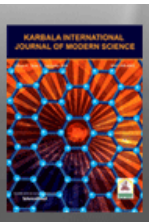
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Abstract

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Keywords

Aquaculture, Immune receptor, LC-HRMS, Rutin, Viral disease

Authors

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RESEARCH PAPER

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Abstract

The viral disease affects the whiteleg shrimp (*Litopenaeus vannamei*) and causes losses. Immunostimulants are recognized as a more environmentally friendly approach to disease management and antimicrobial properties of aquatic organisms. Watercress (*Nasturtium officinale*) is an aquatic plant species that contains antioxidant properties that can protect the organism from disease and boost the immune system. Shrimp rely heavily on innate immunity to combat infectious agents due to their absence of adaptive immunity. Peroxinectin and scavenger receptor class B (SRB) play essential roles in the shrimp defense system. This research employs a bioinformatic to assess the immunostimulatory potential of watercress by targeting peroxinectin and SRB in *L. vannamei*. Based on LC-HRMS analysis, the best value > 90% M/Z of watercress was identified in 7 compounds. Protein homology modeling showed a large number of amino acid residues in the favored region, peroxinectin at 95.12% and SRB at 99.40%. Rutin, one of the 7 identified compounds, has the lowest value of binding affinity, peroxinectin at −9.8 (kcal/mol) and SRB at −7.1 (kcal/mol). Rutin interaction with receptor proteins forms the most hydrogen bonds among other compounds. The RMSF value of both receptor proteins remained below 2.5 Å, implying the sustained stability and cohesion of the ligand targets throughout the simulation. The bioactive compound of watercress *N. officinale*, especially rutin, has shown stable binding to peroxinectin and SRB. This indicates that the compound is potentially an immunostimulant to activate receptor protein, peroxinectin, and scavenger receptor class B and combat viral infection.

Keywords: Aquaculture, Immune receptor, LC-HRMS, Rutin, Viral disease

1. Introduction

Shrimp culture has contributed to economic income. On the contrary, shrimp viruses such as Infectious Myonecrosis Virus (IMNV), White Spot Syndrome Viruses (WSSV), and Taura Syndrome Virus (TSV) are major risks to the sustainability of the shrimp industry, causing vast economic losses and putting people's income at risk [1]. Shrimp have a weak immune response. The shrimp immune system is naturally defended by its innate immune system, which is essential in combating a diverse array of invasive organisms [2].

Shrimp, like all crustaceans, primarily rely on innate immunity to the absence of adaptive immunity. The immune display of this animal also

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includes two important proteins known as peroxinectin and Scavenger Receptor class B (SRB) that work in the shrimp defense system [3]. Peroxinectin is a type of C-type lectin that binds to bacterial cell wall structures and functions as an opsonin. Peroxinectin functions as a PRR and activates the IMD pathway, an important shrimp component in the defense system [4]. Activated peroxinectin, on the other hand, hastens the degranulation and opsonization of emerging pathogens and enhances hemocyte encapsulation or phagocytosis [5]. The ability of scavenger receptor class B to recognize pathogen-associated molecular patterns (PAMPs) and signal pathways for Antimicrobial Peptides (AMPs) as humoral immune effectors is crucial to invertebrate innate immunity [6]. It performs the function of actively destroying microbes [7]. Scavenger receptor class B from shrimp serves as key recognition and signaling receptors for the immune deficiency (IMD) pathway, protecting against microbial pathogens [4]. It binds to a wide variety of ligands, such as viruses and bacteria [8].

Immunostimulants are more eco-friendly means of disease control that also have effects on the growth, viability, and antimicrobial characteristics of aquatic species. In aquaculture, certain plants can also be used in the immunoprophylaxis [9]. *Nasturtium officinale,* or watercress, belonging to the family of aquatic plants, is rich in polyphenolic compounds such as phenolics and flavonoids, which possess antioxidant activity [10,11]. Antioxidant activity may be useful to enhance disease resistance in an organism and improve the immune system [12,13]. Watercress has a significant effect on the immune system, particularly its anti-inflammatory action [14].

In the realm of modern drug discovery and development, bioinformatics techniques have emerged as powerful tools for predicting and analyzing the interactions between bioactive compounds obtained from plants and immune proteins [15]. They have grown simpler and now appear faster as software focused on proteins is rapidly developed. The in silico approach provides a cost-effective and time-saving means to pinpoint potential immunostimulants that specifically interact with immune proteins, thereby transforming the realm of immunopharmacology. This technique has applications in drug design in that it enables the prediction of drug–protein interactions and the effect of these drugs on biological pathways and functions [16].

While advancements have been made in comprehending the functions of peroxynectin and scavenger receptor class B in shrimp immunity, there is still much to uncover about how these proteins interact with other elements, like bioactive compounds from plants. This study uses a bioinformatics approach aims to assess the immunostimulatory potential of watercress (*N. officinale*) by targeting peroxincetin and scavenger receptor class B in whiteleg shrimp (*Litopenaeus vannamei*) to combat viral infection.

2. Material and methods

2.1. Sample preparation of watercress

Watercress *N. officinale* was obtained from the farm at Tawangargo Village, Malang District, East Java Province, Indonesia. The initial stage, watercress extraction, is carried out before LC-HRMS analysis. Watercress extraction aims to remove unwanted substances that may interfere with and improve the quality of analysis [17]. The method used in the extraction is maceration using methanol solvent p.a. about 200 g of watercress simplicial is mixed with 800 ml of methanol (1:4) and left for 24 h at room temperature. The maceration results were filtered using the Whatman filter paper. The solution is then evaporated using a rotary evaporator.

2.2. Liquid chromatography high-resolution mass spectrometry (LC-HRMS) analysis

A liquid chromatography high-resolution mass spectrometry (LC-HRMS) analysis was performed at the central laboratory of life sciences, Brawijaya University. In this experiment, the sample of watercress extract was put into an auto-injector before being put into the LC-HRMS tools, which consist of Thermo Scientific Dionex Ultimate 3000 RSLCnano and a micro flow meter. The separation of the compounds was done using a two-component mobile phase; component A is 0.1% aqueous formic acid, and component B is 0.1% acetonitrile containing formic acid, and a Hypersil GOLD aQ analytical column (50 mm × 1 mm. 1.9 μm). Forty microliters per minute was an analytical flow rate that was implemented during separation, and 40 °C was the column temperature held for the duration of the analytical procedure. The total run time is estimated to be 30 min, and full scans carried a resolution of seventy thousand, while data-dependent analyses were carried out at seventeen and a half thousand resolution. Finally, the resulting data was processed using Compound Discoverer software version 3.2 from Thermo Scientific, including the mzCloud MS/MS library [18].

2.3. Protein homology modeling

Peroxinectin and scavenger receptor class B protein sequences were obtained from the

UniProt database (https://www.uniprot.org/) with ID accession number A0A3R7Q0J4 for Peroxinectin and A0A423TW99 for scavenger receptor class B. Homology modeling was conducted using the Swiss Model web server (https://swissmodel.expasy.org/). Swiss-Model is an online tool focused on automatically creating 3D protein structure models by comparing sequences [19]. Users can input amino acid sequences to generate models through its web interface. The platform automates template selection, alignment, and model-building processes. The quality of the protein's 3D structure was assessed by examining the Ramachandran plot within the Biovia Discovery Studio 2019 software.

2.4. Molecular docking

Molecular docking was used to find possible immunostimulating substances present in watercress. Selection of the most favorable position for the ligand, which has the least amount of binding energy, the ideal configuration was identified. Accurate protein-ligand docking was performed using Autodock Vina Tools [20].

The experiments we did were based on computation, so statistical analysis is included in the tools used. The working principle of this tool is the searching method and scoring function. The results obtained are derived from the computational process of the Autodock Vina. For this procedure, peroxynectin and SRB proteins acted as target molecules. Finally, the identification of prospective ligands with immunostimulatory properties was performed using PyRx 8.0 [21]. The binding sites and interactions with ligands and proteins were resolved with the use of Biovia Discovery Studio 2019 software [22].

2.5. Stability of peroxinectin and scavenger receptor class B

The stability of peroxinectin and scavenger receptor class B (SRB) proteins was performed using the CABS-flex software (accessible at http://biocomp.chem.uw.edu.pl/CABSflex2) server. The protein stability evaluation methodology was carried out by maintaining simulation criteria with 50 cycles and 50 frames of trajectories at a temperature of 1.4 with a duration of 10 ns [23]. Fluctuations in amino acid residues were described based on Root Mean Square Fluctuation (RMSF) values. The highest RMSF value signifies higher flexibility, while the lowest value signifies limited system motion during the simulation process.

3. Results

3.1. Phytochemical identification of watercress (N. officinale) using LC-HRMS

Active compounds of watercress were successfully identified using LC-HRMS and selected on best match value > 90% M/Z (Table 1) so that they are ideal for molecular docking. The compounds can be grouped into three secondary metabolites such as flavonoids, alkaloids, and terpenoids.

3.2. Protein homology modeling

The template used to build the peroxinectin protein model is A0A3R7Q0J4.1.A with 100% identity value, while the template used to build the scavenger receptor class B protein model is A0A423TW99.1.A with 100% identity value. The template selection is based on the highest identity value of several templates produced. The resulting 3-dimensional protein structure on the peroxinectin protein builds a total of 822 amino acid residues, while the scavenger receptor class B protein produces 170 amino acids (Fig. 1).

The results of Ramachandran Plots on peroxinectin protein, the number of amino acid residues in the favored region is 95.12%, and the number of amino acid residues in the outlier region is only 0.85%. The results of Ramachandran plots on scavenger receptor class B protein obtained the number of amino acid residues in the favored region of 99.40% and the outlier region of 0.00% (Fig. 2).

3.3. Molecular docking

The results of molecular tethering in this study produce binding affinity and Root Mean Square Deviation (RMSD) values. The RMSD value is the basis used to give an assessment. The conformation chosen is the conformation that has a binding affinity value with RMSD 0 because that value is the best conformation of the tethering of each ligand. The binding affinity values of ligands from the

Table 1. Phytochemical compounds in watercress (N. officinale).

Compounds	Molecular weight (g/mol)	*m/z* cloud Best Match	Molecular Formula
Kaempferol	286.24	99.2	$C_{15}H_{10}O_6$
Rhamnetin	316.26	99.2	$C_{16}H_{12}O_7$
Rutin	610.50	99.1	$C_{27}H_{30}O_{16}$
Hexadecanamide	255.44	98.9	$C_{16}H_{33}NO$
Quercetin	302.23	98.3	$C_{15}H_{10}O_7$
Sinapinic acid	224.21	92.8	$C_{11}H_{12}O_5$
Andrographolide	305.40	91.5	$C_{20}H_{30}O_5$

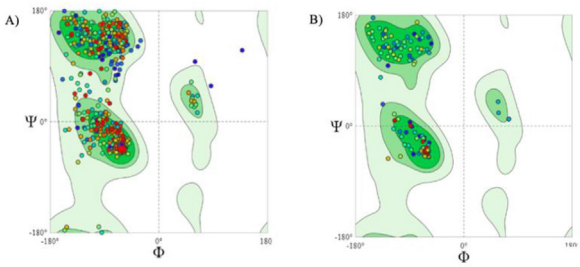

Fig. 1. Protein 3D structure, A) Peroxinectin and B) Scavenger receptor class B.

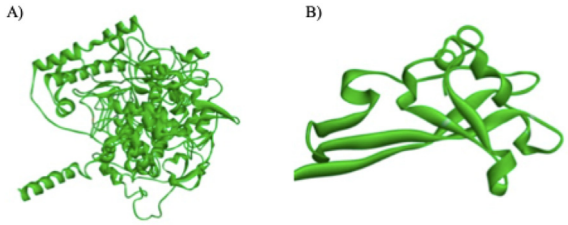

Fig. 2. Results of Ramachandran plots, A) Peroxinectin and B) Scavenger receptor class B.

docking results of watercress *N. officinale* compounds against peroxinectin and scavenger receptor class B are shown in Table 2. The binding affinity value showed that the rutin has the lowest value on both receptor proteins, −9.8 (kcal/mol) for peroxinectin protein, and the binding affinity value for scavenger receptor class B is −7.1 (kcal/mol). The hexadecanamide showed the highest binding affinity after interacting with both receptors, with respective scores of −5.7 (kcal/mol) for peroxinectin protein and −4.7 (kcal/mol) for scavenger receptor class B protein.

3.4. Receptor protein–ligand interaction

The amino acid residue interactions shown between the ligands that have the best affinity values with peroxinectin and scavenger receptor class B are hydrogen bonds, van der Waals, and hydrophobic

Table 2. The value of binding affinity.

Compound	Binding Affinity (kcal/mol)	
	Peroxinectin	Scavenger Receptor class B
Rhamnetin	−8.3	−6.9
Quercetin	−8.6	−6.9
Rutin	**−9.8**	**−7.1**
Kaempferol	−8.1	−6.6
Hexadecanamide	−5.7	−4.7
Sinapinic acid	−6.8	−4.9
Andrographolide	−7.4	−6.5

(Figs. 3 and 4). The rhamnetin, quercetin, kaempferol, and sinapinic acid bind to the active side of the peroxinectin, forming two hydrogen bonds. In contrast, Hexadecanamide and Andrographolide formed a hydrogen bond. On the other hand, the interaction between the rutin and peroxinectin formed five hydrogen bonds (Table 3). The results also show that some ligands form bonds at the same residues, such as quercetin and kaempferol, and have the same type of hydrogen bonds at residues PHE550 and GLU553 when they bind to the peroxinectin. The rhamnetin, quercetin, and kaempferol, when binding to the peroxinectin, form van der Waals bond types at residues ARG450, VAL165,

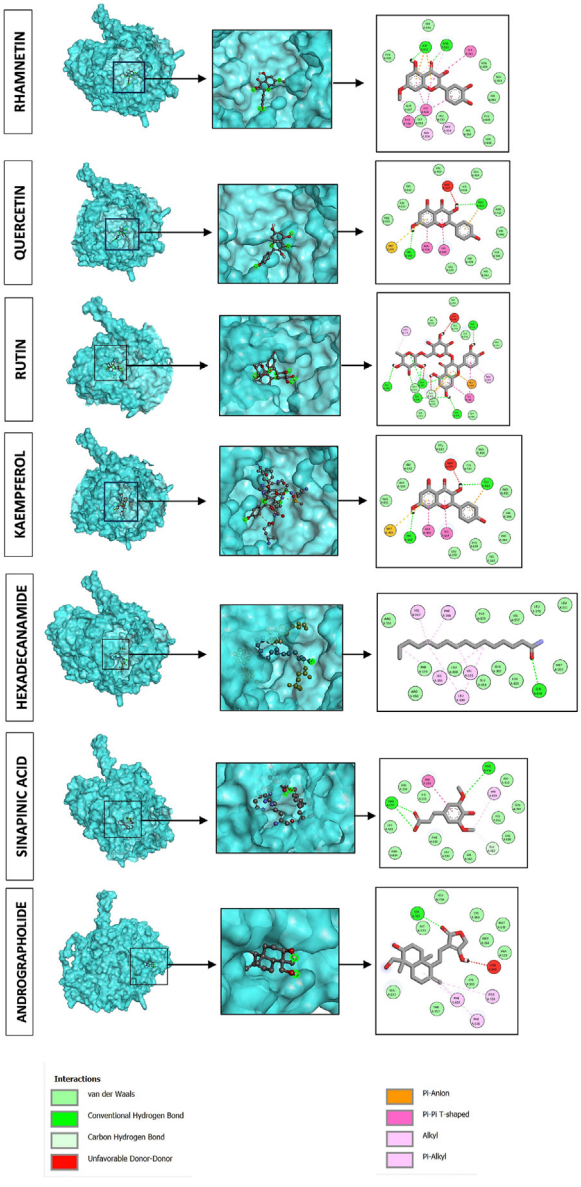

Fig. 3. Visualization of molecular docking of ligands with peroxinectin.

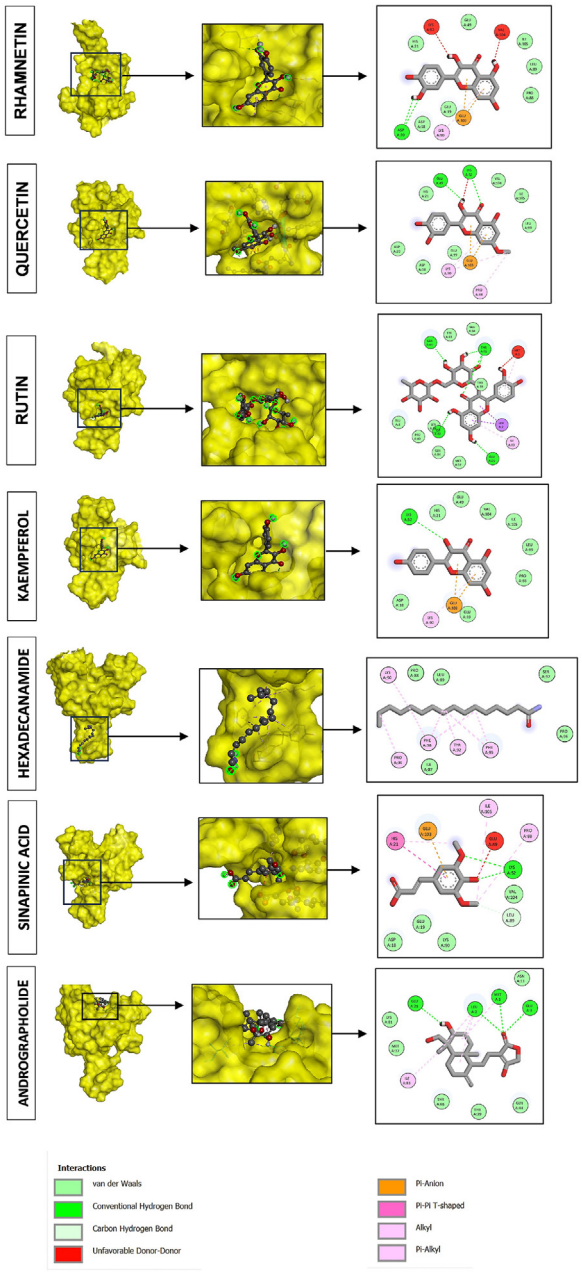

Fig. 4. Visualization of molecular docking of ligands with scavenger receptor class B.

PHE619, HIS162, LEU630, GLY553, and hydrophobic bond types at residues MET303, ALA306, HIS554 (Table 3).

The bonds formed when the scavenger receptor class B interacts with the rhamnetin are two hydrogen bonds, while the quercetin, kaempferol, and sinapinic acid form a hydrogen bond. On the other hand, the interactions between rutin and scavenger receptor class B form 4 hydrogen bonds, likewise the interaction between andrographolide and scavenger receptor class B. However, Rutin has a higher number of van der Waals and hydrophobic bonds than andrographolide. Rutin also has the lowest binding affinity value compared to andrographolide and other compounds. In scavenger receptor class B, the ligands that form the same residue are rhamnetin and kaempferol, which produce a type of hydrogen bond at residue LYS52. The ligands rhamnetin, quercetin, and kaempferol produce the same van der Waals bond type at residues HIS21, ILE105, GLU19, ASP18, and hydrophobic bond type at residues GLU103 and LYS90 (Table 4).

3.5. Stability of peroxinectin and scavenger receptor class B

The stability of protein can be assessed using molecular dynamic simulations. The root mean square fluctuation (RMSF) graph was employed to examine the variations in each residue of peroxinectin and scavenger receptor class B (Fig. 5). Based on the RMSF Result in Fig. 5 shows that almost all the residues are below 2.5 Å. Peroxinectin fluctuated in SER46, PRO100, and ASP567 residues whenever scavenger receptor class B fluctuations occurred in GLN94 and SER170 residues.

4. Discussion

4.1. Phytochemical identification of watercress (N. officinale)

Active compounds that were successfully identified using LC-HRMS were selected based on best match values > 90% *m/z* and have the potential as immunostimulants so that they are ideal for molecular tethering. The active compounds are included in flavonoid, alkaloid, and terpenoid compounds, which have many biological functions. Flavonoids themselves have biological functions such as the ability as anticancer, anti-inflammatory, and antibacterial. Flavonoids can also increase immunity and animal resistance to infection [24]. Alkaloids are secondary metabolites that are widely found in many medicinal plants and are the main source of improving the performance of immune function. Alkaloid class compounds found in various plants can be used as efficient and environmentally friendly immunostimulants to enhance the immune system of aquaculture animals. Another secondary metabolite is terpenoids, where the compound can show its therapeutic activity [25]. Terpenoid class compounds have antiviral, antidiabetic, anti-inflammatory, and immunomodulatory activities. The beneficial effects of terpenoids on the immune system mainly occur in antibody production [26].

Table 3. Ligand interaction with residues on peroxinectin.

Ligand	Type of Interaction		
	Hydrogen Bond	Van Der Waals Bond	Hydrophobic bond
Rhamnetin	ASP310, ARG551	ARG450, GLU453, VAL165, PHE619, LEU618, HIS162, LEU630, GLY553, GLN307, PHE550	HIS311, MET303, ALA306, HIS554, PHE166,
Quercetin	PHE550, GLU453	ARG551, ASP310, GLY553, VAL557, GLN454, HIS311, ARG450, VAL165, PHE166, HIS162, PHE619, LEU630	HIS554, ALA306, MET303
Rutin	GLN206, ALA111, PHE379, ASN378, GLN205	LEU373, VAL113, TRP245, GLN253, SER110, PRO257, GLY390, SER372, PRO251, GLU374	ARG112, ARG254, GLU383, HIS381
Kaempferol	GLU453, PHE550	ARG551, ASP310, GLY553, VAL557, HIS311, GLN454, ARG450, VAL165, PHE166, HIS162, PHE619, LEU630	MET303, GLN307, HIS554, ALA306
Hexadecanamide	GLN454	ARG551, PHE579, VAL557, LEU575, LEU561, ARG450, PHE619, LEU618, GLU453, GLN307, LEU614, MET303	HIS162, PHE166, HIS554, LEU630, VAL165
Sinapinic acid	ARG637, ARG450	ASN634, LEU633, PHE318, HIS162, ASP310, GLN307, HIS311, LEU618, GLU453, VAL165, LEU630, ARG551	ASN365, PRO158, PHE624, PHE626
Andrographolide	SER361	GLU332, GLY333, LEU356, CYS363, MET628, MET364, ALA159, CYS330, THR157	ASN365, PRO158, PHE626, PHE624

Table 4. Ligand interaction with residues on scavenger receptor class B.

Ligand	Type of Interaction		
	Hydrogen Bonding	Van Der Waals Bond	Hydrophobic Bond
Rhamnetin	GLU49, LYS52	HIS21, VAL104, ILE105, LEU89, GLU19, ASP18, ASP20	PRO88, GLU103, LYS90
Quercetin	ASP20	HIS21, GLU49, ILE105, LEU89, PRO88, GLU19, ASP18	LYS90, GLU103
Rutin	LYS82, GLN44, THR65, GLU25	MET37, GUN84, PRO40, SER38, GLU4, TYR43, GLU64, THR39	LEU2, ILE83
Kaempferol	LYS52	HIS21, GLU49, VAL104, ILE105, LEU89, PRO88, GLU19, ASP18	LYS90, GLU103
Hexadecanamide	–	PRO88, LEU89, SER97, PRO96, ILE87	LYS90, PHE95, TYR92, PHE98, PRO34
Sinapinic acid	LYS52	ASP18, VAL104, LEU89, LYS90, GLU19	HIS21, GLU103, ILE105, GLU49, PRO88,
Andrographolide	GLU25, LEU2, MET1, GLU3	MET37, LYS81, ASN11, GLN44, THR39, THR65	ILE83

4.2. Protein homology modeling

The template used in peroxinectin and scavenger receptor class B has a high identity value of 100%. Such a high value can improve the quality of the resulting model, which is highly dependent on the correctness of the identified template and the accuracy of the alignment produced. An identity value of >50% results in an accurate model, while an identity value of <40% results in an error-prone model that requires improvement to the side chains and loops to improve the modeled protein [27].

The quality of protein structure depends on the number of residues in the favored and disallowed regions in Ramachandran Plots. A large number of amino acid residues were obtained in the favored region of both receptor proteins, peroxinectin and scavenger receptor class B, implying the good quality of protein structure. The greater the percentage of amino acid residues in the favored region, the quality of the protein structure is better [28]. The quality of the protein structure can be said to be good if the percentage of amino acid residues in the favored region is >90% [29]. The Ramachandran Plot results also show the number of amino acid residues in the outlier region in the two proteins is very small, about 0.85% in the peroxinectin protein. On the other hand, in the scavenger

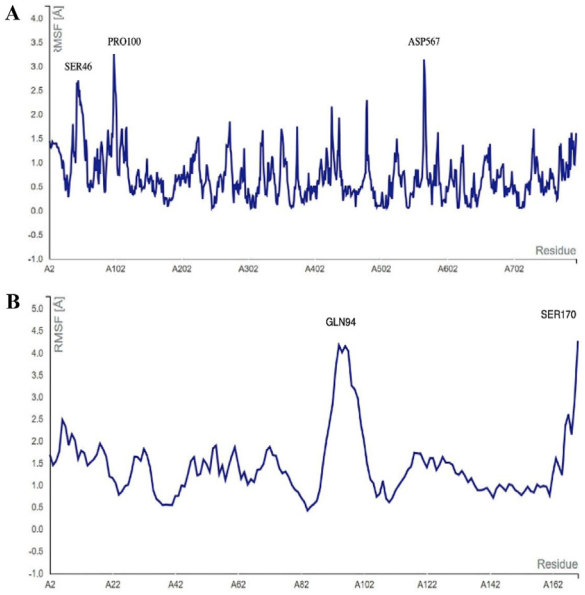

Fig. 5. The value of RSMF, A) Peroxinectin and B) Scavenger receptor class B.

receptor class B protein, it is 0.00%. So, there are very few errors in the structure of the two proteins. The Outlier region indicates an amino acid region that has an unfavorable and less desirable dihedral angle in the protein structure. This can be caused by errors during data processing that cause changes in the protein structure, thus changing the protein activity [30].

4.3. Molecular docking

Molecular docking in this study produces data in the form of binding affinity and Root Mean Square Deviation (RMSD) values. The selected conformation is the conformation that has a binding affinity value with RMSD 0 because that value is the best conformation of the tethering of each ligand. An RMSD value of less than 2 Å is a good value because it indicates a match between the ligand and the active side of the protein [31]. The binding affinity between ligand and protein is one of the main selection criteria in drug discovery, and generally, the molecule should bind tightly to the target protein [32].

The results of molecular tethering between the Rutin test ligand and both receptor proteins showed the lowest binding affinity values of −9.8 (kcal/mol) at the peroxinectin receptor and −7.1 (kcal/mol) at the scavenger receptor class B. The highest binding affinity values were shown between the interaction of the test ligand hexadecanamide with both receptors, namely −5.7 at the peroxinectin and −4.7 (kcal/mol) at the scavenger receptor class B. Binding affinity (ΔGbind) is the energy required by the ligand when interacting with the receptor protein binding site. The smaller the value (ΔGbind), the more stable the ligand binds to the receptor [33]. The rutin test ligand is the most potential ligand to be used as a drug because it has the smallest binding affinity value, so it shows a stable bond when interacting with the receptor protein. The ligand also has a very strong interaction with the receptor protein.

Rutin has the largest molecular weight (MW) value and the highest number of hydrogen bonds compared to the other seven compounds. The magnitude of the MW value of a compound will affect the molecular size and the number of atoms participating in a bond. The large molecular size value and chemical characteristics of a compound will affect the donor and acceptor of hydrogen bonds. So, it will produce more hydrogen bonds when interacting with proteins [34]. The hydrogen bonds in proteins stabilize their complex three-dimensional structures. Carbon-centered hydrogen bonds in proteins help bind substrates and play a role in C−H bonding activity through the bond metathesis [35].

The binding affinity value indicates the energy required for the ligand to bind to the receptor protein. A low binding affinity value indicates that the bond between the ligand and the receptor protein is stronger because the ligand only requires low energy to bind to the receptor protein [36].

4.4. Receptor protein−ligand interaction

The types of bonds resulting from the interaction between the test ligands with peroxinectin and scavenger receptor class B are hydrogen bonds, van der Waals bonds, and hydrophobic bonds. Hydrogen bonding is the most crucial bond when looking for the active side of the receptor protein. This is because proteins are composed of NH and OH groups that can donate H bonds and other groups that accept them, so hydrogen bonds aid the interaction between proteins and ligands by stabilizing the ligand in the binding pocket [37]. The interaction between the rutin with the peroxinectin and scavenger receptor class B was the interaction that generated the most hydrogen bonds. The bonds generated between the peroxinectin and the rutin were five hydrogen bonds, while the interaction between the scavenger receptor class B and the rutin resulted in 4 hydrogen bonds. On the other hand, The interaction between Scavenger receptor class B and andrographolide formed 4 hydrogen

bonds. But these interactions formed van der Waals and hydrophobic bonds less than the Scavenger receptor class B and Rutin. This proves that rutin can form hydrogen bonds, which are very important in the stability between the ligand and the receptor protein with good binding affinity so that it has the potential to be used as a drug.

The resulting bonds other than hydrogen bonds are van der Waals bonds and hydrophobic bonds. van der Waals bonds formed on peroxinectin and scavenger receptor class B occur due to the presence of two adjacent atoms, resulting in a change in the electron charge distribution around the atom. This change in atomic charge distribution causes a force of attraction or repulsion called van der Waals bond. van der Waals bonds play a role in maintaining stability between the receptor protein and ligand during the binding process, but this interaction is weaker than ion and hydrogen bonds [38]. Hydrophobic interactions are interactions that have properties away from the liquid environment and tend to cluster within the globular structure of the protein. Hydrophobic interactions also play a role in determining the stability of the ligand to the receptor protein [39]. The formation of hydrophobic bonds will minimize the interaction of non-polar residues with water. The intensity of these interacting bonds is very important in evaluating the affinity between the protein and the ligand [40]. The interaction results also show that some ligands form bonds to the same residues in the peroxinectin and scavenger receptor class B. Interactions between the same amino acid residues with different ligands can strengthen the bond between the ligand and the protein to increase the stability of the protein [41]. Tyrosine, glycine, serine, and arginine are key amino acids in the quantitative relationship between specific amino acids and binding affinity because they have a strong correlation with binding affinity [42].

4.5. Stability of peroxinectin and scavenger receptor class B

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 [43]. The RMSF for peroxinectin and scavenger receptor class B proteins has lower fluctuation in binding site residues. During the simulation, the protein residue exhibited relatively minor fluctuations. Elevated peaks were predominantly noticed within the loop regions across various countries, signifying greater variability in loops compared to structured regions. Additionally, the overall measurements remained below 2.5 Å, further implying the sustained stability and cohesion of the ligand targets throughout the simulation. RMSF can be used to assess protein structural stability, as changes in RMSF values can indicate changes in protein flexibility and stability [44].

5. Conclusion

Active compounds of watercress *N. officinale* were successfully identified using LC-HRMS with best match >90% M/Z are rhamnetin, quercetin, rutin, kaempferol, hexadecanamide, sinapinic acid, and andrographolide. The bioactive compound of watercress *N. officinale*, especially rutin, has shown stable binding to peroxinectin and SRB. This indicates that the watercress compound has the potential as an immunostimulant to activate receptor protein, peroxinectin, and scavenger receptor class B, improve shrimp immunity, and combat viral infection.

Ethical statement

Ethical approval of this study was obtained from the Health Research Ethics Commission of the Faculty of Medicine, Brawijaya University (No. 315/EC/KEPK/11/2023).

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Conflict of interest

The author declares no conflict of interest.

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