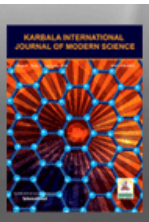
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Abstract

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Keywords

Antiviral; RdRp; Viral Disease; Watercress; White Shrimp

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RESEARCH PAPER

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Abstract

Infectious myonecrosis virus (IMNV) disease causes mass mortality and decreased shrimp production. The RdRp region projects to the interior, where it may function in transcription. The focus of this study was to determine the effect of amino acid polymorphisms from several countries on the structure of RdRp and identify the potential of watercress in inhibiting IMNV by targeting the RdRp protein of IMNV through an in silico approach. The results showed that the structure of the IMNV RdRp protein from Indonesia was similar to Mexico, and the protein structure from India_QDN was identical to India_QIL. Ligand binding affinity values showed that Rhamnetin 3-sophoroside in RdRp samples from Indonesia and India_QDN had the lowest values of −7.8 kcal/mol and −8.7 kcal/mol. Meanwhile, Rhamnazin 3-sophoroside had the lowest binding affinity value of −8.2 kcal/mol in RdRp protein samples from Mexico and India_QIL. The structure of the RdRp protein is still stable after interacting with rhamnetin and rhamnazin as indicated by the RMSD backbone value, ligand structure, and number of hydrogen bonds. Polymorphisms of amino acids from various countries have altered the structure of the RdRp protein of IMNV. The bioactive constituents in watercress *N. officinale*, specifically, rhamnetin and rhamnazin, have shown stable binding to RdRp protein. This suggests that the compound might inhibit the viral activity of the RdRp protein of IMNV from several countries.

Keywords: Antiviral, RdRp, Watercress, Viral disease, White shrimp

1. Introduction

Litopenaeus vannamei is a favorite fishery product in Indonesia. This species is one of the main aquaculture commodities by production in the country [1]. *L. vannamei* culture is susceptible to infections and causes a decline in aquaculture production. A virus is a disease agent that can cause mass mortality and loss in shrimp production.

Several families of DNA or RNA viruses, which include Parvoviridae, Nimaviridae, Roniviridae, and Dicistroviridae are liable for infecting shrimp [2–4]. Particularly, a disease known as infectious myonecrosis (IMN) was recognized during 2002–2003 in

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Brazil. This disease was also reported in Indonesia farms in 2006 [5]. The causative agent responsible for IMNV is an infectious myonecrosis virus (IMNV) which is a double-stranded RNA virus. This virus was tentatively categorized within the Totiviridae family [6].

The genomes of IMNV variants originating in Brazil and Indonesia have been fully sequenced, revealing a remarkable 99.6% similarity at the nucleotide level [6,7]. This high level of identity, along with anecdotal information on the introduction of *Penaeus vannamei* stocks from Brazil, strongly suggests that the disease was introduced from Brazil to Indonesia in 2006 [5] and spread to several countries, including India in 2017 [8]. Analysis of the RNA-dependent RNA polymerase (RdRp) gene coding sequence through phylogenetics demonstrates IMNV's closest relationship to *Giardia lamblia* virus, a member of the Totiviridae family [3,6,9]. The RdRp region projects into the interior, where it can function in the transcription [9]. The RNA-dependent RNA polymerase (RdRp) plays a crucial role in RNA synthesis and lacks a counterpart in host RNA viruses. RdRp becomes a central focal point for antiviral inhibitors [10]. IMNV is a viral disease that has detrimental effects on shrimp cultivation, resulting in substantial economic losses. Regrettably, there are nonexistent vaccines, medications, or treatment methods to prevent or manage IMNV outbreaks in shrimp farming.

Medicinal plants are often employed in medicine and pharmacology. It usually contains several organic compounds, such as polyphenols, aliphatic compounds, and peptides, which have antimicrobial properties. Watercress belongs to the Brassicaceae family, which has antiviral properties [11]. *Nasturtium officinale* was reported to have antiviral, anti-inflammatory, diuretic, expectorant, antidiabetic, hepatoprotective, antihyperlipidemic, and anti-cancer properties [12]. It contains of flavonoids there are quercetin, kaempferol, isorhamnetin, chlorogenic acid, quercetin-3-O-rutinoside, cafeoiltartaric acid and caftaric acid [13]. It is an effective antiviral against bovine coronavirus infection in vitro with a high safety range [14]. Watercress is known as an herb rich in phytochemicals and bioactive components. Bioactive components can maximize the medicinal/therapeutic benefits of the bioactive under investigation, considering safety and toxicity issues. In silico approaches are required to validate the abundant bioactive constituents in medicinal plants [15].

IMNV infection in *L. vannamei* shrimp in several countries is interesting to study. This study focuses on determining the effect of amino acid polymorphisms from several countries on the structure of RdRp and identifying the potential of watercress in inhibiting IMNV by targeting the RdRp protein of IMNV through an Insilico approach.

2. Material and methods

2.1. Data mining of amino acid sequence of RdRp IMNV

The sequence of RdRp IMNV was acquired through data mining from the database server (www.ncbi.nlm.nih.gov) using gene bank IDs, namely QRG24252.1, UBX38638.1, QDN53938.1, QIL87249.1 with the origin country is Indonesia, Mexico, India-sample1 (India_QDN), India sample-2 (India_QIL) respectively.

2.2. Alignment of the amino acid sequence of RdRp IMNV

The alignment of an amino acid sequence by clustalW software via server (www.ebi.ac.uk/Tools/msa/). All the sequences of RdRp were aligned to identify the similarity among the sequences and mutations in specific viral proteins by the Clustal Omega database [16–18].

2.3. Structural modeling and quality analysis of RdRp IMNV

The Swiss-Model web server is utilized to construct a three-dimensional (3D) RdRp model for IMNV, encompassing all atoms. Swiss-Model (http://swissmodel.expasy.org) is an online platform dedicated to the automated comparative modeling of protein structures in three dimensions. It offers multiple degrees of user interaction via its World Wide Web interface, where inputting an amino acid sequence prompts the creation of a 3D model. The server handles template selection, alignment, and model construction entirely through automated processes [19]. Evaluation of the modeling structure's quality involves the use of Ramachandran plots. These plots analyze the amino acid backbone conformations distribution within the protein structure, visually indicating energetically permissible regions for dihedral angles ψ and φ of amino acid residues.

2.4. Structure alignment of RdRp IMNV

Structure alignment involves determining the corresponding amino acid residues. This process

was conducted to pinpoint the correspondences among pairs of amino acid residues within structures, enabling the superposition of these structures to minimize the distance between already identified equivalent residues. This is achieved by discovering a transformation that yields the lowest root-mean-square deviation (RMSD) [20]. The alignment structure of RdRp employed Pymol software, which is displayed in RMSD values.

2.5. Molecular docking and visualization between RdRp of IMNV and watercress N. officinale compound

Determining secondary metabolite compounds from watercress as potential antiviral was employed molecular docking. The optimal arrangement of the ligand, referred to as the best pose in docking, is determined by selecting the ligand conformation with the lowest binding energy. Utilizing Autodock Vina [21], precise protein-ligand docking was conducted. Four representative IMNV RdRp models from various countries were used as docking targets. This process was incorporated into PyRx 8.0 [22] to identify potential ligands that inhibit protein activity. A specialized docking approach was employed by configuring the grid box to encompass only the amino acid binding site of the proteins (refer to Table 1). Examining binding sites and chemical interactions between proteins and ligands was executed using Biovia Discovery Studio 2019 software (Dassault Systèmes Biovia, San Diego, California, USA) [23].

2.6. Molecular dynamics simulation

Molecular dynamics simulations were analyzed using the YASARA 20.12.24 program [24] under the AMBER14 forcefield [25]. The environment setting for the simulations was as follows: temperature 310 K, NaCl 0.9%, pressure 1 bar, pH 7.4. The simulation was performed for 100 ns with automatic saving every 25 ps. The structural flexibility and integrity were analyzed using the root-mean-square deviation (RMSD) of the atomic positions and the root-mean-square fluctuations (RMSF) of the residue positions [26].

3. Result

3.1. Sequence alignment of RdRp protein of IMNV

There was an RdRp IMNV mutation in the sample from Indonesia, which was different from other samples in the amino acid sequence 253, 327, 328, 475, 556, 579, 604, 665. Mutations in the RdRP sample from Mexico also occurred in the amino acid sequences 190, 309, 509, 538, 590, and 606. The amino acid sequences 644 and 715 of the samples from Mexico and Indonesia differed from those from India_QDN and India_QIL; both had mutations (Fig. 1).

3.2. Structural modeling and molecular dynamics simulation of RdRp proteins of IMNV

The RdRp IMNV protein from Indonesia has structural similarities with Mexico, as the protein from India_QDN has structural similarities with India_QIL. However, proteins from India_QDN and India_QIL differ from those from Mexico and Indonesia (Fig. 2A). The placement of the α-helices and β-sheets alters how the protein structure appears. The difference in protein structure of RdRp from Indonesia and India is seen in the position α-helices and β-sheets. The position of the a-helices of the RdRp protein structure from India_QDN and India_QIL is more upward when compared to the RdRp protein from Indonesia. Likewise, the β-sheet position of the RdRp protein structure of India_QDN

Table 1. Amino acid binding site position and grid setting for specific docking of RdRp IMNV.

RdRp	Amino acid binding site	Grid	
		Center	Dimension
Indonesia	Arg205, Tyr266, Ala267, Ser268, Phe269, Asp270, Ser337, Gly346, Asp378	X: 32.7826 Y: −5.1221 Z: 44.2878	X: 24.1540 Y: 18.9396 Z: 19.3218
Mexico	Arg205, Asp265, Tyr266, Ala267, Ser268, Phe269, Asp270, Ser337, Thr342, Gly346, Asp378	X: 30.4528 Y: 4.9994 Z: 35.2343	X: 16.1920 Y: 21.0360 Z: 23.6922
India_QDN	Asp354, Tyr355, Phe358, Asp467	X: 222.8262 Y: 150.0742 Z: 207.6033	X: 17.8840 Y: 16.8890 Z: 11.9300
India_QIL	Lys57, Arg64, Ser127, Asp129, Asp237	X: 2.9940 Y: 4.8685 Z:-9.1380	X: 21.4383 Y: 17.0729 Z: 17.9869

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QRG24252.1      PNDEVETNASNISLLERRAGIELEQLHHINKVKWSRHVRQSYKYLELPKRLGGFGIYRFQ    511
UBX38638.1      PNDEVETNASNISLLERRAGIELQQLHHINKVKWSRHVRQSYKYLELPKRLGGFGIYLFQ    511
QDN53938.1      PNDEVETNASNISLLERRAGIELQQLHHINKVKWSRHVRQSYKYLELPKRLGGFGIYRFQ    600
QIL87249.1      PNDEVETNASNISLLERRAGIELQQLHHINKVKWSRHVRQSYKYLELPKRLGGFGIYRFQ    370
                **********************:**********************************  **

QRG24252.1      GWLPNGKLPLAKKPLVNVEDIHPSQELFLPLSEQQKKILAQVEMINKMQTDDIPGTQKLF    571
UBX38638.1      GWLPNGKLPLAKKPLVNVEDIHPSQETFLPLSEQQKKILAQVEMTNKMQTDDIPGTQKLF    571
QDN53938.1      GWLPNGKLPLAKKPLVNVEDIHPSQELFLPLSEQQKKILAQVEMTNKMQTDDIPGTQKLF    660
QIL87249.1      GWLPNGKLPLAKKPLVNVEDIHPSQELFLPLSEQQKKILAQVEMTNKMQTDDIPGTQKLF    430
                *************************************************************

QRG24252.1      SKEWIQKMRAKKIIWSRNQTIPIHTDHTVRIPRWDEKIKFPRYKSEYILNNKINLTMEQV    631
UBX38638.1      SKEWIQKVRAKKIIWSRNETIPIHTDHTVRIPKWNEKIKFPRYKSEYILNNKINLTMEQV    631
QDN53938.1      SKEWIQKVRAKKIIWSRNQTIPIHTDHTVRIPKWDEKIKFPRYKSEYILNNKINLTMEQV    720
QIL87249.1      SKEWIQKVRAKKIIWSRNQTIPIHTDHTVRIPKWDEKIKFPRYKSEYILNNKINLTMEQV    490
                *******:**********:*************:*:*************************

QRG24252.1      LRQYNLLKEVERYDKDLKVPKLLDILDKWFPVQCSKIKTYESQGFHRTDAINLAVGEIPT    691
UBX38638.1      LRQYNLLKEVERYDKDLKVPKLLDILDKWFPVQSSKIKTYESQGFHRTDAINLAVGEIPT    691
QDN53938.1      LRQYNLLKEVERHDKDLKVPKLLDILDKWFPVQSSKIKTYESQGFHRTDAINLAVGEIPT    780
QIL87249.1      LRQYNLLKEVERHDKDLKVPKLLDILDKWFPVQSSKIKTYESQGFHRTDAINLAVGEIPT    550
                ************:*********************.*************************

QRG24252.1      EPAVKINPILINFVKLHLERQGIRHQRGRNKIAKFIYQKTKQAENMILQSSLQQMYRY      749
UBX38638.1      EPAVKINPILINFVKLHLERQGIRHQRGRNKIAKFIYQKTKQAENMILQSSLQQMYRY      749
QDN53938.1      EPAVKINPILINFVKLHLERQGITHQRGRNKIAKFIYQKTKQAENMILQSSLQQMYRY      838
QIL87249.1      EPAVKINPILINFVKLHLERQGITHQRGRNKIAKFIYQKTKQAENMILQSSLQQMYRY      608
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Fig. 1. The alignment of the amino acid sequence of RdRp from several countries.

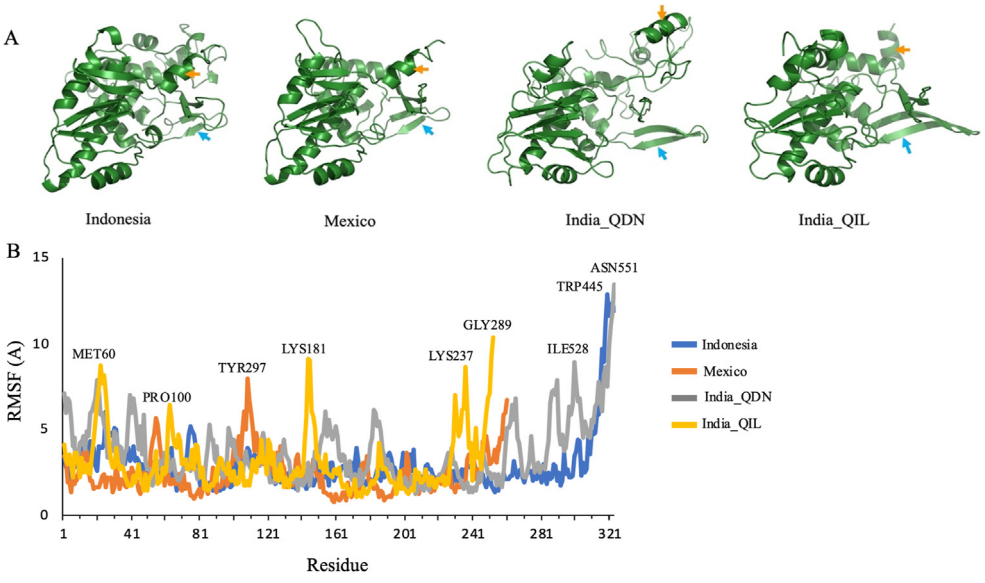

Fig. 2. RdRp protein of IMNV. A) Structural modeling (α-helices in orange arrow and β-sheets in blue arrow), and B) The simulation of molecular dynamics in RSMF value.

and India_QIL is also longer than in Indonesia. Contrarily, the site of the α-helices and β-sheets of the RdRp structure from Indonesia and Mexico is similar.

Molecular dynamics occupy a critical function in studying the structural and dynamic properties of proteins. The root-mean-square fluctuation (RMSF) graph was employed to inspect the variations in each residue of RdRp (Fig. 2B). The RMSF value illustrated the instability of several RdRp residues, including MET60, PRO100, TYR297, LYS181, LYS237, GLY289, ILE528, TRP445, and ASN551. However, those residues were not involved in ligand–protein interaction.

3.3. Structural alignment of four RdRp proteins of IMNV

The RMSD results in Table 2 show that the protein structure of RdRp from Indonesia is similar to RdRp from Mexico, with an RMSD value close to 0, which is 0.864.

However, it has a considerable difference between RdRp from India_QDN and India_QIL with values of 6221 and 2764 (Table 2).

3.4. Molecular docking and visualization between RdRp of IMNV and watercress N. officinale compound

The molecular docking value between RdRp IMNV protein and compounds contained in watercress *N. officinale* is taken from the lowest binding affinity value, and the result is shown in Table 3.

The value of the ligand binding affinity showed that Rhamnetin 3-sophoroside in the RdRp samples from Indonesia and India_QDN had the lowest values, −7.8 kcal/mol, and −8.7 kcal/mol, respectively. Meanwhile, Rhamnazin 3-sophoroside had the lowest binding affinity value of −8.2 kcal/mol in RdRp protein samples from Mexico and India_QIL.

The docking results (Fig. 3) showed that the bioactive compounds contained in watercress *N. officinale* bind to their respective target proteins. The amino acid residues' interactions are van der Waals, hydrogen, and hydrophobic bonds, the result shown in Fig. 3 and Table 4.

Rhamnetin binds to the active site of RdRp IMNV of the sample from Indonesia by forming 10 van der Waals (vdW) bonds and 7 hydrogen bonds, while the sample from India_QDN includes 10 vdW bonds, 8 hydrogen bonds, and 3 hydrophobic bonds. The sample from Mexico actively binds to rhamnazin compounds. It interacts on the RdRp IMNV active site by forming 13 vdW bonds, 6 hydrogen bonds, and 1 hydrophobic bond, while the sample from India_QIL includes 10 vdW, 7 hydrogen bonds, and 2 hydrophobic bonds.

Table 2. The root mean square deviation (RMSD) of four RdRp proteins of IMNV.

RMSD	Indonesia	Mexico	India_QDN	India_QIL
Indonesia	0	0.864	6.221	2.764
Mexico	0.864	0	4.857	2.347
India_QDN	6.221	4.857	0	4.771
India_QIL	2.764	2.347	4.771	0

3.5. Stability of the interaction between RdRp and rhamnetin and rhamnazin

A molecular dynamic (MD) simulation of RdRp proteins and ligands was carried out to analyze the stability of protein structure and protein-ligand interactions (Fig. 4). The RMSD value of the backbone atom illustrates that the RdRp structure looks stable. On the other hand, rhamnetin compounds affect the stability structure of the RdRp India_QDN which showed some augmentation fluctuation but at 50–100 ns had a minimum fluctuation and appeared stable (Fig. 4A). The compound structure was also stable during the simulation, although rhamnazin exhibited the highest RMSD value (Fig. 4B). Simulation results showed that the MD values of hydrogen bonds in the complexes were similar without any significant difference (Fig. 4C).

4. Discussion

Viruses can undergo mutations when adapting to new hosts and environments. IMNV infects the

Table 3. Ligand binding affinity (kcal/mol) between RdRp of IMNV and watercress N. officinale compound.

Ligand	Binding affinity (kcal/mol)			
	Indonesia	Mexico	India_QDN	India_QIL
Sinigrin	−5.6	−6.0	−4.3	−6.4
Benzylglucosinolate	−4.3	−4.2	−6.2	−4.3
2-Phenylethylglucosinolate	−6.3	−5.8	−4.3	−5.6
7-(Methylsulfinyl)heptyl glucosinolate	−3.5	−3.4	−5.0	−3.8
7-Methylthioheptyl glucosinolate	−5.8	−5.8	−3.6	−6.1
8-Methylsulfinyloctyl glucosinolate	−5.9	−5.6	−3.8	−6.3
8-Methylthio-octyl glucosinolate	−6.3	−6.0	−5.7	−6.2
4-Hydroxybenzyl glucosinolate	−6.8	−7.1	−5.2	−7.0
9-Methylthiononyl glucosinolate	−6.4	−6.0	−5.4	−6.4
Benzenepropanenitrile	−3.6	−3.9	−5.0	−4.0
1-Cyano-8-(methylthio)heptane	−6.7	−6.6	−5.9	−6.9
1-Cyano-8-(methylthio)octane	−6.6	−6.9	−5.8	−7.0
Rhamnetin 3-sophoroside	**−7.8**	−8.0	**−8.7**	−8.1
Rhamnazin 3-sophoroside	−7.3	**−8.2**	−8.0	**−8.2**

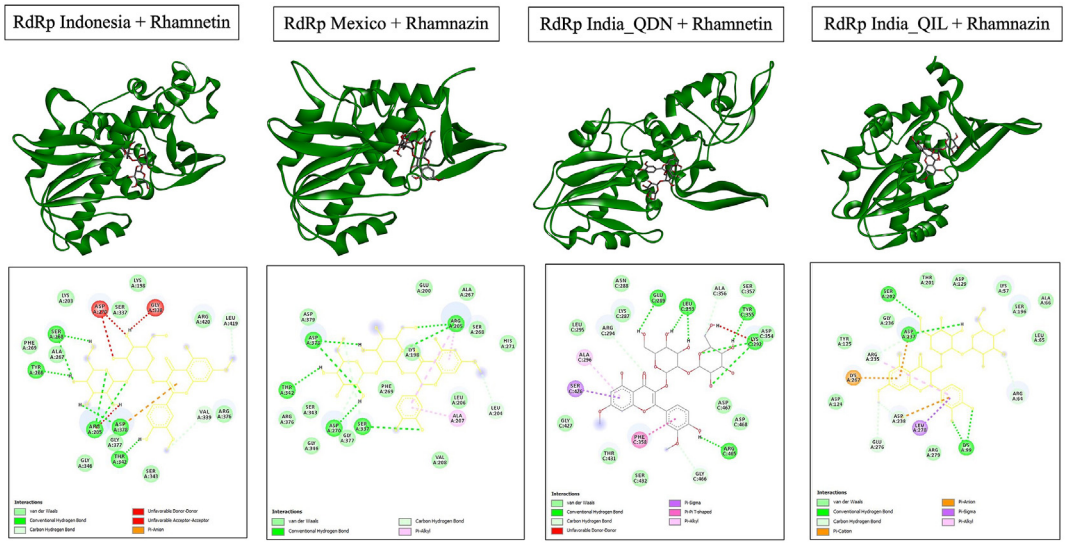

Fig. 3. Visualization of docking results and interaction of RdRp IMNV with watercress N. officinale.

Table 4. The interaction between RdRp IMNV and watercress N. officinale *compounds.*

RdRp sample Origin	Compound	Van der Waals	Interaction Site	
			Hydrogen Bond	Hydrophobic Bond
Indonesia	Rhamnetin 3-sophoroside	LYS198, LYS203, ALA 267, PHE269, SER337, SER343, GLY337, GLY346, ARG376, ARG420	ARG205, TYR266, SER268, THR342, ASP378, VAL339, LEU419	–
Mexico	Rhamnazin 3-sophoroside	LYS198, GLU200, ALA267, SER268, SER 343, LEU206, VAL208, PHE269, HIS271, GLY346, ARG376, GLY377, ASP379	ARG205, ASP270, SER337, THR342, ASP378, LEU204	ALA207
India_QDN	Rhamnetin 3-sophoroside	LYS287, ASN288, LEU295, ASP354, SER357, GLY427, THR431, SER432, ASP467, ASP468	GLU289, LEU293, TYR355, LYS292, ARG465, ARG294, ALA356, GLY466	ALA296, SER426, PHE358
India_QIL	Rhamnazin 3-sophoroside	LYS57, LEU65, ALA66, TYR125, ASP124, ASP129, SER196, THR201, GLY236, ARG279	LYS99, SER202, ASP237, ARG64, ARG235, ASP238, GLU276	LYS267, LEU278

shrimp cells and replicates their RNA after attaching itself to the receptor-mediated endocytosis. Mutations can occur when the virus replicates. Whenever the virus replicates, sometimes the change or mutation is trivial. Still, whenever the virus changes one or more times, it is referred to as a new variant of the original virus. Once inside the cell, the virus hijacks the host's translational machinery to produce viral proteins and replicate its genomic RNA [5]. The virus encodes a major capsid protein (MCP) and an RNA-dependent RNA polymerase (RdRp). After synthesis, the newly formed positive-sense RNA molecules are packaged into nascent virions, forming mature viral particles [9]. Sequence alignment results of RdRp IMNV samples from Indonesia, Mexico, India_QIL, and India_QDN showed mutations. In a short period, de novo diversity is produced by viruses as they adapt to new hosts and environments.

In contrast to viral DNA, viral RNA mutates more quickly [27]. IMNV is a double-stranded (ds) RNA virus [6]. Compared to other DNA viruses, this enables the virus that infects these shrimp to mutate more quickly. On the other hand, the use of drugs facilitates the virus' evolution and resistance [28]. Reverse transcriptase, an error-prone enzyme found in viruses, enables RNA viruses to potentially amass genetic mutations, enhancing their ability to adapt within the host. This poses a greater difficulty in creating effective antiviral treatments for RNA

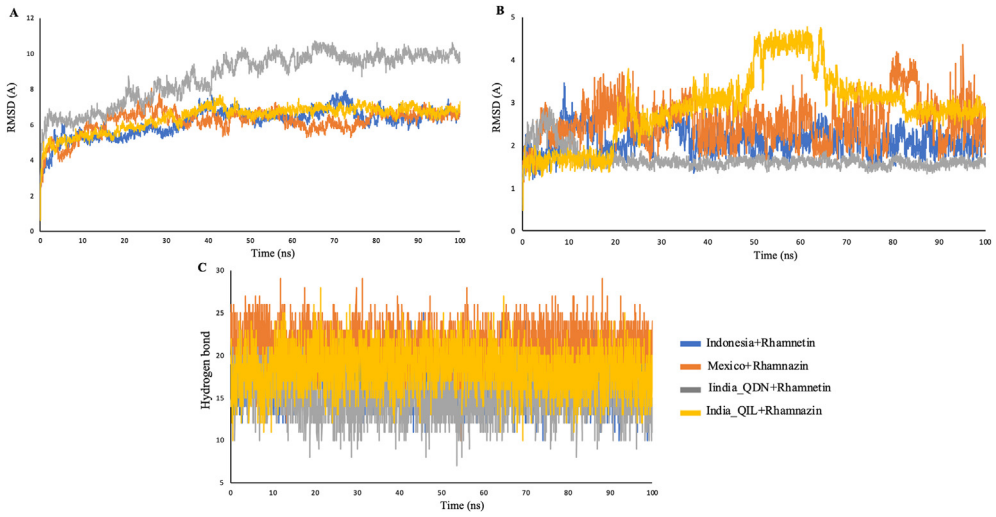

Fig. 4. The structural dynamics of RdRp proteins, ligands, and the interaction among them. A) RMSD of atom backbone. B) RMSD of ligand structure. C) Number of hydrogen bonds of the RdRp protein.

viruses [29]. Furthermore, mutations in RdRp can affect factors such as viral load, virulence, and the accuracy of the replication process [30].

The position of the α-helices and β-sheets in the RdRp protein structure in several countries show the difference in RdRp protein structure between samples from Indonesia, India_QDN, and India_QIL. Mutations, changes in the number of amino acids, and different IMNV virus variants can influence changes in protein structure. Mutations in the virus, such as amino acid substitutions, insertions, or deletions, can lead to changes in the protein sequence, potentially affecting the virus's properties, including its infectivity, transmissibility, and antigenicity [31]. Mutations in viral structural proteins, which can affect viral assembly, pathogenesis, and interactions with the host, are often associated with the emergence of new viral variants [32]. The difference in binding affinity values for four RdRp proteins from several countries is due to differences in amino acid and amino acid structure. The binding affinity of RdRp to various compounds reveals that the P323L mutation does not alter the enzyme's catalytic activity but affects its conformation and substrate binding [33]. A higher frequency of RdRp mutations leads to increased mutation rates and altered binding affinity [34].

The RMSF determines the fluctuation of an atom or group of atoms during the simulation, which is generally used to check the flexibility of residues within a protein during the simulation [35]. The RMSF of RdRp protein showed fluctuations. During the simulation, the protein residues showed relatively small fluctuations. The few elevated peaks were mostly seen within the loop regions in various states, signaling greater variability within the loop compared to the structured areas. The increased residues are not involved in protein-ligand interactions, so those instabilities may not impact the stability of the RdRp structure. RMSF can be used to assess the impact of mutations on protein structural stability, as changes in RMSF values can indicate changes in protein flexibility and strength [36].

The root-mean-square deviation (RMSD) depends on the number of aligned residues [37] to predict the coordinates of the structure protein model correlation with the reference structure [38]. Docking software can forecast empirical orientations, yielding root-mean-square deviations (RMSD) that typically range between 1.5 and 2 Å [39]. The RMSD value of RdRp protein samples from Indonesia and Mexico is lower than those from India_QDN and India_QIL, which is close to zero. This indicates the similarity of the RdRp protein structure between Indonesia and Mexico. RMSD scoring is a technique to determine the accuracy of the protein structure alignment method. RMSD is a reliable indicator of structural similarity during the simulation. As the molecular system approaches equilibrium, protein structures generally stabilize around an average stable conformation, forming a plateau in the RMSD values. A low value of RMSD indicates very high accuracy, and vice versa [35].

The binding affinity value of a ligand needs to be analyzed when carrying out the docking process. Binding affinity is the bond energy required to form a protein-ligand complex affected by the protein-ligand Field's total binding energy [40]. The binding affinity showed the highest affinities to the RdRp viral target. The lowest ligand binding affinity

values are characterized by an increasingly negative value. It indicates a good level of stability of the ligand and protein bonds. Thus, the bonds formed are stronger and maximize the inhibitory activity. The low affinities of other compounds indicate that they do not interact effectively with this viral target [41]. Affinity plays a crucial role in establishing potency. The degree of attachment between a ligand and its receptor characterizes the strength of its interaction [42].

Molecular docking predicts a ligand's binding mode and affinity to a target protein. The interaction between molecular docking and the target protein is significant in drug discovery and structural biology [43]. Molecular docking serves as a tool for pinpointing prospective drug contenders and unraveling the dynamics of protein-ligand interplay. Successful binding of a compound to the target protein signifies its indication as a viable drug candidate. This implies that the compound is predicted to have the potency to inhibit the viral activity of the RdRp protein of IMNV. The accuracy and reliability of molecular docking predictions depend on the target protein structure's quality, the docking algorithm's choice, and the scoring function used to evaluate the binding affinity of the ligand to the protein [44].

The interactions of the amino acid residues show that van der Waals forces are relatively weak attractions due to permanent or induced molecular polarity. In particular, vdW forces contribute more to the binding of covalently bonded benzene than when benzene is physisorbed [45]. Hydrogen bonding involves the interaction of covalently bonded hydrogen atoms with electronegative atoms [46]. Hydrophobic interactions are widespread in water-based biological and technological contexts, occurring between nonpolar substances as a potent and frequently extended-range attraction. The strength of hydrophobic interaction significantly influences the adherence of two interacting surfaces [47]. There are similarities and differences in the intramolecular interactions between samples from Indonesia and samples from Mexico such as van der Waals bonds with residues ALA 267, SER343, and ARG376 and hydrogen bonds with residues ARG205, THR342, and ASP378. The differences in intramolecular interaction in the four RdRp proteins of IMNV are influenced by mutations in the RdRp protein of IMNV. The mutation occurring in the RdRp protein alters the protein's stability and flexibility and disrupts its internal interactions with adjacent molecules [48].

Molecular dynamics simulations provided evidence of stable binding of the compounds to exert inhibitory activity on the IMNV virus (Fig. 4). Rhamnetin and rhamnazine also formed hydrogen bonds, which helped stabilize the interaction [49]. The structure of the RdRp protein is still stable after interacting with rhamnetin and rhamnazin as indicated by the RMSD backbone value, ligand structure, and number of hydrogen bonds. Therefore, the stability of the protein-ligand and its interaction makes it possible to inhibit the replication of IMNV genomic RNA associated with viral infection.

This study suggests that rhamnetin and rhamnazin have promising antiviral properties with their ability to inhibit the activity of RdRp protein, which is involved in IMNV infection. Nevertheless, these in silico forecasts must be approved utilizing in vitro and in vivo approaches. The investigation of watercress compounds, rhamnetin and rhamnazin, as an antiviral agent, will be a breakthrough in shrimp antiviral research.

5. Conclusion

Polymorphisms of amino acids from several countries affected the structure of the RdRp protein of IMNV. The structure of the RdRp IMNV protein from Indonesia is close to Mexico, as well as the structure of the protein from India_QDN, which is related to India_QIL. The bioactive components in watercress *N. officinale*, specifically, rhamnetin and rhamnazin, are promising antiviral agents against IMNV. Furthermore, rhamnetin and rhamnazin have shown stable binding to RdRp protein that is possible to inhibit the replication of IMNV genomic RNA associated with viral infection. This indicates its capacity to inhibit viral activity across multiple countries.

Ethical statement

Ethical approval of this study was obtained from the Health Research Ethics Commission of the Faculty of Medicine, Brawijaya University, with approval number 315/EC/KEPK/11/2023.

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