approved for molecular dynamic simulation analysis.

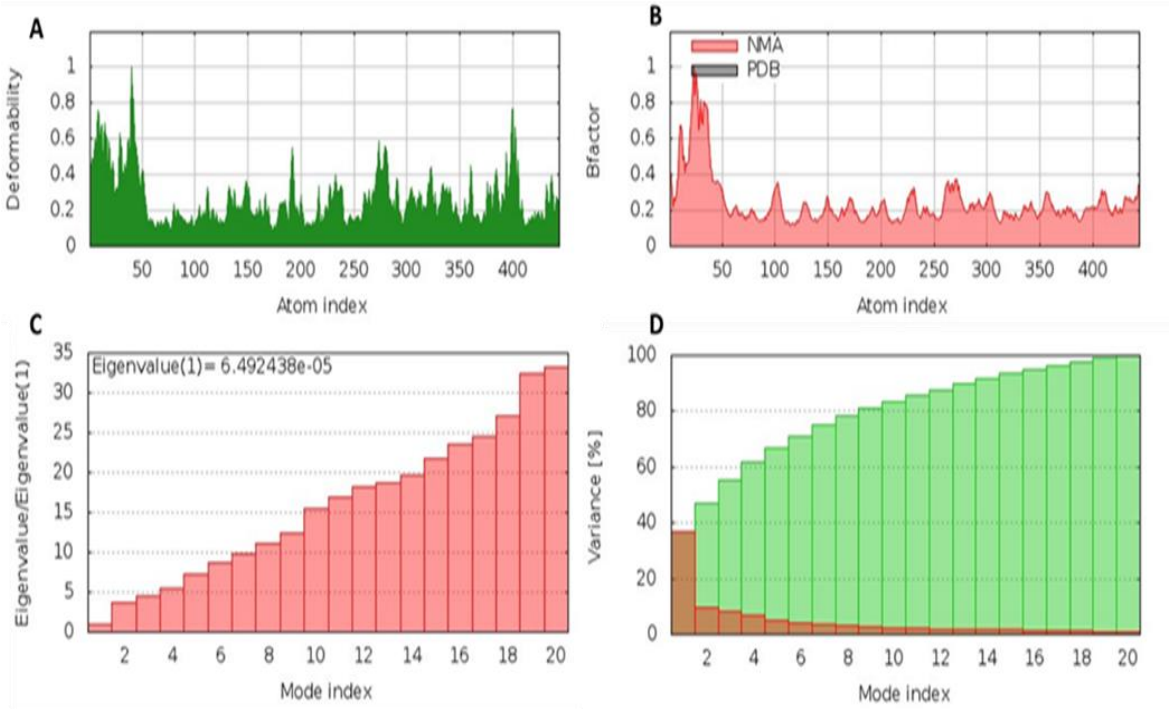


Fig 6. The molecular dynamics simulation of the major capsid protein- CID 12303662 docked complex (A) Deformability simulations on main chains show high deformability in hinges (B) Normal mode analysis generates B-factor values, which measure each atoms uncertainty (C) The eigenvalue of the docked complex, showing the energy required to deform the structure (D) The variance matrix between complex and residue

Discussion

The differential expression patterns of the MCP gene have been reported in numerous viral infections in fish species (Shan et al., 2016; Lu et al., 2013; Huang et al., 2012). According to several studies, Ginger is one of the most beneficial traditional medicinal plants on the planet [45, 46]. From antiquity, almost all components of the plant have medicinal characteristics and have been utilized as traditional medicine or cures for a variety of diseases [47]. It is now regarded as a valuable source of unique natural compounds for the creation of medications to treat a variety of illnesses. 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 looked into the benefits of phytochemicals in disease prevention [32, 48].

Computer-Aided Drug Design (CADD) is one of the most promising tools for the selection of novel compounds against a specific protein as it includes different advanced features and techniques [49]. The CADD approach has minimized the required time and costs involved in the entire drug discovery process making the virtual screening process includes molecular docking, molecular dynamic simulation, ADMET, etc. as integral parts of drug designing [50].

A 3D structure prediction was performed (the lowest energy score was used to choose the best model) and the result was the achievement of the best model among the discovered models. For the Ramachandran plot, we observed a good number of Z-scores (-5.59) and the most favored, accepted, and disallowed regions.

In this study, we identified potential drugs by molecular docking and another process. Initially, the molecular docking process has used to screen the compounds, where the top 3 compounds have been selected with the highest binding affinities of -9.2 to -8.7 kcal/mol from which only one compound was selected for further validation. The RO5 demonstrated the drug-like properties of the selected compounds [51, 52]. Only one compound was found to follow the five 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 [53]. After toxicity testing, we confirmed that the CID 12303662 compound selected is non-toxic or low-toxic.

The compounds were investigated and optimized by a computational DFT-based QM simulation. We retrieved and re-docked the geometry optimized by DFT with the desired protein, and the docking energy was significantly above >9.00 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 compound CID 12303662 was high >3.00 eV which confirms the low reactivity correspondence to the bioactivity of the compound.

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 [50, 53]. Also, it can determine the stability and rigidity of protein-

ligand complexes in a specific artificial environment like the body [50]. The B-factor, main-chain deformability simulation, and eigenvalue of the selected protein – CID 12303662 complex systems indicate the best stability of the compound.

Conclusion

The study is the first to identify potential antibacterial drug candidates targeting major 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 12303662 as a potential drug candidate that will help to inhibit the activity of the major capsid protein. 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 viral infections.

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