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Bioinformatics Studies of Flavonoid Derivatives Compound from Saga Rambat Leaves as an Antipyretic Candidate

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Abstract

This research is backed by the frequent use of herbal plants in the community, one of which is the saga (*Abrus precatory* L.), which is used to reduce body temperature in the Tirtajaya District, Karawang Regency. Saga leaves contain several secondary metabolites with potential antipyretics, one of which is flavonoids. The study aimed to determine the inhibitory activity of flavonoid compounds of saga leaves as inhibitors of COX-2 receptors and IL-1 receptors that reduce fever. The methods used were pharmacokinetic and toxicity studies, molecular docking, and molecular dynamic simulation. The outcomes of molecular docking experiments with seven flavonoid-derived compounds from saga leaves targeting cyclooxygenase-2 (4PH9) receptors revealed that isohemiphloin compounds exhibited the most favorable Gibbs free energy (ΔG) at -7.08 kcal/mol. In the case of interleukin-1 (5R85), cirsimaritin compounds displayed the lowest Gibbs free energy (ΔG) at -7.78 kcal/mol. The analysis of drug screening results indicates that the best compound adheres to four of the five Lipinski rules. Furthermore, the predictions for pharmacokinetics and toxicity are fairly good, as the best compound demonstrates a favorable pharmacokinetic profile and is determined to be non-toxic. These findings collectively suggest that the isohemiphloin compound from saga leaves may be a promising candidate for developing an antipyretic drug, particularly due to its predicted interaction with the cyclooxygenase-2 (4PH9) receptor.

1. Introduction

Fever, defined as an elevated body temperature above the normal range, is typically caused by an infection or an imbalance between heat production and heat dissipation. This increase in temperature is regulated by the hypothalamus. To reduce fever, antipyretic agents are used, which work by lowering the body temperature to its normal range. These agents include both synthetic drugs, such as paracetamol, and native remedies, like saga leaves, known for their antipyretic properties. Antipyretic drugs are categorized into several groups, namely the salicylate group (e.g., aspirin, salicylamide), the para-aminophenol group (e.g., acetaminophen), and the pyrazoline group (e.g., metamizole).

In the Tirtajaya District, Karawang Regency community, saga leaves can be processed by mashing and boiling. Saga is a climbing plant (*Abrus precatorius* L.) with the synonymous name Abrus frutex Rumph and belongs to the Fabaceae family. Saga plant extract is known to be used as an anti-inflammatory and antipyretic drug, which has been tested in vivo [1]. One of the active compounds in saga plant extract is flavonoids, which can be extracted using polar solvents due to their polar nature [2]. The reactivity of flavonoid compounds in saga leaves towards antipyretic receptors can be determined using molecular docking.

In a study, molecular docking was employed for virtual screening to identify potential antipyretic compounds from saga leaves (*Abrus precatorius* L.). This approach focused on several flavonoid molecules, including hemiphloin, (2 R)-naringenin 6-C-β-D-glucopyranoside, isohemiphloin, hispidulin 4'-O-β-D-glucopyranoside, cirsimarin, hispidulin, and cirsimaritin

[3]. These compounds were analyzed as ligands for the target proteins COX-2 and IL-1, which are crucial in developing fever. Paracetamol was used as a positive control in this research. The molecular docking method was selected for its efficiency and cost-effectiveness, facilitating rapid study progression. The structural-based virtual screening was made possible by the availability of three-dimensional structures of COX-2 and IL-1 in the protein data bank, allowing for precise anchoring of the molecules in the research.

2. Experimental

2.1. Equipment and Materials

The equipment utilized were software and hardware devices. The software was MarvinSketch, AutodockTools 1.5.7, Discovery Studio version 21.1, Molegro Molecule Viewer, Desmond software for academics, and other supporting programs based on online servers such as pkCSM, Protein Data Bank (PDB), PubChem, SAVES, and Lipinski's Rule of Five. The hardware used was a personal computer with the specification of Intel® Core 5i, 8.00 GB of RAM (Random Access Memory), 64-bit operating system Windows 10, and operating system of Linux Ubuntu 18.04.5 LTS. The materials used were seven flavonoid compounds from saga leaves downloaded from PubChem, COX-2 receptor (4PH9), and IL-1 Beta receptor (5R85).

2.2. Procedures

2.2.1. Preparation of Ligand Structure

Seven flavonoid compounds (can be seen in Figure 1) were depicted using Marvin Sketch software. All flavonoid compounds were protonated at pH 7.4 to obtain a structure corresponding to human pH. Geometric optimization was then used to obtain the most stable conformation by obtaining the most stable molecule and the lowest potential energy through Marvin Sketch software [4]. Then, the optimized flavonoid compounds were saved in .GDP file format. Subsequently, these flavonoid compounds were converted into the .pdbqt format using the AutodockTools-1.5.7 software.

2.2.2. Identification of Target Receptor

The receptor profile was analyzed by entering the codes 4PH9 (cyclooxygenase-2) and 5R85 (Interleukin-1Beta) in https://www.ebi.ac.uk/pdbsum. The analysis revealed that the proteins used aligned with the Ramachandran and ERRAT plot parameters. The overall quality factor parameters for the receptor can be seen on the page www.doe-mbi.ucla.edu/errat.

2.2.3. Preparation of Receptors with Native Ligands

The process of receptor preparation began with the isolation of the macromolecular chain responsible for binding to the target. The protein structures of cyclooxygenase-2 (4PH9) and Interleukin-1Beta (5R85) were downloaded from the Protein Data Bank (PDB). To compensate for the missing residue in the 5R85 receptor, receptor modeling was performed using https://swissmodel.expasy.org/. For separating from their native ligands using Molegro Molecular Viewer (MMV) software and saved in the .pdb file format. This separation of native ligands prevents potential inhibition of the test compounds when they interact with the receptor [5].

The native ligand-receptor was cleaned from solvents and other residues using Molegro Molecular Viewer (MMV). Removal of water molecules aims to reduce the inhibitory effect of water molecules on the ligand when interacting with the binding site. The binding site is a macromolecule's active site where the ligand reaction occurs [6].

AutodockTools 1.5.7 software was employed to remove water molecules and add hydrogen atoms. After removing water molecules and separating native ligands, hydrogen atoms were added to the receptor to adjust the docking process to approach the body's pH. This process aims to determine the hydrogen bonds that occur when the ligand interacts with the target receptor [7]. Then, the structures were saved in .pdbqt file format.

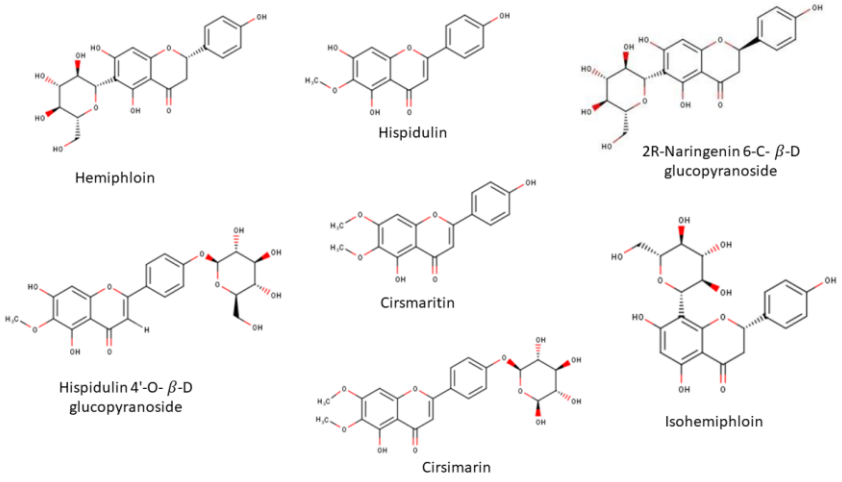

Figure 1. The flavonoid structures of saga leaves

2.2.4. Validation Receptor Docking Method

The validation stage of the docking method employed AutodockTools software to view the RMSD (Root Mean Square Deviation) parameters. The grid parameters of the 4PH9 receptor (grid point size xyz = 40 × 40 × 40, grid central point xyz = 13.578 × 23.024 × 25.205 and grid point spacing = 0.375 Å). The grid parameters of the 5R85 receptor (grid point size xyz = 40 × 40 × 40; grid central point xyz= 39.191 × 2.757 × 73.410 and grid point spacing = 0.375 Å). The RMSD parameter has a requirement of ≤ 2 Å. The smaller the RMSD value, the closer the position of the native ligand resulting from docking is to the native ligand resulting from crystallography [4].

2.2.5. Virtual Screening and Docking Ligand Test Against Target Receptor

Virtual screening was performed using Autodock software to screen flavonoid compounds for their optimal affinity with COX-2 (with grid box parameter X = 13.578, Y = 23.024, and Z = 25.205 Å) and IL-1 Beta receptors (grid box parameter X = 39.191, Y = 2.757, and Z= 73.41 Å). This research employed 20 Lamarckian Genetic Algorithm (LGA) conformations to rank the top-performing flavonoid compounds based on their affinity for proteins, as indicated by Gibbs free energy (ΔG) and inhibition constant (Ki). The ΔG reflects the strength of the interaction between the protein and the ligand. When the ΔG value becomes more negative (lower), the compound requires less energy for binding. This, in turn, indicates that the compound has a higher potential to interact strongly and form stable bonds with the target protein [8].

2.2.6. Molecular Dynamics Simulation

Molecular dynamics simulation is a process that can predict each atom or other movement in a molecular system at any time. This simulation was carried out using Desmond software with several stages, namely receptor and ligand preparation (receptors are prepared by obtaining and refining their 3D structures, including removing extraneous elements and adding missing atoms. Ligands are similarly prepared by acquiring their 3D structures, optimizing geometry, and assigning appropriate charges. The receptor and ligand are then combined, ensuring correct positioning of the ligand in the receptor's active site, followed by a pre-dynamic minimization to rectify any structural anomalies, thus readying the system for the simulation), molecular dynamics simulation and continuing with creating a builder system and determining an orthorhombic box with a buffer of dimensions 10 Å x 10 Å x 10 Å. Then, minimization was carried out using the T1P3P water model and 0.15 M NaCl. Energy minimization was done for 20 ns with a temperature of 300 K and standard pressure (1.01235 bar).

2.2.7. Lipinski's Rule of Five

Lipinski's Rule of Five is an in-silico observation to analyze the similarity of the chemical structure of a drug candidate to be made into an oral dosage form. These rules stipulate that drug molecules must have a relative molecular mass < 500 g/mol, Log P value < 5, bond donor < 5, hydrogen bond acceptor < 10, and molar refractory between 40-130 [9].

2.2.8. Prediction Pharmacokinetics and Toxicity

The pharmacokinetic analysis can predict the ADMET of the test compound and can prevent possible problems from occurring [10]. The pharmacokinetic interaction mechanism is a process that can elucidate the journey of a drug within the body, encompassing stages such as absorption, distribution, metabolism, excretion, and potential toxicity [11]. In this research, predictions regarding the absorption, distribution, metabolism, excretion, and toxicity of flavonoid compounds were conducted using pKCSM, which was accessed on the page www.biosig.unimelb.edu.au/pkcsm/prediction. The analysis involved the assessment of specific parameters, including Caco-2 permeability (>0.90), human intestinal absorption (<30%), medium distribution volume (VDss) (<-0.15), and high (>0.45), Brain Barrier Bload (BBB) Log BB (<-1), and renal OCT substrate.

3. Results and Discussion

3.1. Preparation Ligand Structure

Seven flavonoid compounds were protonated to achieve a state corresponding to blood pH, typically around 7.4. Their conformations were optimized to position the ligand in the most stable configuration, facilitating interaction with the receptor. From these seven conformations, the one with the lowest energy was chosen to advance to the next stage of the study.

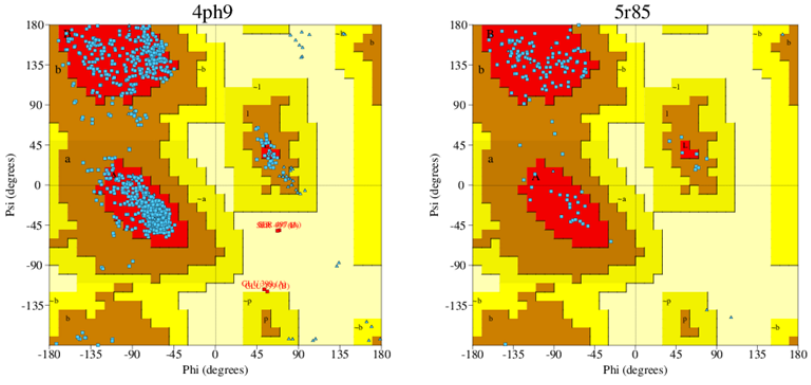

Figure 2. Ramachandran plots of (a) cyclooxygenase-2 (4PH9) receptor, (b) Interleukin-1Beta (5R85) receptor

Table 1. Validation results of receptor docking method

GDP code	Grid box			RMSD (Å)
	X	Y	Z	
4PH9	13.578	23.024	25.205	0.85
5R85	39.191	2.757	73.41	1.81

3.2. Identification Target Receptor

The receptor was analyzed using the Ramachandran Plot and ERRAT (Figure 2) via the www.ebi.ac.uk/pdbsum online server. A receptor is said to be good and stable if it is in the most preferred area >50% and the disallowed area <0.8%.

The analysis of the Ramachandran plot reveals that the cyclooxygenase-2 receptor (4PH9) has a high percentage of amino acids in the most favored regions, specifically 89.4%, with a minimal presence of amino acids in the disallowed regions at 0.4%. Similarly, the interleukin-1 receptor (5R85) demonstrates a robust protein structure, with 88.8% of its amino acids falling within the most favored regions and 0.0% in the disallowed regions. These findings suggest that the cyclooxygenase-2 (4PH9) and interleukin-1 (5R85) receptors possess stable protein structures.

The best model was selected based on the valid q-mean score, using Prochek via www.doe-mbi.ucla.edu/errat. The ERRAT program is a validation tool for assessing the quality of 3D protein structures determined via crystallography. It determines the protein structure's reliability by examining the residues within a defined cutoff distance of 3.5 between different pairs of atoms [12].

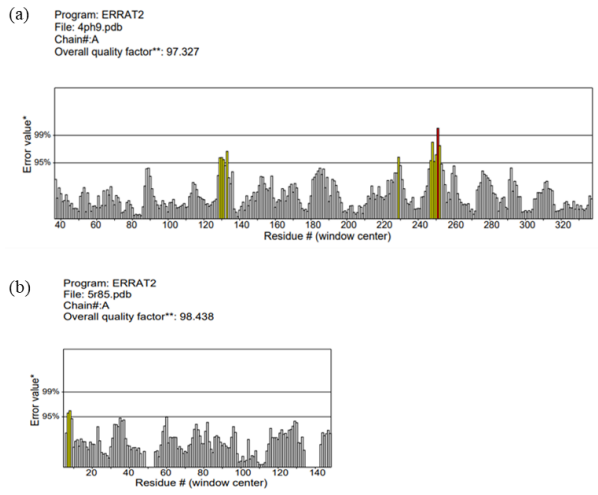

Figure 3. ERRAT analysis results

Based on the analysis of the q-mean score and overall quality factor (Figure 3), the 4PH9 receptor demonstrates an overall quality factor value of 97.327%, while the 5R85 receptor has an overall quality factor value of 98.438%. Hence, both of these receptors have an overall quality factor value within the range of 97%, which is considered acceptable for both receptors.

3.3. Validation of Receptor Docking Method

The docking method was validated using AutodockTools 1.5.7 software. This validation method involved redocking the ligands, including isohemiphloin, hemphloin, cirsimarin, cirsimaritin, hispidulin, hypidulin, 4'-O-β-D glucopyranoside, and 2R-Naringenin 6-C-β-D glucopyranoside, to the active site of the receptor. This method is said to be valid if the mark Root Mean Square Deviation (RMSD) has a condition of ≤2 Å (Angstrom) [4]. Validation results for each receptor show that each RMSD value receptor ≤ 2 Å, so the docking method can be defined to be valid (Table 1). The structured overlay of crystallography and the redocking result can be seen in Figure 4.

3.4. Virtual Screening and Docking Test Against Ligands Target Receptor

Molecular docking experiments involved evaluating the interactions of seven flavonoid compounds with the cyclooxygenase-2 receptor (4PH9) and the interleukin-1 receptor (5R85). Among these compounds, only one emerged as the best candidate for reducing fever and exhibited the strongest interaction with the cyclooxygenase-2 receptor (4PH9), which was the isohemiphloin compound. Similarly, for the interleukin-1 receptor (5R85), the most promising interaction was observed with the cirsimaritin compound. The detailed results of the molecular docking experiments using Autodock can be found in Table 2.

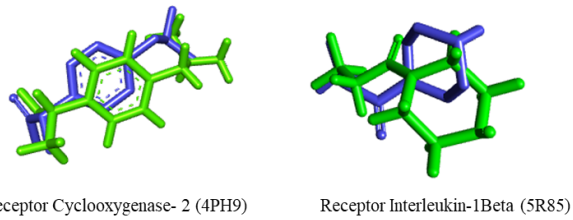

Figure 4. The structure overlay of crystallography and redocking result

Table 2. Results of molecular docking of flavonoid compounds against cyclooxygenase-2 (4PH9) receptor and Interleukin-1Beta (5R85) receptor using Autodock tools

Receptor	Compound	Binding energy (kcal/mol)
4PH9	Isohemiphloin	-7.08
5R85	Cirsimaritin	-7.78

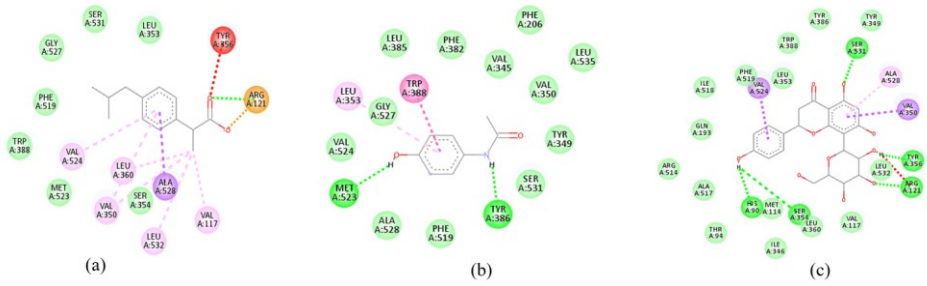

Figure 5. The 2D visualization of (a) native ligands, (b) paracetamol, and (c) isohemiphloin at the 4PH9 receptor

3.5. Visualization of Docking Results

The visualization of docking results was carried out using Discovery Studio software, and the results are presented in Figures 5 (2D 4PH9), 6 (3D 4PH9), 7 (2D 5R85), and 8 (3D 5R85). According to Table 3, the affinity between the ligands and the receptor can be assessed. The best flavonoid compounds exhibit ΔG values lower than the reference antipyretic on the market, paracetamol. For the 4PH9 receptor, the isohemiphloin compound demonstrates a ΔG value of -7.08 kcal/mol and a Ki value of 6.45 µM, whereas paracetamol has a ΔG of -5.20 kcal/mol and a Ki value of 154.84 µM.

Similarly, for the 5R85 receptor, the cirsimaritin compound displays a ΔG value of -7.78 kcal/mol and a Ki value of 1.98 µM. Paracetamol exhibits a ΔG of -5.46 kcal/mol and a Ki value of 98.89 µM. These results suggest that isohemiphloin and cirsimaritin have a more stable interaction and greater potential as antipyretic drugs than the market-available compound paracetamol. This is primarily due to their lower ΔG and Ki values, indicating their potential for stronger and more effective binding to the respective receptors.

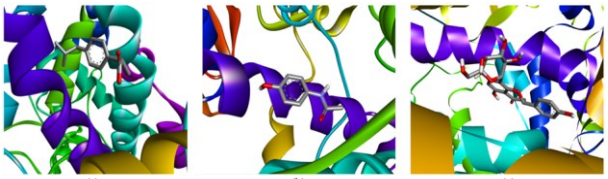

Figure 6. The 3D visualization of (a) native ligands, (b) paracetamol, and (c) isohemiphloin at the 4PH9 receptor

In the case of the interaction between cyclooxygenase-2 receptor (4PH9) with native ligands, the comparator (paracetamol) and the test compound (isohemiphloin) showed that there is interaction between native ligands with amino acid residue with 15 bonds (as in Table 3) and those without own bond hydrogen. Then, the paracetamol with amino acid residue has 16 bonds and a total of 2 bonds hydrogen (Met523 and Tyr386). Then, on the test ligand (isohemiphloin) with residue, amino acids have 23 bonds and a total of 5 bonds hydrogen (His90, Ser354, Tyr356, Arg121, and Ser531).

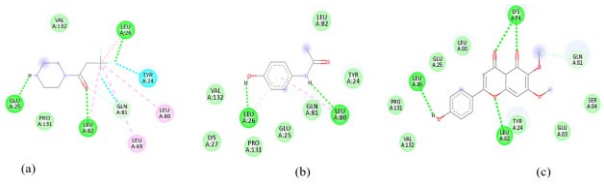

Figure 7. The 2D visualization of (a) native ligands, (b) paracetamol, and (c) isohemiphloin at the 5R85 receptor

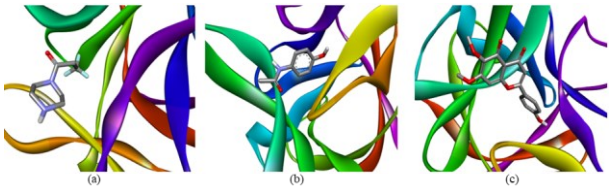

Figure 8. The 3D visualization of (a) native ligands, (b) paracetamol, and (c) isohemiphloin at the 5R85 receptor

Table 3. Analysis of molecular docking results of paracetamol and compounds selected for cyclooxygenase-2 receptor

Compound	ΔG (kcal/mol)	Ki (µM)	Hydrogen bond	Hydrophobic bond
Native ligands	-7.08	6.49	-	Leu353, Ser531, Gly527, Phe519, Trp388, Met523, Ser354, Val524, Leu360, Val350, Leu532, Val117, Ala528, Tyr356, Arg121
Paracetamol	-5.20	154.84	Met523, Tyr386	Phe206, Leu535, Leu385, Phe382, Val345, Val350, Tyr349, Ser531, Phe519, Ala528, Val524, Gly527, Leu353, Trp388
Isohemiphloin	-7.08	6.45	His90, Ser354, Tyr356, Arg121, Ser531	Tyr349, Tyr386, Trp388, Leu353, Phe519, Ile518, Gln193, Arg514, Ala517, Thr94, Ile346, Met114, Leu360, Val117, Leu532, Val524, Val350, Ala528

Table 4. Analysis of molecular docking results of paracetamol and compounds selected to interleukin-1 receptor

Compound	ΔG (kcal/mol)	Ki (μM)	Hydrogen bond	Hydrophobic interaction
Native ligands	-4.79	305.78	Leu26, Leu82, Glu25, Gln81	Val132, Tyr24, Leu80, Leu69, Pro131
Paracetamol	-5.46	98.89	Leu 26, Leu80	Leu82, Tyr24, Gln81, Glu25, Pro131, Lys27, Val132
Cirsimaritin	-7.78	1.98	Leu26, Lys74, Leu82, Gln81	Leu80, Glu25, Pro131, Val132, Tyr24, Glu83, Ser84

In the interaction study involving the interleukin-1 receptor (5R85) with native ligands, paracetamol, and the test compound cirsimaritin, it was observed that the native ligands formed nine bonds with the amino acid residues of the receptor, including a total of four hydrogen bonds (Leu26, Leu82, Glu25, and Gln81) (Table 4). Then, the paracetamol with amino acid residue has nine bonds and a total of 2 hydrogen bonds (Leu 26 and Leu80). Then, on the test ligand (cirsimaritin) with amino acid residue has 11 bonds and a total of 4 bonds hydrogen (Leu26, Lys74, Leu82, and Gln81). Leu26 also interacts with hydrogen bonds with the native ligand or paracetamol.

3.6. Molecular Dynamics Simulation

Molecular dynamics simulation is a process that can predict each atom or other movement in a molecular system at any time. A protein molecule can be considered stable if it shows protein stability because it can maintain a position bound with ligands. Whereas enhancement RMSD fluctuations are too tall, the bond of released proteins exists so protein can be denatured. RMSF (Root Mean Square Fluctuation) graph can be used to assess the stability of interactions between ligands and amino acid residues.

The RMSD graph (Figure 9) shows the RMSD results of the molecular dynamics of the 4PH9 receptor ligand, the compound isohemiphloin, and paracetamol. The compound isohemiphloin displays stability throughout the molecular dynamics simulation spanning 12-20 nanoseconds. Based on the RMSD graph, it can be inferred that during the 20 ns molecular dynamics simulation, isohemiphloin exhibits the most stable interaction compared to native ligands and reference compounds. This result is supported by a mean value of RMSD during 20 ns simulation (Table 5).

Based on Figure 10, the three 4PH9 ligand-protein complexes, fluctuations occur in a common area. However, the ligand complex with the lowest fluctuation is isohemiphloin, with an average RMSF value of 1.07. The charts provide evidence that isohemiphloin exhibits a higher degree of interaction stability when compared to both native ligands and other compounds. Additionally, during the molecular dynamics simulation, interactions between the compounds and the 4PH9 receptor can be observed, as depicted in Figures 11 and 12.

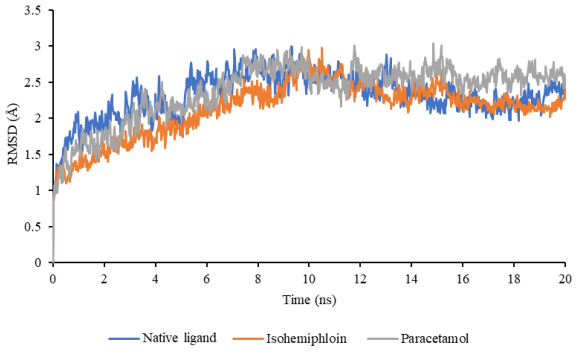

Figure 9. The RMSD results of 4PH9 molecule

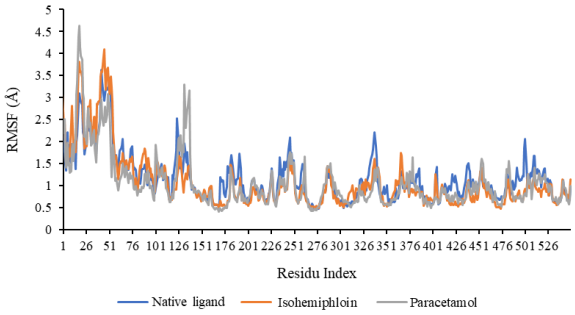

Figure 10. The RMSF results of 4PH9 molecule

Table 5. Average RMSD and RMSF values for the 4PH9 complex

System complex	RMSD	RMSF
Native ligand-4PH9	2.30	1.71
Isohemiphloin	2.12	1.07
Paracetamol	2.37	1.09

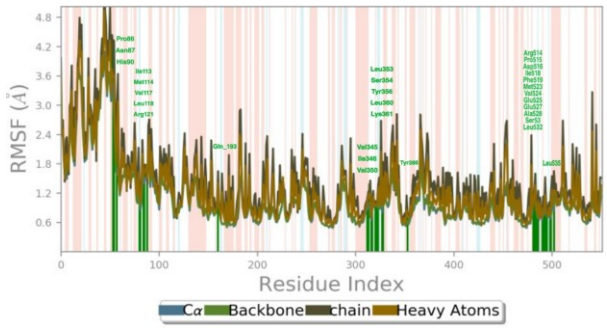

Figure 11. Protein RMSF graph and contact residue

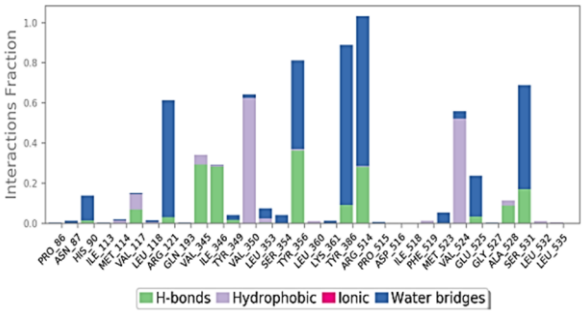

Figure 12. Interaction residue contact with compound isohemiphloin

Figures 11 and 12 show the residue in contact with the isohemiphloin compound. These residues interact via hydrogen bonds (HIS_90, VAL_117, ARG_121, VAL_ 345, ILE_346, TYR_349, TYR_356, TYR_386, ARG_514, GLU_ 525, ALA_528, SER_531), hydrophobic (MET_114, LEU_118, VAL_350, LEU_353, LEU_360, PHE_519, VAL_524, LEU_532, LEU_535), and water bridges (PRO_86, ASN_87, ILE_113, GLN_193, ESR_354, LYS_361, PRO_515, MET_523, GLY_527) were identified. Interactions between isohemiphloin ligands with amino acid residue for 20 ns include hydrophobic interaction, bonding hydrogen, and water bridges.

The RMSD graph (Figure 13) shows the RMSD results of the molecular dynamics of the 5R85 receptor ligand, the compound cirsimaritin, and paracetamol. The compound paracetamol demonstrates stability during the molecular dynamics simulation, ranging from 8-14 nanoseconds. According to the RMSD graph, it can be concluded that during a 20 ns molecular dynamics simulation, paracetamol exhibits the most stable interaction when compared to native ligands and test ligands. This result is supported by the mean value of RMSD during 20 ns simulation (Table 6).

Table 6. Average RMSD and RMSF values for the 5R85 complex

System complex	RMSD	RMSF
Native ligand-4PH9	1.38	0.79
Isohemiphloin	1.53	0.86
Paracetamol	1.26	0.79

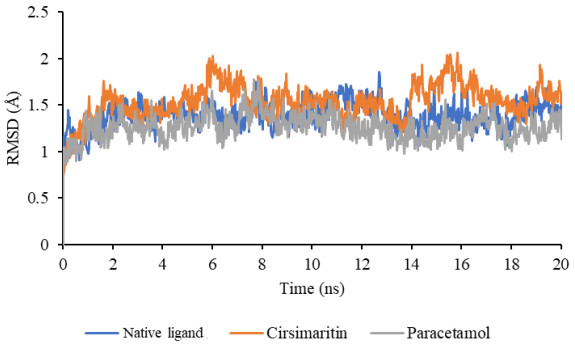

Figure 13. The RMSD results of molecules on receptors code 5R85

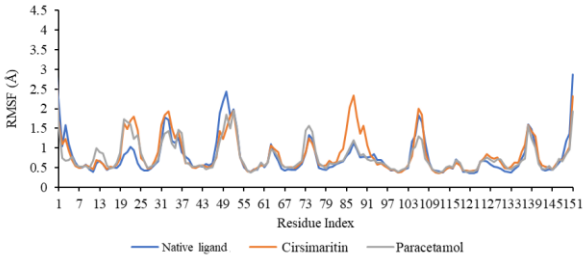

Figure 14. The RMSF results of molecules on receptors code 5R85

Based on Figure 14, the three 5R85 ligand-protein complexes have fluctuations in the same area. However, the ligand complex with the lowest fluctuation was paracetamol, with an average RMSF value of 0.79. The average value of the compound cirsimaritin is big compared to native ligands and reference ligands; the average value at the time physical docking is carried out is stable, but at times, dynamics molecular get results nature No stable. During simulation dynamics, molecular can also look at interactions between compounds with 5R85 receptors, as in Figures 15 and Figure 16.

Figures 15 and 16 show the contact residue with the cirsimaritin compound. These residues interact via hydrogen bonds (TYR_24, GLU_25, LEU_26, LYS_74, LYS_77, LEU_80, GLN_81, LEU_82, VAL_ 1 32), hydrophobic (MET_20, PRO_23, LEU_67, PRO_131), and water bridges (LYS_65, ASP_75, THR_79). , GLU_83, SER_84, LEU_134) were identified. Interactions between isohemiphloin ligands with amino acid residue for 20 ns include hydrophobic interaction, bonding hydrogen, and water bridges.

3.7. Lipinski's Rule of Five

These regulations specify that pharmaceutical compounds should adhere to specific criteria, including a relative molecular mass below 500 g/mol, a Log P value less than 5, fewer than 5 bond donors, fewer than 10 hydrogen bond acceptors, and a molar refractivity within the range of 40-130. Characterizing a compound as a potential drug candidate involves evaluating a diverse array of properties that determine its suitability for pharmaceutical use [9].

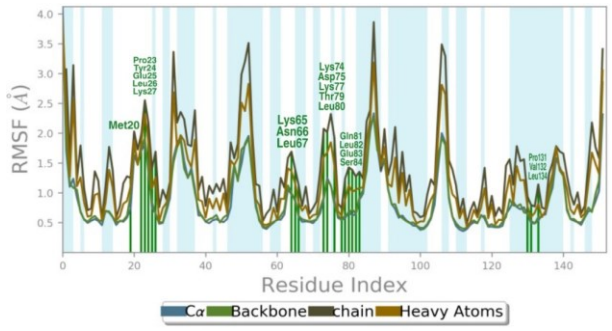

Figure 15. Protein RMSF graph and residue contacts

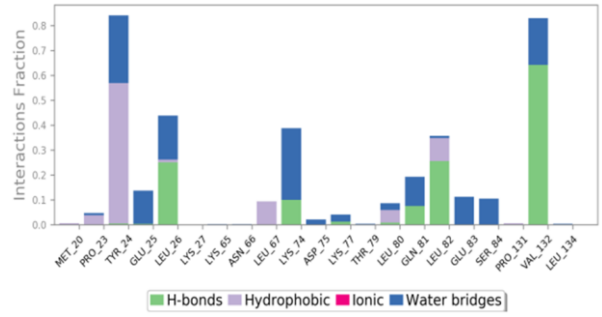

Figure 16. Contact residue interaction with compound cirsimaritin

Molecular weight must be below 500 g/mol to enter the cell membrane. However, if the molecular weight is more than 500 g/mol, the drug cannot diffuse across the cell membrane, disrupting the drug distribution process [13]. The results of the hydrogen donor and acceptor values are related to the biological activity of the drug molecule. When hydrogen bonds are associated with drug molecules, it will affect the chemical-physical properties of the compound, such as melting point, boiling point, solubility in water, ability to chelate, and acidity [14].

The results of the Log P value (partition coefficient) are related to the ability of a drug compound to have hydrophobic molecular characteristics or dissolve in oil. On pharmacokinetic grounds, drugs that will be absorbed orally must pass through the lipid bilayer in the intestinal epithelium to make the delivery system efficient. The drug must be hydrophobic enough to penetrate the lipid bilayer. If the drug does not have hydrophobic properties, it will not be able to penetrate the lipid bilayer and will cause the drug to become toxic in the body [13]. Molar refractory is the total result of the polarizability of drug molecules strongly influenced by temperature, refractive index, and pressure. Polarizability is the simplicity of a molecule in forming a temporary dipole or inducing a molecule [14].

Based on Lipinski's Rule of Five from Table 7, the flavonoid compounds from *Saga Rambat* leaves generally demonstrate good potential as oral drug candidates. Most compounds adhered to the criteria of molecular weight, hydrogen bond donors and acceptors, and Log P values, indicating favorable absorption, distribution, metabolism, and excretion (ADME) properties. This alignment with Lipinski's rules, combined with their predicted pharmacokinetic profiles and non-toxic nature, suggests that these compounds, particularly isohemiphloin, are promising candidates for further development as antipyretic drugs. Their molecular properties are conducive to effective oral administration, enhancing their potential utility in therapeutic applications.

3.8. Prediction Pharmacokinetics and Toxicity

Pharmacokinetic and toxicity properties were assessed using the pKCSM website (Table 8). According to Table 8, all tested compounds exhibit favorable Caco-2 permeability. A predicted value greater than 80% is desirable for HIA, while a value below 30% suggests reduced absorption [15]. The data indicates that most flavonoid compounds fall within the well-absorbed range of 50-95% for HIA. However, isohemiphloin and Hispidulin 4'-O-β-D-glucopyranoside have HIA percentages in the lower range, specifically between 35-48%.

Table 7. Results of drug scan testing on flavonoid compounds

No.	Compound name	Parameter					Accepted
		Molecular weight	Hydrogen donor	Hydrogen acceptor	Log P/Lipopility	Molar refraction	
		< 500 g/mol	< 5	< 10	<5	40-130	
1	Cirsimarin	462	5	11	0.161799	110.116455	No
2	Cirsmaritin	313	1	6	1.9517	79.822777	Yes
3	Hispidulin	299	2	6	1.8633	75.442581	Yes
4	Hispidulin 4'-O-β-D glucopyranoside	461	5	11	-0.6636	108.172966	No
5	Hemiphloin	433	6	10	-0.88171	101.311760	Yes
6	Isohemiphloin	434	7	10	0.0248	102.915047	Yes
7	2R-Naringenin 6-C-β-D glucopyranoside	433	6	10	-0.8871	101.311760	Yes

Table 8. Results of pharmacokinetic testing for flavonoid compounds

No.	Compound name	Parameter							
		Absorption		Distribution		Metabolism		Excretion	
		$Caco_2$	Human Intestinal Absorption	VDss	BBB	CYP3A 4 substrate	CYP3A 4 inhibitors	Total clearance	Renal OCT2 substrate
1	Cirsimarin	0.237	54.616	-0.289	-1.689	No	No	0.554	No
2	Cirsimaritin	1.022	93.987	0.001	-0.59	Yes	No	0.587	No
3	Hispidulin	-0.045	84.654	0.37	-1.12	Yes	No	0.531	No
4	Hispidulin 4'-O-β-D-glucopyranoside	0.278	47.921	0.173	-1.631	No	No	0.497	No
5	Hemiphloin	0.573	51.082	0.807	-1.2	No	No	0.232	No
6	Isohemiphloin	0.424	38.767	0.685	-1.127	No	No	0.238	No
7	2R-Naringenin 6-C-β-D glucopyranoside	0.573	51.082	0.807	-1.2	No	No	0.232	No

Notes: Classification: Caco2 >0.90 = high; HIA <30 = low; VDss ≤0.447 = low, VDss >0.447 = high; BBB >0.3 = well distributed in the brain, BBB <-1 = poorly distributed in the brain; SSP >-2 = distributed both in the SSP, SSP <-3 = difficult to distribute in the SSP

The distribution properties were assessed using VDss and BBB values. According to pKCSM predictions, compounds with a low volume of distribution have VDss values below -0.15, while high values are above 0.45. From the data in Table 8, the VDss values range from -0.289 to 0.807. Hemiphloin, isohemiphloin, and 2R-Naringenin 6-C-β-D glucopyranoside have high VDss values among the seven compounds. This suggests that these compounds can be evenly distributed, resulting in an equal distribution throughout the body. Additionally, pKCSM predictions indicate that compounds can readily penetrate the blood-brain barrier if their BBB value exceeds 0.3, whereas they find it challenging to penetrate if the BBB value falls below -1 [15]. In Table 8, all the compounds have BBB values that allow them to penetrate the blood-brain barrier.

The test results assessed the metabolism involving the CYP3A4 enzyme, which can act as a substrate or inhibitor of CYP3A4 enzymes. These compounds can either enhance or impede enzyme activity, potentially affecting the performance of cytochrome P450 enzymes. Based on Table 8, the research findings reveal that five flavonoid compounds do not undergo liver metabolism. As a result, these compounds are unlikely to induce hepatotoxic effects. In contrast, the remaining two compounds are associated with hepatotoxic properties, suggesting their metabolism may adversely affect the liver.

The test results included assessments of excretion, particularly the parameters of total clearance and renal OCT2. The results are related to total clearance, which is significant in determining the dosage needed to reach steady-state concentration [16]. The data from Table 8 indicates the CLTOT (total clearance) values for different flavonoid compounds, such as cirsimarin, cirsimaritin, hispidulin, Hispidulin 4'-O-β-D-glucopyranoside, hemiphloin, isohemiphloin, and 2R-Naringenin 6-C-β-D glucopyranoside. CLTOT values can be used to predict the rate of compound excretion. The results from the seven flavonoid compounds indicate that none of these compounds have the potential to cause interactions with OCT2 inhibitors. This implies that the compounds are not predicted to be substrates for OCT2, thereby reducing the likelihood of unwanted interactions [17].

The AMES toxicity test is a method that is often used to assess the mutagenic potential of compounds using bacteria [16]. Based on the prediction results listed in Table 9, there is a toxic flavonoid compound in the AMES toxicity parameter, namely hemiphloin. The oral rat acute toxicity (LD_{50}) test is carried out to determine the standard of acute toxicity by assessing the relative toxicity of the types of compounds. LD_{50} is the number of doses that, according to data, can kill 50% of experimental animals [18]. The greater the LD_{50} dose of the test compound, the lower the toxicity. A hepatotoxicity test is a reaction caused by accumulating dangerous drug properties in the liver. The toxicity testing results on hepatotoxicity parameters were obtained from the seven flavonoid compounds, none toxic or poisonous to the liver.

Table 9. Toxicity test results for flavonoid compounds

No	Compound name	Parameter		
		AMES toxicity	LD_{50} (mol/kg)	Hepatotoxicity
1	Cirsimarin	No	2.772	No
2	Cirsimaritin	No	2.254	No
3	Hispidulin	No	2.402	No
4	Hispidulin 4'-O-β-D-glucopyranoside	No	2.647	No
5	Hemiphloin	Yes	2.826	No
6	Isohemiphloin	No	2.907	No
7	2R-Naringenin 6-C-β-D glucopyranoside	No	2.826	No

4. Conclusion

Among the seven flavonoid derivative compounds, the isohemiphloin exhibits favorable pharmacokinetic properties, while another is non-mutagenic, non- carcinogenic, and non-hepatotoxic. Furthermore, this compound is selected to interact with the 4PH9 receptor and another with the 5R85 receptor. Through molecular dynamics simulations, the compound isohemiphloin is identified as the best ligand for the 4PH9 receptor, forming the most stable binding with this protein.

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