

Identification of natural bioactive compounds against rainbow trout viral hemorrhagic septicemia virus (VHSV) by targeting NV protein (R116S) : A computational drug design approach

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Abstract

Fish of all ages are prone to infection, with fry and juveniles being the most sensitive. The viral hemorrhagic septicemia virus (VHSV) is one of the most fatal infectious fish infections, causing severe mortality in a variety of marine fish species including rainbow trout, with a death rate of up to 100% in young fish. This fatality is caused by a virus from the Rhabdoviridae family. However, there is no obvious way to control the spread of this virus due to a lack of effective therapeutic drugs or vaccines. VHSV has been discovered to employ NV protein (R116S) to enter the host cell and cause disease in Rainbow trout.

The NV inhibition may prevent virus budding and virion maturation and might be used to build an antiviral therapeutic candidate. Therefore, the research sought to find prospective natural antiviral drug candidates from the four different marine algae, viz., *Halymenia dilatata zanardini*, *Chlorococcum humicola*, *Caulerpa peltata*, and *P. gymnospora* that would be able to inhibit the virus's budding and virion maturation process by blocking the virus's NV protein activity. Following a homology modeling technique, the protein's 3D structure was identified and refined, and then validated. The improved protein structure was then used to simulate molecular docking.

The binding affinity of all the compounds was estimated using the docking technique, and the top three inhibitory compounds (PubChem CID: 124120485, 33934, and 610132) were chosen for further ADME and toxicity properties. The ADME and toxicity analyses demonstrated that the compounds were effective and nontoxic with the targeted protein. The data suggest that the selected three phytochemicals from *H. dilatata zanardini*, *C. humicola*, *C. peltata*, and *P. gymnospora* may be important VHSV inhibitors in rainbow trout, which could be studied further in the lab.

Introduction

Rainbow trout (*Oncorhynchus mykiss*) is produced commercially in many nations across the world because to its fast growth and high market value due to the high quality of its flesh(Jamal et al., 2020; Sumon et al., 2022). However, rainbow trout can be affected by a variety of viral pathogens, which can have profound economic effects on farms and the industry through reduced growth and mortality. Viral hemorrhagic septicemia virus (VHSV) is a most deadly infectious fish pathogens leading to high mortality in a large panel of marine fish species including Rainbow trout with a mortality rate as high as 100% in juvenile fish (Baillon et al., 2020; Kesterson et al., 2020).

Several studies have found that the Non virion (NV) protein plays an important biological role in either viral replication or the pathogenicity of the VHSV virus. (Baillon et al., 2017; Baillon et al., 2020; Biacchesi et al., 2017; Thoulouze et al., 2004). VHSV or Piscine novi rhabdo virus is a bullet-shaped, encapsulated virion that contains a non-segmented, negative sense, single stranded RNA molecule that belongs to the Rhabdoviridae family of the Mononegavirales order (Schütze et al., 1996). Rhabdoviruses encode five structural proteins with conserved functions in the order 3'-N-P-M-G-NV-L-5' (Dietzgen et al., 2017). Viral RNA is tightly encapsidated with a nucleoprotein (N), a polymerase-associated phosphoprotein (P), and a large RNA-dependent RNA polymerase (L), forming the helical ribonucleoprotein complex (RNP). A matrix protein (M) participates in budding and interacts with the RNP and viral envelope (Ke et al., 2017). As a last step, the viral surface glycoprotein (G) is involved in the entry process, making it a distinct target for neutralizing and protective antibodies. The VHSV genome, on the other hand, has an additional gene located between the G and L genes that codes for a small non-structural NV (Non-Virion) protein. It was dem Ilon et al. (Baillon et al., 2020) revealed 38 single amino acid polymorphisms (SAPs) dispersed over the rainbow trout genome, which may be modulating the pathogenicity of the virus and serving as a molecular marker for the virulence of the virus.

Marine organisms, particularly algae, produce a wide range of compounds having pharmacological activity, such as anticancer, antibacterial, antifungal, antiviral, anti-inflammatory, and others, and are potential sources of novel therapeutic agents (Pérez et al., 2016). Metabolites of green, brown, and red marine algae may be beneficial in suppressing bacteria, viruses, and fungus.

Algal fractions or purified algae components, for instance, have anticoagulant (Wijesinghe et al., 2011), antiviral (Damonte et al., 2004), antioxidant (Cox et al., 2010), anticancer (Namvar et al., 2013) and antiinflammatory (Kazłowska et al., 2010) properties.

Marine algae, *Halymenia dilatata zanardini* (Jainab et al., 2019), *Chlorococcum humicola* (Kavitha & Palani, 2016), *Caulerpa peltata*, and *P. gymnospora* (Murugan & Iyer, 2014) have been utilized for medicinal and other purposes (fish feed, food additives etc.). Several studies confirmed that these algae have rich of bioactive compounds. Algae, sponges, fungus, seaweed, corals, and other marine flora are plentiful and have pharmacologically active novel chemical signatures that are capable of providing new antimicrobial compounds (Wali et al., 2019). Functional bioactive compounds derived from marine plants have proven to be a valuable source of many therapeutic drugs, and they have benefited mankind in treating numerous diseases (Egbuna et al., 2019).

The selection of small bioactive molecules and investigation of their interactions with the targeted protein are required for the development of targeted therapeutics for the fish industry (Aljahdali et al., 2021). Computer aided drug design (CADD) or in-silico methodologies are increasingly important in drug discovery, particularly in the cost-effective identification of promising drug candidates (Brogi et al., 2020).

In-silico methods, such as molecular dynamics, homology modeling, and molecular docking have been used to find a compound with the best properties for a specific target. With molecular docking-based scoring functions, compounds that are most effective against specific targets can be identified, and their interactions can be documented (Torres et al., 2019).

By integrating early ADMET (absorption, distribution, metabolism, excretion and toxicity) profiling of compound, their efficacy and toxicity can be easily predicted, and MD simulations confirm a compound's affinity to the targeted protein (Tareq Hassan Khan, 2010). Developing new drugs can be challenging, but there is a need to advance investigations to identify bioactive compounds by targeting novel protein classes. Therefore, we intend to apply an in-silico approach to test the new drug candidates against the VHSV virus that targets non-virion (NV) protein.

2. Materials and methods

2.1. Protein structure prediction through homology modeling

The amino acid (aa) sequence of the NV protein found in Nervous necrosis virus (NNV) was retrieved from the NCBI database and downloaded in FASTA format. This was done in order to study the structure of the NV protein. The retrieved sequence was sent on 30 April 2022 to the prominent online web portal Iterative Threading Assembly Refinement (<https://zhanglab.dcmb.med.umich.edu/ITASSER/>) in order to ensure or anticipate the three-dimensional (3D) structure of the intended protein(Roy et al., 2010). Top five protein structure models were developed by I-TASSER, which also offered the C-score, TM-score value as well as the root mean square deviation (RMSD) of protein structure. The best protein 3D structure was selected based on the C-score and downloaded as a PDB file. A higher C-score implies that the protein model had a wide range of confidence from positive to negative values.

2.2. Protein structure refinement and validation

The 3D structure of proteins was refined via the GalaxyWeb server. The structural validity is a vital step in homology modeling, which relies on empirically confirmed 3D protein structures. PyMol v2.3.4 software is used to visualize the improved structure. The Ramachandran plot score function was utilized to validate the improved model. Furthermore, the 3D structure is evaluated using the Ramachandran plot score (drug design) and z-score value, which determines the standard deviation via the primary value (Ho et al., 2003).

2.3. Protein and ligand preparation

To construct and develop the protein's 3D structure, the following criteria were applied: water, metal ions, and cofactors were eliminated; polar hydrogen atoms were inserted; nonpolar hydrogen was combined; and gasteiger charges were calculated using AutoDockTools. IMPPAT database was utilized to identify the compounds of the selected plants(Mohanraj et al., 2018). A total of 40 natural phytochemical compounds was retrieved from *Halymenia dilatata zanardini*, *Chlorococcum humicola*, *Caulerpa peltata*, and *P. gymnospora*. To configure and reduce energy for molecules chosen from the database, the Universal Force Field (UFF) designed for each ligand was employed.

2.4. Binding site identification and grid box generation

The NV protein structure has been uploaded to the CASTp 3.0 server (<http://sts.bioe.uic.edu/>) and examined on 2022 in order to estimate the active site residues. Different active pockets of the protein were detected by the server, and the first active pocket was chosen based on its surface area (SA) and volume.

To visualize the binding pocket of the protein, the active pocket and their corresponding amino acid residues were extracted using BIOVA Discovery Studio Visualization Tool 16.1.0. The binding site residues generated by the server were then utilized to select the grid box for molecular docking simulations.

2.5. Molecular docking simulation

PyRx is an open-source tool for virtual screening that is largely utilized in CADD procedures. It is capable of screening libraries of compounds to determine how well they perform against a specified therapeutic target(Dallakyan & Olson, 2015).

PyRx includes AutoDock 4 and AutoDock Vina docking wizards with an intuitive user interface, making it a more reliable CADD tool. In this experiment, the AutoDock Vina molecular docking wizard was used to find the best way for the protein and ligand to bind.

2.6. ADME analysis

The selected compounds were also subjected to ADME analysis. SwissADME software was used to predict the absorption, distribution, metabolism, and excretion (ADME) characteristics of all of the selected substances(Daina et al., 2017). Based on Lipinski's criteria, this software can validate the drug-likeness of ligands.

2.7. Toxicity test

To discover the harmful effects of chemicals on humans, animals, plants, and the environment, their toxicity must be assessed. In silico toxicology analyzes, simulates, visualizes, or predicts chemical toxicity using computational methods(Raies & Bajic, 2016). ProTox-II was utilized in this research to investigate acute toxicity, hepatotoxicity, cytotoxicity, carcinogenicity, mutagenicity, and immunotoxicity of the substances.

3. Results

3.1. Protein 3D structure, refinement, and validation

In this study, homology modelling was used to generate NV protein structure. A best 3D protein structure of NV protein was selected from top five models of protein provided by the I-TASSER server. The selected best protein structure has lowest C-score is -4.03. The NV protein structure after refinement has a GDTHA score of 0.8934, an RMSD value of 0.618, and MolProbity value of 2.417. Prior to refinement, Using the Ramachandran plot, the NV protein had 83.7 percent, 11.3 percent, and 4.7 percent residues in the favorable, allowed, and disallowed regions, whereas the refined NV protein model had 83.962%, 11.321%, and 4.717% residues in the favorable, allowed, and disallowed regions, respectively. (Fig 1A). Similarly, the crude Exportin1 model has a Z-score value of 4.2, which has improved to -4.66 after refinement (Fig 1B).

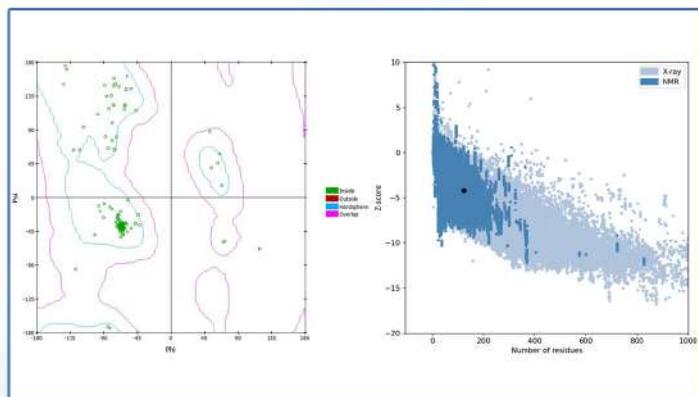


Fig. 1. Validation of the 3D structure of the NV protein. (A) The Ramachandran plot statistics represent the most favorable, accepted, a disallowed region with a percentage of 83.962%, 11.321%, and 4.717% respectively, and (B) the Z-score of refine NV protein -4.66.

3.2. Protein phytochemical and protein preparation

A total of forty phytochemical compounds of *C. candelleana* and *H. fomes* plants were retrieved from the popular database IMPPAT and stored in a 2D (SDF) file format. Compounds were prepared, optimized, and then converted into pdbqt files for further analysis during the ligand preparation process. The protein was optimized and prepared for docking using the AutoDock tool, and then saved in the pdbqt format.

3.3. Binding site active site identification and receptor grid generation

Enzyme active sites (AS) possess a specific shape that permits them to bind with a specific substrate and undergo a chemical reaction. The study first identified AS of the NV protein peptide from CASTpi server then the combined binding position of the active site was retrieved (Fig 2). Analysis of the active pocket of the protein helped to retrieve the binding site residue of the protein (Fig 2). Active site pocket analysis revealed binding site position at ILE3, GLN4, LEU71, ARG74, PRO101, GLY102, PHE104, SER107 residual positions that have been represented in ball shape with different colors red, pink, light and deep green, yellow and blue colors as shown in Figure structure has been uploaded to the CASTp 3.0 server (<http://sts.bioe.uic.edu/>) and examined on 2022 in order to estimate the active site residues.

Different active pockets of the protein were detected by the server, and the first active pocket was chosen based on its surface area (SA) and volume. To visualize the binding pocket of the protein, the active pocket and their corresponding amino acid residues were extracted using BIOVA Discovery Studio Visualization Tool 16.1.0. The binding site residues generated by the server were then utilized to select the grid box for molecular docking simulations.



Fig. 2. Showing the active site and correspondence binding site of the NV protein. Ball shapes with red, pink, green, yellow, and blue colors, respectively, with their binding site position of the NV protein.

3.4. Protein molecular docking simulation

In this study, homology modelling was used to generate NV protein structure. Forty phytochemical compounds were utilized for molecular docking process by using PyRx tools AutoDock Vina wizard. The binding affinity showed a distributed range from -3.2 and -8 after molecular docking of phytochemicals compound. The top ten percentage phytochemical compounds have been chosen from the 40 compounds based on the capacity of top binding affinity. The best three compounds, which are namely 3-methoxy-(3.β)-cholest-5-ene(CID124120485), Diisooctyl phthalate (CID33934), and Anthracene, 9,10-diethyl-9,10-dihydro- (CID60132) have been selected based on their docking score -8 kcal/mol, -7.3 kcal/mol, and -6.9 kcal/mol and further evaluated through different screening methods. The best three compounds selected based on molecular docking score are listed in Table 1 and docking scores for all comA best 3D protein structure of NV protein was selected from top five models of protein provided by the I-TASSER server. Theselected best protein structure has lowest C-score is -4.03. The NV protein structure after refinement has a GDTHA score of 0.8934, an RMSD value of 0.618, and MolProbityvalue of 2.417. Prior to refinement, Using the Ramachandran plot, the NV protein had 83.7 percent, 11.3 percent, and 4.7 percent residues in the favorable, allowed, and disallowed regions, whereas the refined the NV protein model had 83.962%, 11.321%, and 4.717%residues in the favorable, allowed, and disallowed regions, respectively (Fig 3). Similarly, the crude Exportin1 model has a Z-score value of 4.2, which has improved to -4.66 after refinement (Fig 4).

Pubchem ID	Compound Name	Docking Score	Molecular Formula	Molecular Weight
124120485	(3R,8R,9R,10S,13S,14R,17S)-3-methoxy-10,13-dimethyl-17-[(2S)-6-methylheptan-2-yl]-2,3,4,7,8,9,11,12,14,15,16,17-dodecahydro-1H-cyclopentaphenanthrene	-8	C ₂₈ H ₄₈ O	400.7
33934	Diisooctyl phthalate	-7.3	C ₂₄ H ₃₈ O ₄	390.6
60132	Anthracene, 9,10-diethyl-9,10-dihydro-	-6.9	C ₁₈ H ₂₀	236.4

Table 1. List of selected three compounds identified based on molecular docking score (kcal/mol) and their chemical name, formula, and correspondence PubChem CID

3.5. Protein-Ligand interaction analysis

The NV protein with the highest binding score generating compounds was chosen and retrieved to study their interaction.Using the BIOVIA Discovery Studio Visualizer tool, the interaction created between the three selected ligands and the target protein was evaluated. According to the findings, the molecule CID 124120485 establishes a number of hydrogen and hydrophobic bonds with the targeted NV protein. The hydrogen bonds found to formed at SER8position, where the hydrophobic bonds from at the position LEU71, ILE106, ILE75, ARG74, LEU78, and ILE3 position is shown in Fig 3 and the bond types are listed in Table 2.

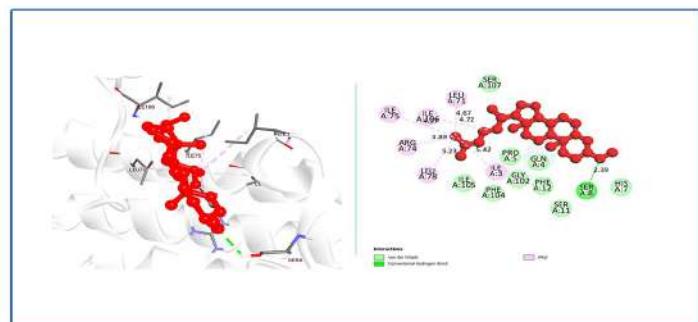


Fig. 3. Shown the interaction between the compound CID124120485 and the NV protein. Left side indicate 3D interaction and the right portion indicates 2D interaction of the protein ligands complex.

PubChem CID	Residue	Distance	CategoryD	Type
CID 124120485	SER8	2.39	Hydrogen Bond	Conv-H-Bond
	LEU71	4.67	Hydrophobic	Pi-Alkyl
	ILE106	4.97	Hydrophobic	Pi-Alkyl
	ILE75	4.72	Hydrophobic	Pi-Alkyl
	ARG74	3.89	Hydrophobic	Pi-Alkyl
	LEU78	5.23	Hydrophobic	Pi-Alkyl
	ILE3	5.42	Hydrophobic	Pi-Alkyl
CID 33934	THR115	3.05	Hydrogen Bond	Conv-H-Bond
	PHE48	4.96	Hydrophobic	Pi-Pi Tshaped
	PHE48	4.51	Hydrophobic	Pi-Pi Tshaped
	PHE48	5.11	Hydrophobic	Pi-Pi Tshaped
	ALA44	3.70	Hydrophobic	Pi-Pi Tshaped
	PHE47	5.22	Hydrophobic	Pi-Pi Tshaped
CID 600132	ILE3	3.89	Hydrophobic	Alkyl
	PRO5	4.91	Hydrophobic	Pi-Alkyl
	PRO5	4.14	Hydrophobic	Pi-Alkyl
	PHE12	4.67	Hydrophobic	Pi-Alkyl
	ILE106	4.48	Hydrophobic	Pi-Alkyl

Table 2. List of bonding interactions between selected four phytochemical with the NV protein.

In the case of compound CID33934,it has been observed to form several hydrophobic bonds in the PHE48, ALA44, PHE47, and ILE3residual position. One conventional hydrogen bond was found to form at the position of THR115 AA position as shown in Figure 4 and are listed in the Table 2.

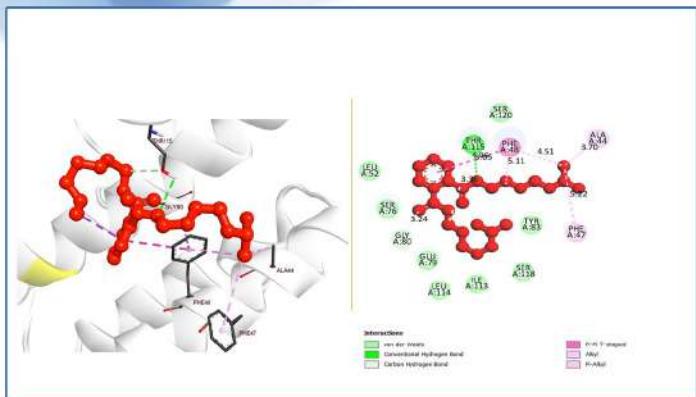


Fig. 4. Shown the interaction between the compound CID33934 and the NV protein. Left side indicate 3D interaction and the right portion indicates 2D interaction of the protein -ligands complex.

A total of 14 hydrophobic bonds were also found to form with the compounds CID600132, including alkyl bonds in the ILE3, PRO5, PRO5, PHE12, and ILE106 residual positions as depicted in Figure 5 and listed in Table 2.

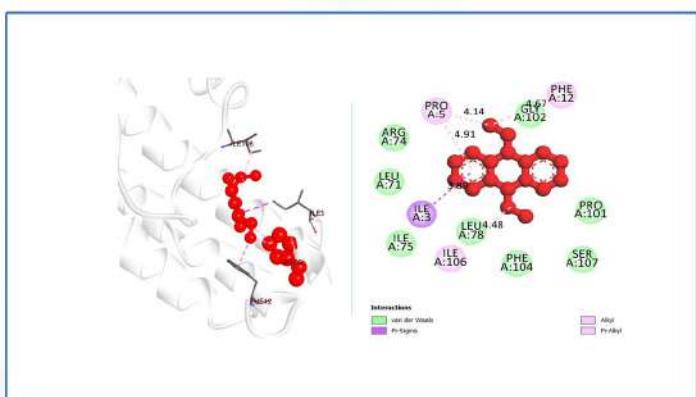


Fig. 5. Shown the interaction between the compound CID600132 and the NV protein. Left side indicate 3D interaction and the right portion indicates 2D interaction of the protein -ligands complex.

3.6. ADME analysis

The SwissADME server was used to analyze the ADME properties (hydrophilic nature, solubility, pharmacokinetics, medicinal chemistry, and drug-likeness feature) of three compounds (CID124120485, CID33934, and CID 610132). The lipophilicity of the druglike compounds allows them to dissolve in fats, oils, and nonpolar solvents. Pharmacophore characteristics have demonstrated that the molecule can be employed as an effective and druggable in the study. All of the compounds have retained optimal pharmacokinetics properties as summarized in Table 3.

Properties	Parameters	CID 124120485	CID 33934	CID 610132
Lipophilicity	MW (g/mol)	400.68 g/mol	390.6g/mol	236.35
	Heavy atoms	29	28	18
	Arom. heavy atoms	0	6	12
	Rotatable bonds	6	16	2
	H-bond acceptors	1	4	0
	H-bond donors	0	0	0
	Molar Refractivity	128.35	116.30	78.13
	Log Po/w	8.04	6.43	5.08
	Log S (ESOL)	-7.65	-6.66	-5.18
	GI absorption	Low	High	Low
Water solubility	Lipinski, Violation	Yes	Yes	Yes
Pharmacokinetics	Synth. accessibility	6.03	3.41	3.22
Drug likeness				
Medi. chemistry				

Table 3. List of pharmacokinetics includes ADME properties of the selected three compounds. The lists also present different physicochemical properties of the three compounds.

3.7. Toxicity prediction

The study employed the ProTox-II website to evaluate the chemical's toxicity because it's quick, cheap, and doesn't require any ethical concerns. The three compounds selected previously through different screening processes, PubChem CID: 124120485, 33934, and 610132, have been submitted to the ProTox-II web server, which determines the oral toxicity, hepatotoxicity, cytotoxicity, carcinogenicity, and mutagenicity of the compounds listed in Table 4. There was no evidence of oral toxicity or organ toxicity in any of the compounds.

Endpoint	Target	CID 124120485	CID 33934	CID 610132
Organ toxicity	Hepatotoxicity	Inactive	Inactive	Inactive
Toxicity endpoints	Carcinogenicity	Inactive	Light active	Inactive
	Immunotoxicity	Active	Inactive	Inactive
	Mutagenicity	Inactive	Inactive	Inactive
	Cytotoxicity	Inactive	Inactive	Inactive
	LD50 (mg/kg)			
	Toxicity class	4	3	5
Tox21-Nuclear receptor signaling pathways	Androgen Receptor (AR)	Inactive	Inactive	Inactive
Tox21-Stress response pathway	Aryl hydrocarbon Receptor (AhR)	Inactive	Inactive	Inactive
	Heat shock factor response element	Inactive	Inactive	Inactive
Fathead minnow LC50 (96 h)	-Log10(mol/L)	6.49	5.96	5.73
48-h Daphnia magna LC50	-Log10(mol/L)	6.07	5.22	5.61
Developmental toxicity	value	0.85	0.75	0.89
Oral rat LD50	mg/kg	841.35	959.71	27861.66
Bioaccumulation factor	Log10	2.9	0.87	1.40

Table 4. List of the drug-induced toxicity profile includes hepatotoxicity, carcinogenicity, immunotoxicity, mutagenicity, cytotoxicity of selected three compounds.

4.Discussion

The viral hemorrhagic septicemia virus (VHSV) is a prominent viral disease agent for farmed rainbow trout that has resulted in substantial economic losses in the aquaculture industry around the world. The NV protein (i.e., NV116) demonstrated a strong role in the virulence of VHSV in rainbow trout. The importance of position 116 at the C-terminal of the NV protein in virulence was validated by Baillon et al. (2020). The NV protein occupies this position within an intrinsically disordered region. Multifunctional viral proteins contain intrinsically disordered regions that interact with several binding partners for signaling, regulation, and control functions in the infected cell. This research focused on inhibiting the virus's NV protein in attempt to find a novel and effective antiviral drug candidate that can be utilized to treat VHSV infections. Recently, marine scientists have become interested in the commercial potential of marine algae, the effects of climate change on the marine environment, the decline of water quality in some marine habitats, the spread of diseases among marine organisms, and biodiversity studies of algal species. (Fantanalgo, 2018). Several studies have found that *Halymenia dilatata zanardini*, *Chlorococcum humicola*, *Caulerpa peltata*, and *P. gymnospora* are among the most beneficial traditional medicinal algae (Jainab et al., 2019; Kavitha & Palani, 2016; Murugan & Iyer, 2014). These are a source of unique natural ingredients for immunostimulants to treat a range of diseases.

Computational approaches are increasingly becoming more popular, acknowledged, and applied in the drug discovery and development process (Kapetanovic, 2008). We used Computer-Aided Drug Design (CADD) which is one of the most promising techniques for finding novel compounds that target a specific protein since it incorporates so many advanced characteristics and methodologies (Sastry et al., 2013). CADD saves time; it is quick and cost-effective in the overall drug discovery process, which includes molecular docking, molecular dynamic simulation, ADMET, and other as vital aspects of drug designing. CADD can identify the specific target molecule based on its behavior and the mechanism of ligand binding. The molecular docking method can be used to describe the atomic interaction between a small molecule and a protein, allowing us to define small molecule behavior in target protein binding sites and elucidate crucial biochemical processes (McConkey et al., 2002). MD simulations, on the other hand, disclose the mechanics of protein-ligand interaction.

As a result, small molecule candidates can be identified as potential therapeutic candidates for the treatment of a certain disease.

In this study, protein structure was initially predicted using Homology Modeling, and then revised and validated using a popular online web service (including, I-TESSER, GalaxyRefine). The best protein 3D structure with the lowest c-score was used as the best model generated from I-TESSER. After refining from the Galaxy Refine server, the model quality was enhanced, and the final refined model exhibited 95.2% in the most favored region of the Ramachandran plot and 0% in the disallowed region, indicating excellent model quality (Rani & Pooja, 2018). While the protein model's Z-score was -4.2 prior to refinement, it increased to -4.66 after refinement. We identified potential drug-like compounds from *Halymenia dilatata zanardini*, *Chlorococcum humicola*, *Caulerpa peltata*, and *P. gymnospora* using molecular docking and an in-silico techniques. The 40 natural phytochemical compounds of *Halymenia dilatata zanardini*, *Chlorococcum humicola*, *Caulerpa peltata*, and *P. gymnospora* from the The Indian Medicinal Plants, Phytochemistry, and Therapeutics (IMPPAT) database were primarily screened using the molecular docking method. The top three compounds, PubChem CID: 124120485, 33934, and 610132, were chosen for further validation because they had the highest binding affinities, which were -8, -7.9 and -6.7 kcal/mol, respectively.

The top three selected compounds, PubChem CID: 124120485, 33934, and 610132 were chosen for further validation because they had the highest binding affinities of -8, -7.9, and -6.7 kcal/mol, respectively. The selected compounds' drug-like qualities were demonstrated by Lipinski's rule of five (RO5) (Lipinski, 2004). Three selected compounds were found to follow the five Lipinski guidelines of drug-likeness attributes. The substance that has good ADME capabilities has been subjected to additional toxicity testing in order to determine the potentially hazardous impacts it may have on both humans and animals (Aljahdali et al., 2021).

After conducting toxicity tests, we verified that the three selected compounds are non-toxic or low-toxic.

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