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RESEARCH PAPER

Unearthing Nature's Defenders: A Computational Quest to Harness Plant Metabolites against the White Spot Syndrome Virus (WSSV) of Shrimp

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Abstract

This study addresses the global threat posed by the *White Spot Syndrome Virus* (*WSSV*) to the aquaculture sector, causing substantial economic losses and impacting food security. The research explores a novel strategy using plant metabolites with proven antiviral properties as an eco-friendly alternative to traditional chemical treatments. Employing molecular docking and several bioinformatics tools, 63 plant metabolites are screened for their potential to inhibit *WSSV*. Hyperoside, Quercetin, and Rutin emerge as top candidates, demonstrating the highest binding affinities with crucial viral proteins. The investigation focuses the molecular interactions between these plant metabolites and *WSSV*, identifying binding sites and drug surface hotspots. Biological property analysis confirms the suitability of these compounds as *WSSV* inhibitors without adverse effects. The study not only offers a promising environmentally friendly approach to combat *WSSV* but also contributes valuable insights into natural product-based antiviral therapies in aquaculture. By unveiling the potential of plant metabolites, this research lays the foundation for innovative strategies to protect global aquaculture and mitigate viral diseases in diverse aquaculture settings. Further experimental verification through *in vitro* and *in vivo* studies is strongly recommended to validate these encouraging findings.

Introduction

In 1992, *White Spot Syndrome Virus* (*WSSV*) arose in shrimp farms in northern Taiwan, bringing sickness and enormous mortality (Lightner et al., 2012). Since its discovery in 1992, *White Spot Syndrome Virus* (*WSSV*) has emerged as the most dangerous viral agent in shrimp aquaculture (Afsharnasab et al., 2014). Due to the large host range of *WSSV*, which includes freshwater *shrimp*, *crayfish*, *crabs*, *spiny lobsters*, and *penaeids* of both salt and brackish water, it also poses a threat to the natural marine ecology (Reddy et al., 2013). The novel pathogenic agent, which was found in an epidemic in Japan towards the end of 1993, quickly spread to other countries that raised shrimp. In China, 80% of the losses in shrimp production from farms were caused by *WSSV*

(Talukder et al., 2021). The growth of shrimp farming is threatened by *WSSV*'s spread to other nations that produce shrimp. Thailand's total lost export revenue is estimated to have been around $1 billion USD in 1996 and 1997 due to the white-spot syndrome virus (*WSSV*), which caused far worse peak losses (Subasinghe et al., 2000). The virus caused numerous fatalities in some Ecuadorian farms in February 1999. Over the past 20 years, Bangladesh's shrimp aquaculture production has suffered greatly from WSD outbreaks. When the first WSD outbreak was detected in 1994 at a semi-intensive shrimp farm in Cox's Bazar, it swiftly expanded to Khulna and other Bangladeshi shrimp farming districts (Talukder et al., 2021). Furthermore, since 2007, Bangladesh has seen recurring WSD attacks, with the number of victims increasing on a daily basis. Some farmers shifted from raising *Penaeus monodon* to *Macrobrachium rosenbergii* after the first outbreak of WSD, which caused enormous financial losses. Others altered their farming practices from semi-intensive to extensive farming (Ramaswamy et al., 2013).Wild crustaceans off the French Mediterranean coast were found to contain *WSSV* in 2002. The growth of shrimp aquaculture could be hampered by the presence of *WSSV* in the Mediterranean, particularly in North African nations (Bateman et al., 2012). *White Spot Syndrome Virus* (*WSSV*), a double-stranded DNA virus and the sole representative of the genus *Whispovirus* and family *Nimaviridae*, is the culprit behind WSD (Dashtiannasab, 2020). According to the initial clinical signs, the *WSSV* virus was known as the following: *penaeid* rod shaped DNA virus (PRDV), rod shaped nuclear virus of *Penaeus japonicus* (RV- PJ), *hypodermal* and *haematopoietic necrosis baculovirus* (HHNBV), *systemic ectodermal* and *mesodermal baculovirus* (SEMBV), and White Spot Disease (WSD) (Wang et al., 2007). The circular dsDNA genome of *WSSV*, which is roughly 300 kb in size, is one of the largest animal viral genomes to have been completely sequenced. There are homologues for just 6% of the 184 putative ORFs encoded by the virus genome in databases that are open to the public (van Hulten, Witteveldt, Peters, et al., 2001); The majority of these genes encode enzymes involved in protein modification, DNA replication, and nucleotide metabolism. White spots (0.5-3.0 mm in diameter) may appear suddenly on the exoskeleton, appendages, and internal surface of the epidermis of *WSSV*-infected shrimp (Sánchez-Paz, 2010). Since these spots are not always present and because similar spots can be caused by certain bacteria, excessive alkalinity, and stress, they are not considered a reliable indicator for a preliminary diagnosis of this condition. Additional signs of *WSSV* include lethargy, a sharp reduction in appetite, redness throughout the body and on the appendages, and loose cuticles. Shrimp raised for food die rapidly, with a cumulative death rate that frequently ranges from 90% to 100%. 3-5 days after infection (Rahman et al., 2007). All tissues of mesodermal and ectodermal origin, such as the lymphoid organ, the cuticular epithelium, and the subcutaneous connective tissues, are affected by *WSSV* infection. Infected nuclei develop hypertrophy, marginalized chromatin, and inclusion bodies that stain highly eosinophillic in early infection and basophilic in more advanced infection. Rod-shaped, enveloped, non-occluded virions were visible when sections and viral suspensions were examined under an electron microscope. The nucleocapsid appeared to be made up of rings of subunits stacked one on top of the other (Leu et al., 2005). The last three phases of a virus' life cycle are entry (directly or through host mechanisms like endocytosis) into the host cell, uncoating of the genome, replication, and particle assembly and release, which has been extensively investigated. Escobedo-Bonilla and colleagues proposed a model of *WSSV* life cycle and morphogenesis (Verbruggen et al., 2016). The virus and its host engage in a variety of molecular interactions during these periods. At first a virus enters into the host cell. When *WSSV* proteins engage with host receptors, Clathrin-mediated endocytosis is induced. Then, *WSSV* passes via endosomes. As cells mature, the pH drops, signaling viruses to leave the endosomes. VP28 and Rab7 are most likely interacting at this time. It is unclear how *WSSV* penetrates the nuclear envelope (Huang et al., 2013). In shrimp farming, plant compounds are emerging as possible therapies for *White Spot Syndrome Virus* (WWSV). Their allure stems from their safety, long-term viability, and antiviral potency. Plant chemicals, unlike manmade medications, are biodegradable and less likely to leave hazardous residues in the environment or on shrimp. They have targeted antiviral characteristics that efficiently attack WWSV without causing harm to the aquaculture system. Furthermore, because of their lower development costs, they are more accessible and inexpensive. Using plant-based remedies corresponds with regulatory trends in aquaculture that encourage natural, eco-friendly solutions. To summarize, plant chemicals provide a safer, more sustainable, and cost-effective strategy to treating WSSV in shrimp farming, making them a more appealing therapeutic option. In this study we have identified potential plant compounds that could be novel medication for the *WSSV* treatment. An *insilico* approach using Molecular docking, molecular dynamics (MD) simulation, Physiochemical and Toxicity assessment were performed aiming to screen novel candidates. Molecular docking and various *insilico* based screening tools has been become an effective strategy for identification and development of novel promising compounds as drug candidates (Ferreira et al., 2015). We identified potential plant compounds that could be novel drug candidates for *WSSV* treatment in this study. An *insilico* approach was used to screen novel candidates, which included Molecular docking, Molecular dynamics simulation, physiochemical analysis, and Toxicity assessment.

Materials and Methods

The methodology involved in this present study have been demonstrated in Figure 1**.**

Choosing of Key Target Proteins

Following a review of the literature, three common and critical proteins required for the survival of the *White Spot Syndrome Virus* were chosen for investigation. *White Spot Syndrome Virus* (*WSSV*) produces a large amount of viral protein 9 (VP9) early in infection. VP9 is a nonstructural protein. The polymers of VP9 dimers have a DNA mimic-like appearance, according to the crystal structure of VP9 and alters the cellular higher-order chromatin structure, which could be a potential strategy adopted by WSSV to facilitate its replication (Liu et al., 2006; Tan et al., 2020; Verbruggen et al., 2016). At least 35 different proteins make up the *WSSV* viral envelope, with VP28 and VP26 being the most prevalent and making up around 60% of the envelope (Valdez et al., 2014). In the initial phases of systemic *WSSV* infection in shrimp, VP28 might be significant (van Hulten, Witteveldt, Snippe, et al., 2001). Additionally, data suggests that *WSSV* VP28 functions as an attachment protein during the infection process, securing the virus to shrimp cells and permitting cytoplasmic entry. Because of some potential glycosylation sites, VP28 is thought to potentially have a significant role in receptor recognition at the shrimp cell surface (Tran et al., 2022). VP26, a viral nucleocapsid component, may aid *WSSV* movement through interactions with actin or cellular actin-binding proteins, toward the nucleus (Sánchez-Paz, 2010; Zhu et al., 2018). The tegument protein VP26, which functions as a stromal-like junction protein between the viral envelope and the nucleocapsid, could potentially facilitate the cytoskeleton-mediated transfer of the *WSSV* nucleocapsid to the host nucleus (Chang et al., 2008;Liao et al., 2021).

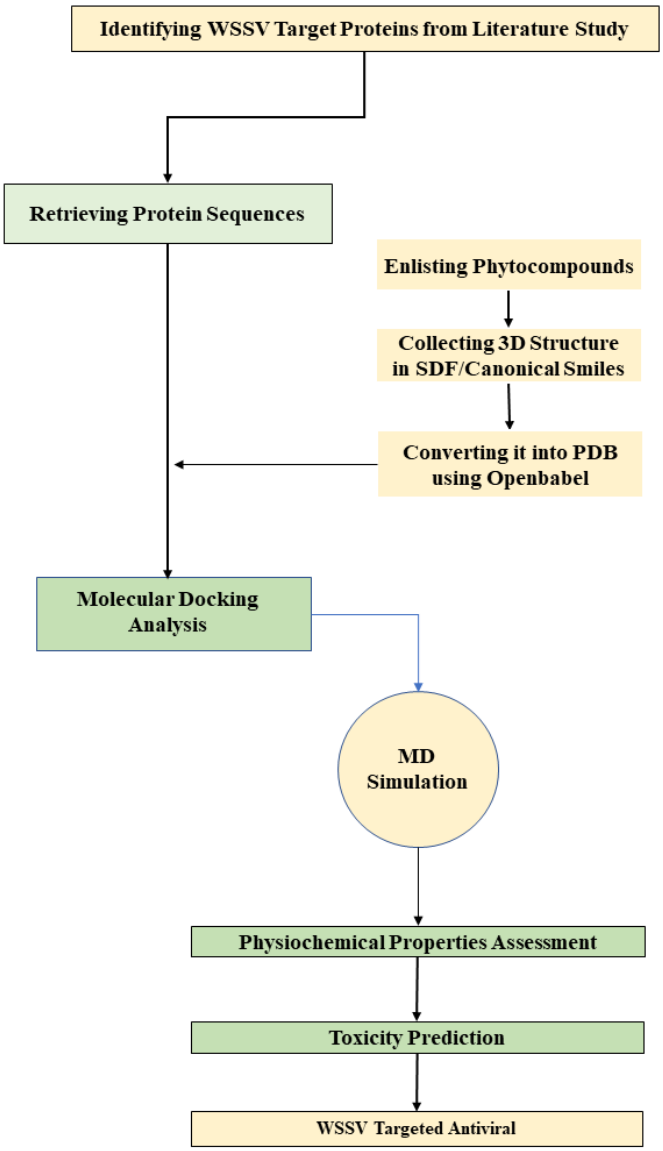

Figure 1. Schematic representation of present study methodology.

Obtaining the Target Protein

The structures of several proteins were available in the RCSB Protein Data Bank (https://www.rcsb.org/). The Protein Data Bank (PDB) (Berman et al., 2000) library of the 3D structure is a vital resource for academics and researchers working in basic biology, health, energy, and biotechnology (Chang et al., 2008). For structural data on biological macromolecules, the Protein Data Bank is the only available global repository (Burley et al., 2017). The PDB formatted structures of a few chosen proteins are retrieved.

Preparation of Protein and Prediction of Active Sites of the Protein

The target protein was further purified by eliminating extraneous water, ligands, metals, and ions using the BIOVIA Discovery Studio Visualizer tool (Gogoi et al., 2021; Sharma et al., 2021). BIOVIA Discovery Studio allows for the visualization, profiling, and analysis of many chemical library sources in order to develop and improve compound selection (Sharma et al., 2019). The Swiss-Pdbviewer has been used for the energy minimization of the proteins (Guex and Peitsch, 1997). The produced proteins were then visualized using the UCSF Chimera Software. CastP server has been utilized for prediction of the active sites of the protein (Tian et al., 2018).

Structure Listing and Collection of Metabolites

A selection of secondary metabolites with antiviral activity from various plants (63) was made after IMPPART database search (Supplementary Table S1). These metabolites, compounds, and inhibitors might be used therapeutically to treat viral disease. After reviewing the literature, we used the reference ligands Acyclovir to compare the binding affinities of our listed metabolites (Huang et al., 2022). The PubChem database (https://pubchem.ncbi.nlm.nih.gov/) and IMPPAT (Indian Medicinal Plants, Phytochemistry And Therapeutics) (https://cb.imsc.res.in/imppat/) were used to retrieve the metabolite structure in SDF (3D) format or smile format (Kim, 2021; Kim et al., 2016; Mohanraj et al., 2018; Vivek-Ananth et al., 2023). Open Babel v2.3 was used to create the PDB format for the structures from SDF format / canonical smile format (O'Boyle et al., 2011).

Identifying the Binding Affinity of Metabolites and Inhibitors

Molecular docking was performed to examine the binding affinity between metabolites and proteins (Anantharam et al., 2022), a crucial tool for researching interactions between tiny ligands and macro molecules (Jakhar et al., 2020; Tao et al., 2020). Consequently, the molecular docking analysis of the selected ligands with our target proteins was performed using PyRx package (El Aissouq et al., 2021; Kondapuram et al., 2021). Using the protein's PDB files as inputs, and protein data bank (PDBQT) files were generated for the proteins. Prior to docking, all 63 compounds and reference drugs were energy minimized and transferred to PDBQT format. For the docking procedure, Vina-Wizard was used (Supplementary Table S2-S4). The PyMOL v2.0 program was also used to analyze binding sites (DeLano, 2002).

Molecular Dynamics (MD) Simulation

The connection of a ligand to its target receptor as it relates to binding is best understood through the use of molecular dynamics (MD) simulations. Using a 2 ns MD simulation using the Desmond program and the OPLS3e force field (Kumar et al., 2023; Zala et al., 2023), The best-docked complex's stability was assessed in this study. The system design for the Desmond MD simulations was methodical and used a six-step relaxation methodology (Fatima et al., 2022). The system builder in Desmond made it more straightforward to create a solvated environment for docked complexes. The protein complex was created using a protein preparation wizard, and it was then further optimized using PROKA pH in order to improve hydrogen bonding interactions. TIP3P parameters were used to model the simulation environment (Marcisz & Samsonov, 2023; Mu et al., 2021). The simulation box was orthorhombic in form, with dimensions of 10×10×10. Na+ and Cl- ions were introduced to the system's surroundings to neutralize it. To assess several aspects of the system's behavior, such as Protein-Ligand interactions and Root Mean Square Deviation (RMSD and RMSF), a 2 ns MD simulation was run. Desmond's Simulation Interaction Diagram (SID) was utilized to determine the ligand's stability and binding orientation based on the MD trajectories.

Physiochemical Properties Assessment

The physiochemical properties of the top compound were evaluated in SwissADME server (Daina et al., 2017). When predicting the physiochemical properties of the compounds, especially in the early stages when there is a large number of compounds to test but a limited supply of actual compounds, *insilico* methods have been proposed as a solid substitute for experimental techniques. The prediction is consider to be a establish protocol to check the feasibility risk assessment of a compound (Wang & Urban, 2004). Using the server, predictions were created after the top compounds were uploaded in SDF format and converted to SMILES.

Toxicity Testing

The overall toxicity of the top metabolites was then predicted using a web-based server known as pkCSM

(https://biosig.lab.uq.edu.au/pkcsm/prediction). This method is effective for predicting toxicity aspects since It foundation is a graph-based signature system that shows patterns of molecular distance (Pires et al., 2015).

Results

Retrieval of Target Proteins and Preparation

The RCSB PDB database yielded the proteins VP9, VP26, and VP28, which were identified by PDB IDs 2GJI, 2EDM, and 2ED6, respectively. The retrieved proteins have been modified and prepared using BIOVIA Discovery Studio. Each target proteins had hetatms like ligands, ions etc. These hetatms were removed for protein preparation. The proteins were then viewed in three dimensions using the UCSF Chimera software, which produced the three-dimensional structure shown in Figure 2**.**

Predicting Active Site of Proteins

The active site of three target protein were analyzed using CastP 3.0 server. Vp9 contain active residue of *MET1, ALA2, THR3, PHE4, GLN5, THR6, ASP7, ALA8, ASP9, PHE10, LEU11, LEU12, VAL13, GLY14, ASP15, ASP16, THR17, SEER18, ARG19, TYR20, GLU21, GLU22, VAL23, MET24, LYS25, THR26, PHE27, ASP28, THR29, VAL30, GLU31, ALA32, VAL33, ARG34, LYS35, SER36, ASP37, LEU38, ASP39,ASP40, ARG41, VAL42, TYR43, MET44, VAL45, CYS46, LEU47, LYS48, GLN49, SER51, GLY50, THR52, PHE53, VAL54, LRU55, ASN56, GLY57, ILE59, GLU60, GLU61, LEU62, ARG63, LEU64, LEU65,THR66,GLY67,ASP68,SER69,THR70,LEU71,GLU72 ,ILE73,GLN74PRO75, MET76, ILE77, VAL78, PRO79.*

VP26 contains *ASP7, ALA8, ASP9, GLU31, ALA32, ARG34, MET44, CYS46, LEU47, LYS48, GLN49, GLY50, SER51, MET76, ILE77, VAL78, PRO79, THR80* and VP28 contain *ASP49, GLU50, ASN51, LEU52, ARG53, PHE72, AYS131, ASN133, THR135, GLY136, MET137, GLN138, MET139, VAL140, PRO141, SER153, ASN154, THR155, SER156, PHE158, PRO160, VAL161, SER162, TYR193, VAL194, HIS195, THR197, SER199, GLY200, THR201* as active site.

Collection of Metabolites and Ligand Preparation

Following a search of IMPPART database, 63 antiviral metabolites were identified from various plants. These compounds, metabolites, and inhibitors may be employed as therapeutic candidates to treat different types of viral infections. Following the generation of the metabolites, the PubChem database (pubchem.ncbi.nlm.nih.gov) and IMPPART database were queried for the SDF/smile format of all metabolites. The structures were converted to PDB format using Open Babel v2.3.

Binding Affinity Analysis

Docking interactions of 63 metabolites with three key proteins were studied to understand the underlying mechanism of antiviral activity against *WSSV*. Metabolites were used as ligands, and the chosen receptors were proteins. Among all the metabolites Rutin, Quercitrin and Hyperoside showed highest

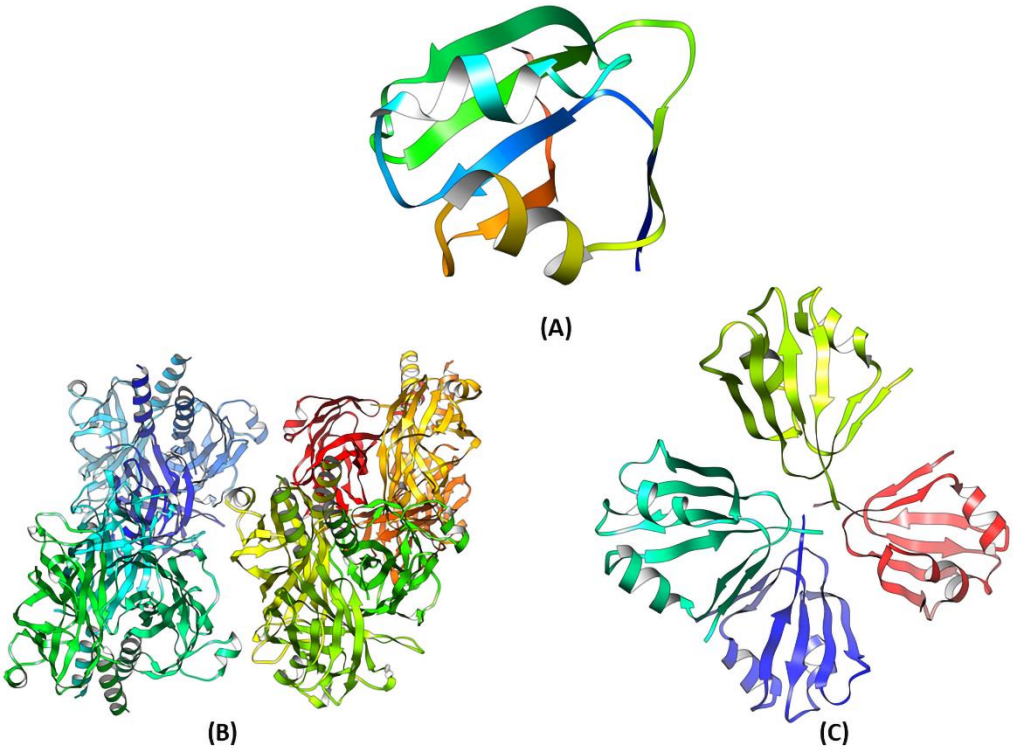

Figure 2. Visualization of target protein A. VP9, B. VP26 and C. VP28.

affinity for binding across all target proteins (Table 1). Among the top metabolites Hyperoside showed highest binding energy of -9.6 kcal/ mol with VP28 protein. In VP26, Quercetin revealed -8.9 kcal/mol as the maximum binding affinity. All the top metabolites outperform the reference drug Acyclovir.

Exploring the Binding Sites

Binding sites were evaluated using the PyMol program, as shown in Table 1. Among the top metabolites Hyperoside with VP28 protein that showed the highest binding affinity was binded with maximum active site of the receptor. It contains 10 binding residues in the complex with residue of *ASN51, GLU50, LEU52, ARG53, PRO55, PHE72, THR201, PRO160, SER162, VAL70* where acyclovir only showed 6 binding sites. Again, Rutin with VP26 showed the maximum binding site hotspot of 8 residue including *SER51, GLY50, LYS48, THR80, PRO79, GLU31, ASP9, ILE77* but our reference drug only showed 4 binding site residues with VP26. In Vp9; THR*26, PHE27, ASP28* are common for maximum of metabolites and reference drug. For VP26 it is *GLU31, ASP9, LYS48, MET76* and *SER162, THR201, ARG53, ASN51 and GLU50* for VP28. These residues are the hotspot binding site of our target proteins. This binding site residues are shown in Figure 3-4-5.

Molecular Dynamics Simulation

Among the top metabolites Rutin showed consistent results with all the target proteins. So, the stability of the compound Rutin was analyzed against VP9, VP26, VP28 complex. In the Vp9-Rutin complex the Highest RMSD of protein backbone was 3.8 Å, the average RMSD was 1.9 Å. The ligand Rutin showed a little fluctuation till 0.50 ns then it showed a good stability with the protein. The highest RMSD for ligand was 4.0 Å and the avg. RMSD was 2.5 Å. In Vp26-Rutin complex the Highest RMSD was 2.4 Å for the backbone

Table 1. Binding affinity of top metabolites against target proteins

Protein	Metabolites	Docking Score	Binding site residue
Vp9	Hyperoside	-6.7	*GLY57, PHE27, THR52, ASP28*
	Quercetin	-6	*THR26, PHE27, ASP28, THR29, THR52, VAL54*
	Rutin	-6.6	*GLU61, PHE27, ASP28, THR29*
	Acyclovir (Control)	-5.1	*THR26, PHE27, THR29, ASP28, THR52*
VP26	Hyperoside	-7.9	*PRO79, SER51, GLN49, LYS48, ASP9, ILE77, VAL76*
	Quercetin	-8.9	*GLU31, LYS48, GLN49, VAL78, MET76, ASP9*
	Rutin	-8.2	*SER51, GLY50, LYS48, THR80, PRO79, GLU31, ASP9, ILE77*
	Acyclovir (Control)	-6.4	*GLU31, ASP9, LYS48, MET76*
VP28	Hyperoside	-9.6	*ASN51, GLU50, LEU52, ARG53, PRO55, PHE72, THR201, PRO160, SER162, VAL70*
	Quercetin	-7.7	*GLU50, ASN51, ARG53, THR201*
	Rutin	-8.3	*SER162, PRO160, ARG53, THR201, GLU50*
	Acyclovir (Control)	-4.9	*SER162, SER199, THR201, ARG53, ASN51, GLU50*

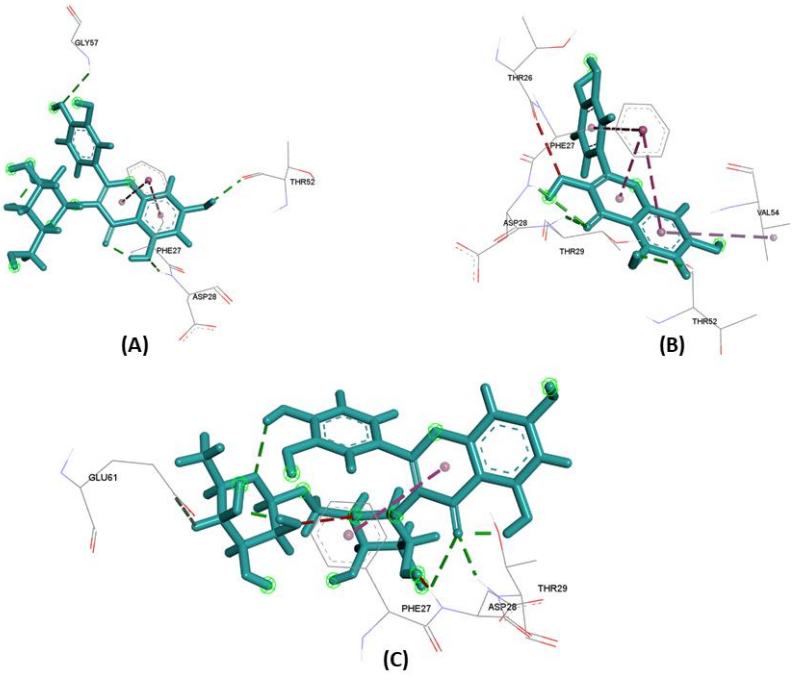

Figure 3. Binding site residue of VP9 with A. Hyperoside B. Quercetin and C. Rutin

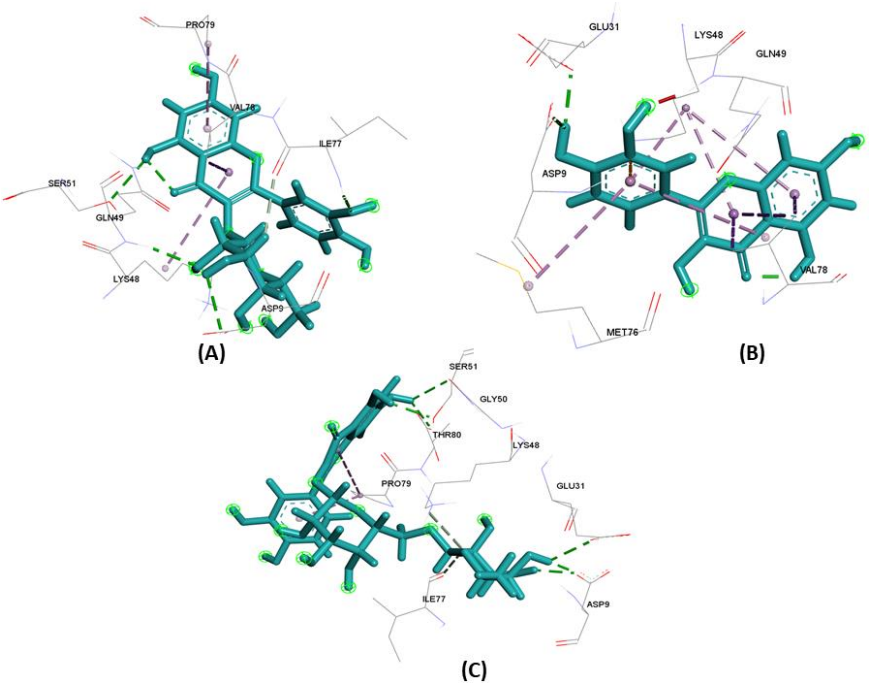

Figure 4. Binding site residue of VP26 with A. Hyperoside B. Quercetin and C. Rutin

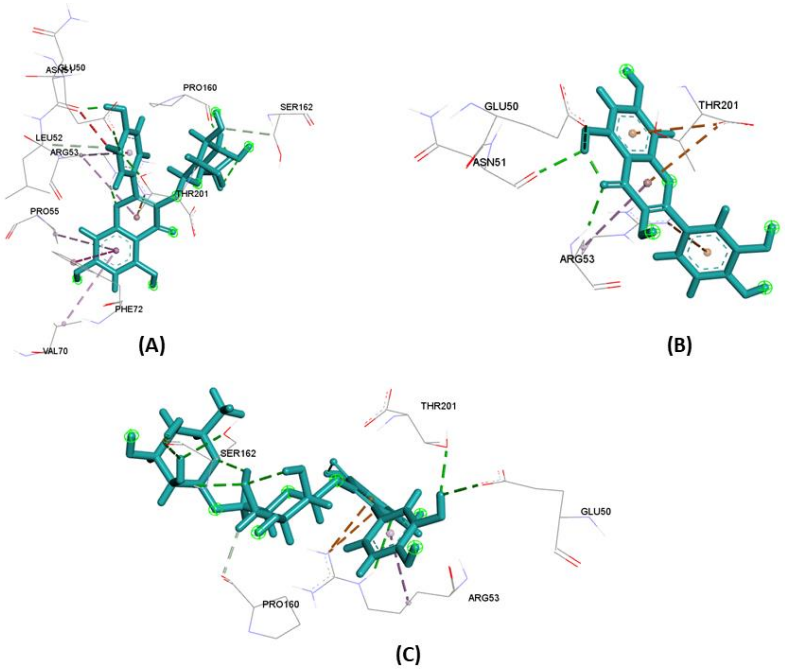

Figure 5. Binding site residue of VP28 with A. Hyperoside B. Quercetin and C. Rutin

and avg. RMSD was 2.0. The ligand showed much fluctuation than vp9. The highest RMSD was 4.6 and the avg. RMSD was 3.6. The VP28-Rutin complex showed a great fluctuation as complex till 1.60ns than it showed a good stability with the protein. The avg RMSD of the protein backbone was 2.0 Å and the avg. RMSD of the ligand was 2.4 Å. In most of the analysis the RMSD was below 4 Å indicating our complexes to be a stable one (Figure 6). The RMSF analysis of each of the complex was done and no abnormal fluctuation was observed in the analysis. The ligand protein contact and the fluctuation is shown in Figure 7. The Highest RMSF for Vp9 was 4.0 Å, Vp26 showed a highest RMSF of 3.0 in residue 80 and VP28 showed a highest RMSF of 3.25 Å after residue index 175. The protein-ligand contact analysis showed the H-bond for vp9 in residue specially *THR26, ASP28, THR29, THR52, VAL54, ASN56, GLY57, GLY58*, in Vp26 the H-bond was observed in *A:ASP9, A:PRO79, A:THR80, D:GLN49, D:GLY50, D:SER51* and *K:GLU50, K:ASN51, K:SER74, L:ASN133, L:PRO160, L:SER162, L:THR201* FOR VP28 (Figure 8). Figure 9 represents the residues that interacted for least 30% time in the 2ns MD simulation.

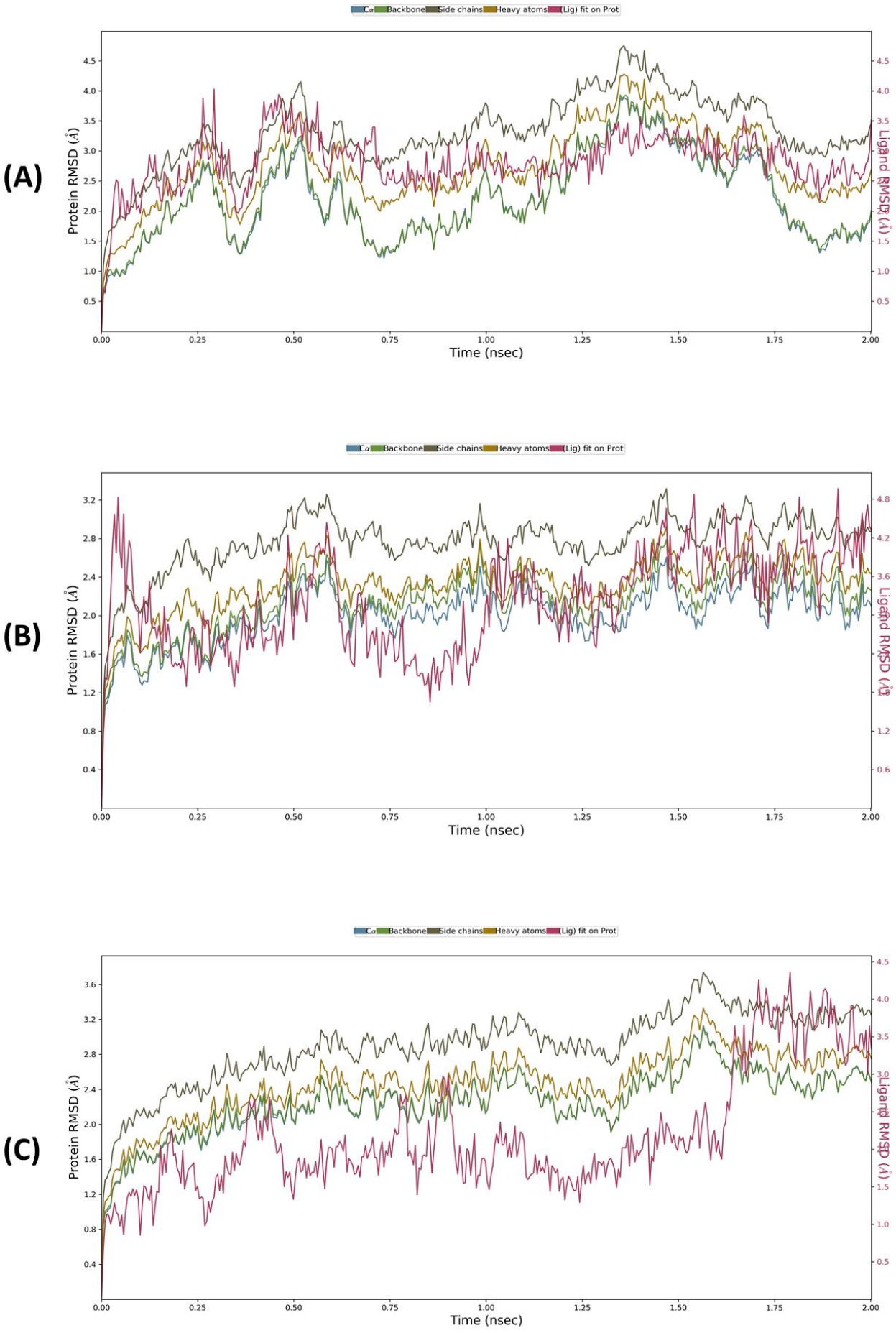

Figure 6. RMSD analysis of A. Vp9-rutin, B. VP26-rutin and C. VP28-rutin complex

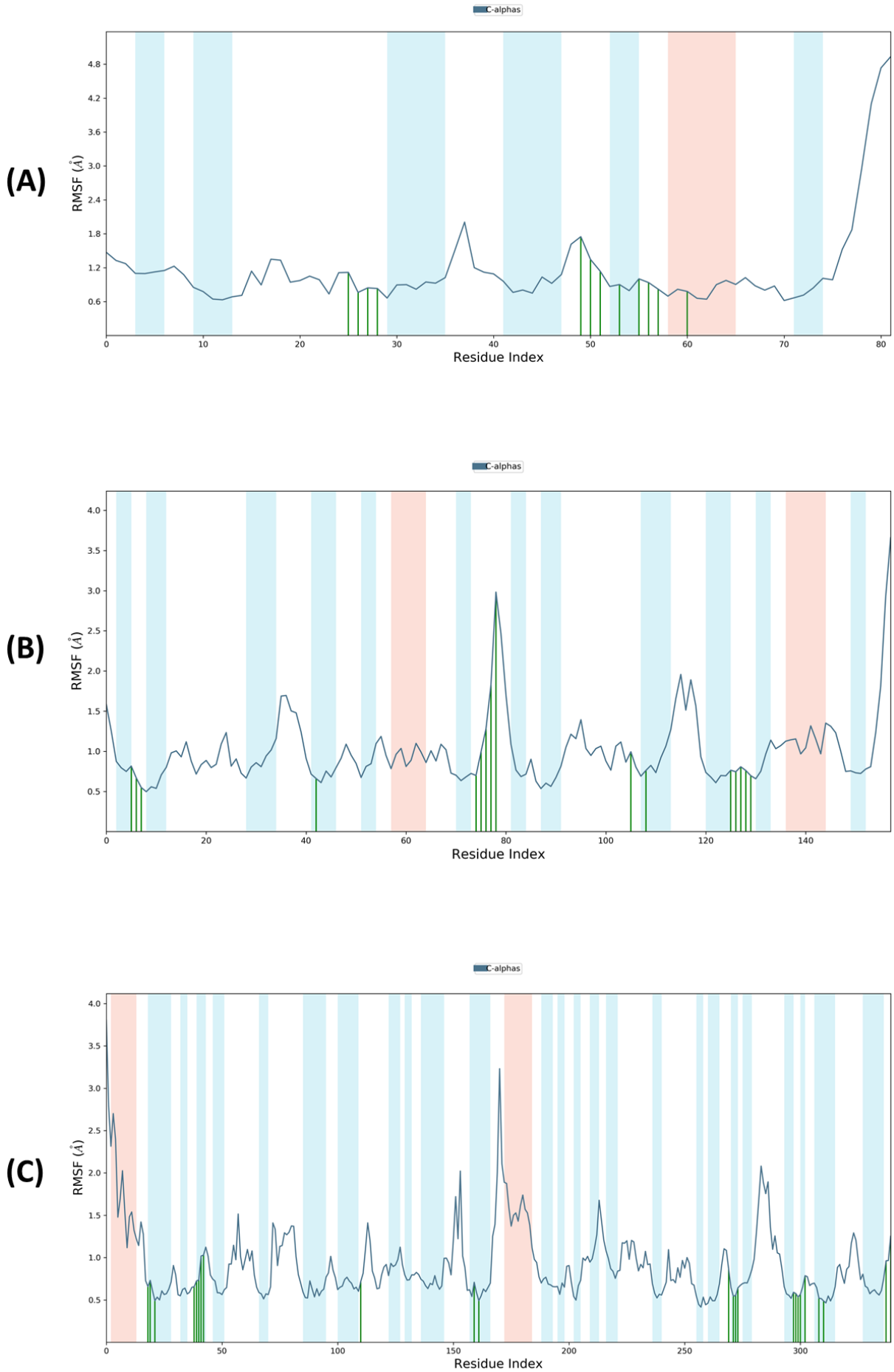

Figure 7. RMSF analysis of A. Vp9-rutin, B. VP26-rutin and C. VP28-rutin complex

Physiochemical Properties Analysis

The online SwissADME was utilized to compute the physiochemical parameters of the nominated compounds (Table 2). The molecular weight of top candidates was below 615Da. Though the H- bond acceptor was more than 10, it didn't exceed 16. The MR value was between 78.03 to 141.38. All of the compound was shown soluble in Esol class, indicating them to be a good soluble compound as drug candidate. The Bioavailability score of the top candidates indicating the good bioavailability in nature.

Toxicity Assessment

The toxicity parameters of the top compounds were analyzed. All of our predicted drugs were analyzed to be non-toxic (Table 3). They showed negative results in AMES toxicity. They also showed negative results as Skin Sensitization and hepatotoxicity. The LD50 value was predicted to be between 0.49 to 0.57.

Discussion

A highly contagious and virulent pathogen, the *White Spot Syndrome Virus* (*WSSV*) mainly affects crustaceans, particularly shrimp and prawns (Pradeep et al., 2012). It is thought to be among the illnesses that harm aquaculture the most, causing large financial losses. The virus is distinguished by its rapid spread and the appearance of distinct white spots on the exoskeletons of infected crustaceans, giving rise to the term "White Spot Syndrome." *WSSV* can quickly spread once introduced into a shrimp farm or aquatic environment, decimating entire populations and posing a significant threat to the global seafood supply chain (Rodgers et al., 2011). There is no known treatment for *WSSV* at this time. In this study three essential proteins for causing the disease was targeted. They were VP9, VP26 and VP28. A total of 63 plant metabolites were collected and screened to find novel best compound for the treatment of *WSSV*. Acyclovir was considered as a reference drug for the *WSSV* treatment (Huang et al.,

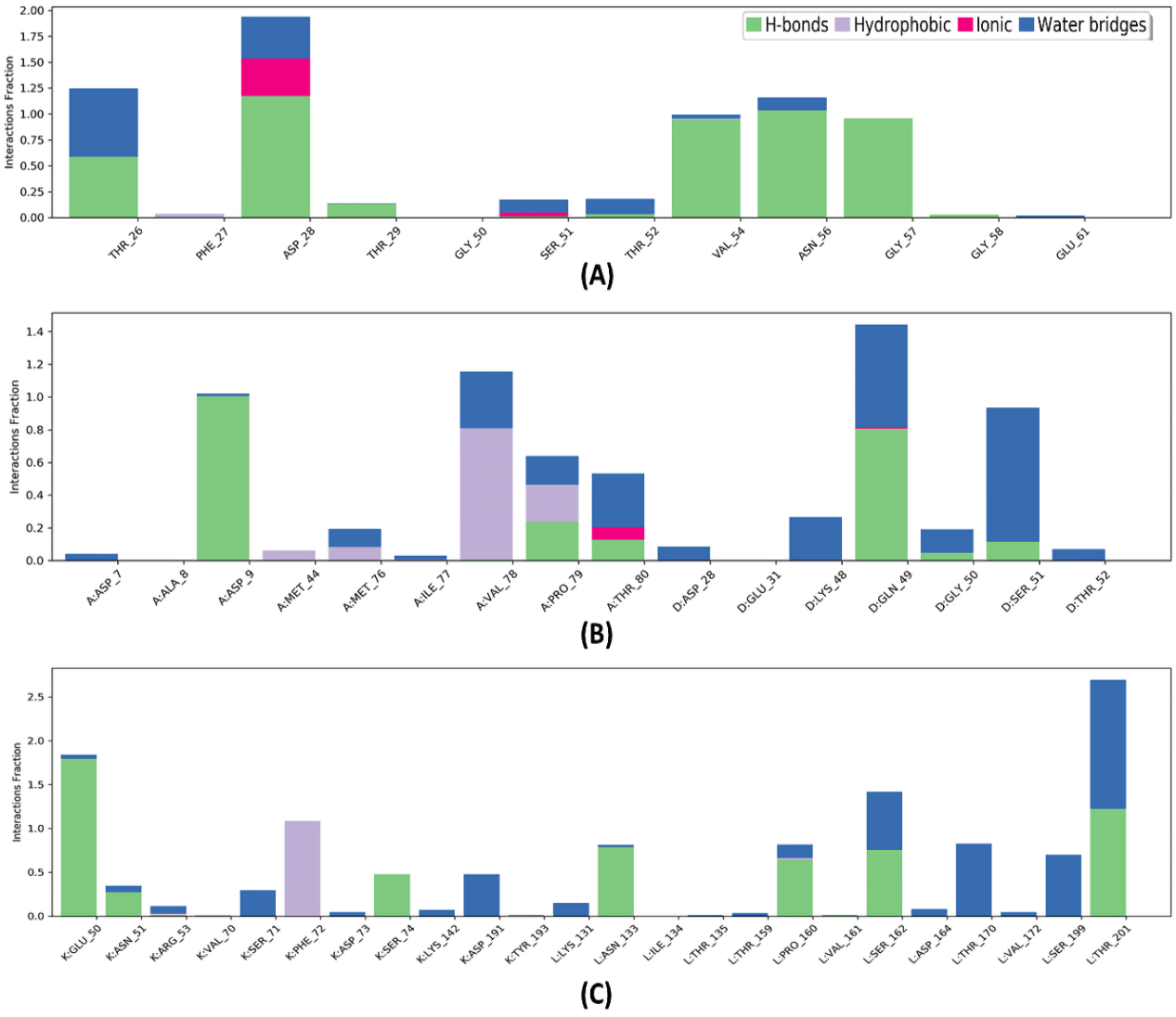

Figure 8. Protein-Ligand Contact map in 2ns MD simulation run A. Vp9-rutin, B. VP26-rutin and C. VP28-rutin complex.

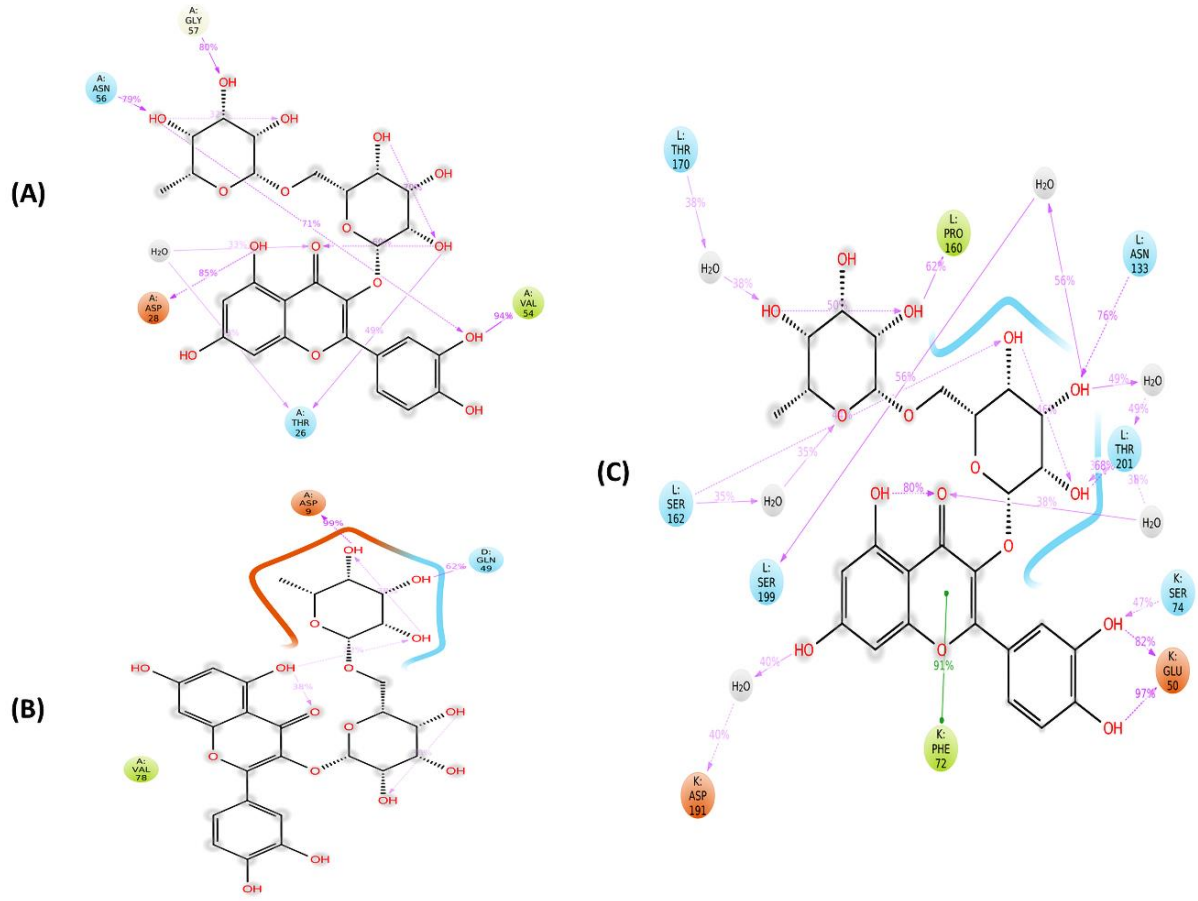

Figure 9. Protein-ligand interaction during Molecular dynamics simulation A. Vp9-rutin, B. VP26-rutin and C. VP28-rutin complex.

Table 2. Physiochemical Properties of top metabolites

Molecule	***Hyperoside***	***Quercetin***	***Rutin***
Formula	$C_{21}H_{20}O_{12}$	$C_{15}H_{10}O_7$	$C_{27}H_{30}O_{16}$
MW	*464.38*	*302.24*	*610.52*
Heavy atoms	*33*	*22*	*43*
Aromatic heavy atoms	*16*	*16*	*16*
Fraction Csp3	*0.29*	*0*	*0.44*
Rotatable bonds	*4*	*1*	*6*
H-bond acceptors	*12*	*7*	*16*
H-bond donors	*8*	*5*	*10*
MR	*110.16*	*78.03*	*141.38*
TPSA	*210.51*	*131.36*	*269.43*
iLOGP	*2.11*	*1.63*	*1.58*
XLOGP3	*0.36*	*1.54*	*-0.33*
WLOGP	*-0.54*	*1.99*	*-1.69*
MLOGP	*-2.59*	*-0.56*	*-3.89*
Silicos-IT Log P	*-0.59*	*1.54*	*-2.11*
Consensus Log P	*-0.25*	*1.23*	*-1.29*
ESOL Log S	*-3.04*	*-3.16*	*-3.3*
ESOL Solubility (mg/ml)	*4.23E-01*	*2.11E-01*	*3.08E-01*
ESOL Solubility (mol/l)	*9.10E-04*	*6.98E-04*	*5.05E-04*
ESOL Class	*Soluble*	*Soluble*	*Soluble*
Ali Log S	*-4.35*	*-3.91*	*-4.87*
Ali Solubility (mg/ml)	*2.10E-02*	*3.74E-02*	*0.0083*
Ali Solubility (mol/l)	*4.51E-05*	*1.24E-04*	*1.36E-05*
Ali Class	*Moderately soluble*	*Soluble*	*Moderately soluble*
Silicos-IT LogSw	*-1.51*	*-3.24*	*-0.29*
Silicos-IT Solubility (mg/ml)	*1.43E+01*	*1.73E-01*	*3.15E+02*
Silicos-IT Solubility (mol/l)	*3.08E-02*	*5.73E-04*	*5.15E-01*
Silicos-IT class	*Soluble*	*Soluble*	*Soluble*
Bioavailability Score	*0.17*	*0.55*	*0.17*
Synthetic Accessibility	*5.32*	*3.23*	*6.52*

Table 3. Toxicity assessment of predicted metabolites

Toxicity Parameters	Top Screened Compound		
	Hyperoside	Quercetin	Rutin
AMES Toxicity	No	No	No
Max. Tolerated Dose (log mg/kg/day)	0.569	0.499	0.452
hERG I inhibitor	No	No	No
Oral Rat Acute Toxicity, LD_{50} (mol/kg)	2.541	2.471	2.491
Oral Rat Chronic Toxicity, LOAEL (logmg/kg_bw/day)	4.417	2.612	3.673
Hepatotoxicity	No	No	No
Skin Sensitisation	No	No	No
Minnow Toxicity (log mM)	8.061	3.721	7.677

2022), which allowed us to compare our novel metabolites whether they contain higher potential. The molecular docking analysis revealed that, among 63 compounds Hyperoside, Quercetin and Rutin outperformed our reference drug in case of all the target proteins. Acyclovir showed only the highest binding energy of -6.4 kcal/mol with VP26 where all of our predicted metabolites outperformed the number. Hyperoside showed -9.6 kcal/mol, Quercetin showed -7.7 kcal/mol and Rutin showed -8.3 kcal/mol with VP28. Among all these metabolites Hyperoside showed the highest binding energy with Vp28 which was -9.6 kcal/mol. Hyperoside also showed the highest number of binding site with VP28. It showed 12 binding hotspots consist of *ASN51, GLU50, LEU52, ARG53, PRO55, PHE72, THR201, PRO160, SER162, VAL70.* The higher the binding site indicates the higher chance of a strong complex with target. Acyclovir showed only a maximum of 6 binding site residue which is also outperformed by our novel compounds. This active site where resemble with the previously predicted active site of the predicted protein using CASTp 3.0 server. The higher binding energy and binding site residue confirming our metabolites to be a strong candidate. Rutin showed consistence higher result with all three targets. That's why Rutin was further analyzed with VP9, VP26 and VP28 for MD simulation. The MD simulation was performed for 2ns using Desmond Software. Rutin showed a good stability with all three target proteins. The lower RMSD indicates a good stability of a complex (Lokhande et al., 2022). The RMSD value for all the complex was less than 4 Å. Most of the complex showed a stability with the ligand compound and protein backbone. Among the three complex VP9 showed less RMSD and a better stability with Rutin. The average RMSD of VP9-Rutin complex was 2.5 Å. The RMSF analysis didn't show any significant fluctuation. So overall all three complex was observed to be stable in 2ns MD simulation run (Figure 6). The vp9-rutin also showed highest number of H-bond in the complex. It showed 8 residues namely *THR26, ASP28, THR29, THR52, VAL54, ASN56, GLY57, GLY58* as H-bond interaction. The docking result was justified because of a stability of the complex in MD simulation. Furthermore, all the top metabolites were further checked for toxicity analysis and its drug candidacy ability against *WSSV*. Molecular weight is an important factor for drug. The bioavailability score was between 0.17 to 0.55. Log S values of -3.5 to -6.5 are acceptable ranges for these compounds (Daina et al., 2014). Our top metabolites showed a value from 2.11 to 1.58 which is close to the acceptable range. The Log S value was also between -3.3 to -3.04. All the compounds showed to have a good solubility in water. So, the compounds could be easily given as a treatment. The logP value was also less than 5 for all the metabolites. Also, our target proteins were showed to be Non-toxic in all the toxicity analysis. They showed no AMES toxicity, No skin irradiation and No hepatotoxicity. Blockage of the heart hERG channel is unexpected and can result in deadly arrhythmias. None of our drug candidates showed to inhibit the hERG I inhibitor so it isn't harmful to humans. So, these compounds are more likely to be safe for Aquatic animal and environment. Hyperoside, Quercetin and Rutin could be novel therapeutics against *WSSV*.

Conclusion

In this computational quest to harness plant metabolites against the *White Spot Syndrome Virus* (*WSSV*), we have embarked on an innovative journey that holds the potential to revolutionize antiviral strategies in aquaculture and beyond. The threat posed by *WSSV* to the global aquaculture industry has necessitated the exploration of alternative, sustainable, and environmentally friendly solutions. Our study's Hyperoside, Quercetin and Rutin, with their prominent binding affinities against the core proteins of *WSSV* offers a promising avenue for such solutions. This computational quest represents a crucial step towards a greener, more resilient future in the battle against *WSSV* and beyond.

Data Availability

The supplementary data that support the findings of this study are available from the corresponding author upon reasonable request.

Ethical Statement

Not Applicable.

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Author Contribution

Angkur Chowdhury: Conceptualization, Data Curation, Formal Analysis, Investigation, Methodology, Supervision, Visualization and Writing-original draft, Writing-review and editing.

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Saila Kabir Falguni: Formal Analysis, Investigation, Methodology, Writing -original draft, Writing-review and editing.

Md. Abdullah-Al-Mamun: Methodology, Supervision, Visualization and Writing -original draft, Writing -review and editing.

Rakibul Hasan Md. Rabbi: Formal Analysis, Investigation, Methodology, Writing -original draft, Writing-review and editing.

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Anik Banik: Formal Analysis, Investigation, Methodology, Writing -original draft.

Conflict of Interest

The authors declare that they have no conflict of interests.

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