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Molecular Docking Test of Procoagulant Compounds in Biwa Leaves (*Eriobotrya japonica* (Thunb.) Lindl.) Against Factor VIII Deficiency in the Genetic Disease Hemophilia-A

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Abstract

Background: *The treatment of Hemophilia A remains chalenging, primarily the risk of inhibitor development and the high cost of recombinant Factor VIII treatment, necessitating the development of adjuvant therapeutic agents to enhance its effectiveness. This situation necessitates innovation in the form of developing adjunctive therapeutic agents aimed at improving the effectiveness and outcomes of existing therapies.* **Methodology:** *To address this need, this study was designed using an in silico virtual screening method with PyRx software and AutoDock Vina. All stages were conducted computationally, followed by molecular docking simulations to map the interactions and binding affinities between candidate compounds and the predetermined molecular target, von Willebrand factor Domain A3, in order to identify the most promising candidate compounds.* **Findings:** *The study successfully identified two compounds, namely Kaempferol and Phenolic acid, which showed the strongest interaction and the best stability profile against the von Willebrand factor A3 domain. The binding free energy (ΔG) value for Kaempferol was -7.9 kcal/mol and for Phenolic acid -4.4 kcal/mol, with a stability value (RMSD) of 0.0 Å for both compounds. The affinity value of Kaempferol is better than that of the reference compound (Coumarin, ΔG = -5.9 kcal/mol), while Phenolic Acid shows a low value. This strong binding affinity indicates the potential of both compounds in stabilizing the interaction of Factor VIII with the A3 domain of von Willebrand factor, which can support hemostatic function. These findings concluded that Kaempferol and Phenolic Acid are worthy of further development as candidate adjunct therapies for Hemophilia A. Therefore, further studies are highly recommended to validate these findings through molecular dynamics simulations, in vitro and in vivo tests to confirm the biological activity and pharmacokinetic (ADME) profile of these compounds.* **Contribution:** *This study introduces the bioactive compounds Kaempferol and Phenolic acid from Biwa leaves as new candidates for modulating the A3 domain of von Willebrand factor, offering a potential and largely unexplored adjunctive strategy in Hemophilia A treatment.*

Keywords: *A3 Domain of von Willebrand Factor; Biwa Bioactive Compounds; Eriobotrya japonica; Hemophilia A; Molecular Docking*

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INTRODUCTION

Hemophilia A is a bleeding disorder caused by a deficiency of Factor VIII in the blood. Recent data shows that the number of hemophilia A patients recorded globally is still far from the actual estimate, with the World Federation of Hemophilia reporting only about 22% of the total estimated number of patients recorded on 2022 (Coffin et al., 2024). This phenomenon of underdiagnosis, coupled with the high cost and availability of conventional therapies, reinforces the urgency of finding more affordable and effective alternative or complementary therapies. Darman & Bahraen (2023) revealed a global estimate of 400,000 people with hemophilia. In Indonesia, the Himpunan Masyarakat Hemofilia Indonesia (HMHI) recorded 2,098 patients by the end of 2018. This figure is thought to represent only 10% of the actual estimate. The actual number is estimated to be between 20,000 and 25,000 cases. According to Putri & Devi (2022), hemophilia is classified based on severity. The classification is divided into mild, moderate, and severe. The severity level is determined by residual factor activity. Mild hemophilia (5-40% activity) generally triggers post traumatic/major surgical bleeding. Moderate hemophilia (1-<5%) causes sporadic spontaneous bleeding. Severe hemophilia (<1%) is characterized by spontaneous intra articular or intramuscular bleeding.

The debate regarding plasma-derived Factor VIII versus recombinant Factor VIII as a therapeutic source began around the turn of the millennium. Clinicians were particularly concerned that recombinant Factor VIII might be more immunogenic than plasma-derived Factor VIII in previously untreated patients, prompting discussions about long term safety, product selection, and clinical trial design (Oldenburg et al., 2015). Therefore, the search for natural compounds that can support adjunctive therapy for hemophilia A has become an attractive alternative to develop. Biwa leaves (*Eriobotrya japonica*) are known to contain various active compounds, such as flavonoids, triterpenoids, and phenolic acid, which have the potential to have procoagulant effects (Sagar et al., 2020). Several flavonoid compounds, such as quercitrin and kaempferol, have been reported to have broad biological activities, including in the modulation of blood clotting processes (Chen & Chen, 2013). This makes biwa leaves a promising source of natural compounds for development as an adjunct therapy for hemophilia A. Although previous studies have identified various bioactive compounds from plants, studies specifically evaluating the potential of compounds from biwa leaves *(Eriobotrya japonica*) on A3 Domain of von Willebrand Factor as an adjunct therapy for hemophilia A are still limited.

In the hemostasis system, von Willebrand Factor (vWF) plays a crucial role in protecting Factor VIII from degradation and aiding platelet adhesion to damaged blood vessels (Pipe et al., 2016). Domain A3 holds a central role as a critical locus in interaction and stabilization. Recent research using molecular docking and dynamic simulations has successfully mapped key residues in Domain A3. The A3 domain side of von Willebrand Factor, which binds directly to FVIII, is a potential target for modulating FVIII activity. Based on this understanding, in silico docking studies are now directed at screening small compounds or peptides that can stabilize this interaction with higher binding affinity as a long lasting therapeutic strategy (Jacquemin et al., 2003).

Using molecular docking, researchers can computationally predict interactions between active compounds and target proteins before conducting experimental tests. Molecular docking is an important in silico approach for predicting inteactions between small molecules or ligands and target proteins. In the context of hemophilia A, this method allows the identification of compounds that can bind to the A3 Domain of von Willebrand Factor, thereby potentially increasing FVIII stability and supporting hemostatic function (Desai et al., 2022).

This study aims to identify bioactive compounds in Biwa leaves that have potential as therapeutic candidates, evaluated through molecular docking simulations against the A3 Domain von Willebrand Factor (PDB: 1AO3) to investigate the possible interactions between these compounds and the target protein. This study is expected to contribute to the discovery of new natural compound candidates as adjunctive therapies for Hemophilia A through the modulation of the A3 Domain von Willebrand Factor.

METHOD

This research was conducted from July to August 2025. The tools used included a Zyrex Notebook Kintamani series laptop with an AMD APU A9-9400 dual-core processor with 4 GB DDR4 memory, as well as a number of supporting software such as PubChem, PASS Online, ProTox-3.0, SwissADME, STRING-db, PyMol, PyRx equipped with Autodock Vina, and Protein Plus. The research materials included the three dimensional structures of active compounds from biwa leaves: Triterpenoids, Phenolic acid, Quercitrin, Kaempferol, and Neohesperidoside, along with the comparative compound Coumarin. These candidate compounds were selected based on a literature review reporting the content of these compounds in Biwa leaves (*Eriobotrya japonica*). Compound structures were obtained from the PubChem database and target macromolecular structures were prepared using Swiss Target Prediction.

Ligand Preparation

The initial stage of the procedure involved the preparation and characterization of bioactive compounds through the download of 3D structures from PubChem in *.sdf format, which were then analyzed for biological activity using PASS Online, toxicity using ProTox 3.0, and suitability as drug candidates based on Lipinski's rules using SwissADME.

Target Protein Preparation

The structure of the A3 Domian of von Willebrand Factor was downloaded from the Protein Data Bank (PDB). Protein preparation involved structure purification by removing all water molecules (H_2O) and irrelevant ions using PyMol software.

Docking Protocol

Molecular docking simulations were performed using PyRx software integrated with AutoDock Vina. All ligands were converted to *.pdb format. The binding site was determined based on literature identifying the natural binding location of Factor VIII on the A3 Domain of von Willebrand Factor. The grid box was set with dimensions

of 20Å x 20Å x 20Å and centered at coordinates (x = 10.5Å, y = -2.3Å, z = 15.8Å) to cover the entire potential binding site (Trott & Olson, 2010).

Validation

The docking method was validated by redocking a known standard compound that binds to the A3 Domain of von Willebrand Factor. Validation was considered successful if the Root Mean Square Deviation (RMSD) between the redocked pose and the reference protein structure was less than 2.0 Å.

RESULTS AND DISCUSSION

Target Protein Energy Value

This study analyzed molecular docking results through binding affinity and RMSD values. Binding affinity measures the strength of the bond between the drug and the receptor. A more negative binding affinity value indicates a stronger ligand affinity to the receptor, while a more positive value indicates a weaker affinity (Nugroho & Fauzi, 2024).

Kaempferol emerged as the most promising candidate because it combines strong binding affinity (-7.9 kcal/mol and RMSD 0.0 Å) with a good drug suitability profile with a TPSA value of 111.13 Å². Phenolic acid has moderate affinity (-4.4 kcal/mol and RMSD 0.0 Å) but low TPSA (20.23 Å²) and meet Lipinski's rules, making it potentially highly suitable for oral administration. Quercitrin and Triterpenoid show very strong affinity but have oral development constraints. Quercitrin has a large TPSA value of 190.28 Å² and violates Lipinski's rules, while Triterpenoid has a Molecular Weight >500 g/mol. Neohesperidoside has a similar pattern to Quercitrin, with strong affinity but high TPSA and violates Lipinski's rules. Coumarin as a control, has moderate affinity (-5.9 kcal/mol) and a good suitability profile.

Coumarin

The results of testing the binding energy values of target proteins on Coumarin compounds are as follows on table 1. Based on the test results, the affinity energy between the coumarin compound and A3 Domain of Von Willebrand Factor (PDB: 1AO3) has a binding affinity value between -5.9 and -5.6 kcal/mol to the target protein A3 Domain of von Willebrand Factor. The best interaction is shown at an energy of -5.9 kcal/mol with an RMSD of 0.0 Å, indicating a stable ligand position. In contrast, the energy of -5.6 kcal/mol shows a weaker interaction with an RMSD of up to 7.638 Å, indicating a large and less stable shift in the ligand position. At an energy of -5.7 kcal/mol, conformational variations were still found with an RMSD of around 2-3 Å, which was relatively stable compared to other conformations. Thus, coumarin at an energy of -5.9 kcal/mol was the most stable and strongest conformation in interacting with the target protein.

Table 1. Coumarin binding energy values on the A3 Domain of vWF (PDB: 1AO3)

Binding Affinity (kcal/mol)	RMSD/lb (Å)	RMSD/ub (Å)
-5.9	0.0	0.0
-5.8	4.43	4,708
-5.7	3,113	3,808
-5.7	3,661	4,692
-5.7	2,565	2,997
-5.7	8,103	8,402
-5.6	3,495	4,411
-5.6	3,467	3,804
-5.6	7,447	7,638

Coumarin, as the control compound in this study, showed stable binding affinity values with hydrogen interactions at specific residues of the coagulation protein domain. This interaction pattern is consistent with the known properties of coumarin, which has anticoagulant activity through vitamin K antagonism and modulation of the coagulation pathway. Lu et al., (2022) reports that coumarin derivatives can inhibit ADP induced platelet activation, supporting the interpretation that coumarin does indeed play a direct role in the hemostasis system. Additionally, an in silico study by Verawati et al., (2024) shows that coumarin and its derivatives have good binding affinity to target proteins through hydrogen and hydrophobic interactions. This reinforces the research findings that coumarin can serve as a relevant control compound, both biologically and pharmacologically in docking simulations against coagulation protein domains.

Triterpenoid

The results of testing the binding energy values of target proteins on Triterpenoid compounds are as follows on table 2. Based on the test results, the affinity energy between the triterpenoid compound and A3 Domain of von Willebrand Factor (PDB: 1AO3) shows a value of -8.7 kcal/mol, indicating a strong bond affinity. RMSD analysis shows that in the best conformation (RMSD/ub = 0 Å and RMSD/lb = 0 Å), the interaction between triterpenoid and domain A3 is very stable, indicating an optimal binding position. Other variations with higher RMSD, such as 4.035 Å/2.673 Å, are still considered stable although not as optimal as the first pose. Conversely, conformations with very high RMSD, for example >20 Å, show weak bonds and are biologically irrelevant. Therefore, these poses are considered biologically irrelevant and are not used as a basis for assessing procoagulant potential. Thus, only poses with low RMSD are worthy of interpretation.

Research Rosyid et al., (2016) on bengle rhizomes (*Zingiber cassumunar*) successfully isolated triterpenoid compounds with significant antibacterial activity, proving that this group of compounds plays an important role in biological interactions through hydrophobic bonding mechanisms. This supports the docking results that triterpenoids from biwa leaves can stabilize target protein bonds. Triterpenoid compounds, which are commonly found in various Indonesian medicinal plants, are

reported to have a spectrum of biological activities, including modulation of cellular processes through anti inflammatory effects and membrane stabilization.

Table 2. Triterpenoid binding energy values on the A3 Domain of vWF (PDB: 1AO3)

Binding Affinity (kcal/mol)	RMSD/lb (Å)	RMSD/ub (Å)
-8.7	0.0	0.0
-8.1	18,082	21,265
-7.6	2,673	4,035
-7.4	9,132	15,062
-7.2	3,906	8,925
-7.1	21,358	25,806
-7.0	20,773	2.58
-6.9	22,371	25,198
-6.9	4,861	9,126

Phenolic Acid

The results of testing the binding energy values of target proteins on Phenolic acid compounds are as follows on table 3. The docking test results show that phenolic acid compounds have binding affinity values ranging from -4.4 to -3.9 kcal/mol to the target protein Domain A3 von Willebrand factor (PDB: 1AO3). The best interaction is shown at a value of -4.4 kcal/mol with RMSD/ub and RMSD/lb = 0.0 Å. The RMSD value of 0.0 Å in this best pose indicates that the docking pose is identical to the pose used as the initial reference in the simulation, indicating consistency in finding the optimal binding pose. However, long-term biological stability needs to be confirmed by molecular dynamics simulations. Conversely, the value of -3.9 kcal/mol shows a high RMSD (>33 Å), resulting in a weak and un ly stable interaction. In other variations, such as -4.3 kcal/mol, stable conformations with low RMSD were still found, although there were also poses with large shifts. Thus, phenolic acid have the potential to bind well to target proteins, especially in conformations with the lowest energy of -4.4 kcal/mol and low RMSD.

Table 3. Phenolic acid binding energy values on the A3 Domain of vWF (PDB: 1AO3)

Binding Affinity (kcal/mol)	RMSD/lb (Å)	RMSD/ub (Å)
-4.4	0.0	0.0
-4.3	1,246	1,962
-4.3	22,363	22,807
-4.1	29,148	29,853
-4.0	29,316	30,303
-4.0	29,806	30,796
-4.0	29,264	30,276
-3.9	33,298	33,477
-3.9	32,979	33,201

Phenolic acid are simple aromatic compounds that have one hydroxyl group (–OH) on the benzene ring. The hydroxyl group can act as a hydrogen bond donor or

acceptor, especially to polar amino acid residues in the active site of proteins. Meanwhile, the aromatic structure of benzene supports hydrophobic interactions with nonpolar residues around the active site. Due to their relatively small and flexible molecular size, phenolics in docking can usually adapt well to the active site of proteins (Irawan et al., 2025).

Phenolic acid have relatively low binding affinity (-4.4 kcal/mol), but still form stable interactions at the active site of the target protein. Indonesian research shows a positive correlation between total phenolic content and antioxidant activity in various medicinal plant extracts, demonstrating the contribution of phenols in maintaining molecular stability (Da'i et al., 2012). This reinforces the finding that, despite their simplicity, phenols still play an important pharmacological role through hydrogen bonding.

Quercitrin

The results of testing the binding energy values of target proteins on Quercitrin compounds are as follows on table 4. Based on the test results, the binding affinity between the Quercitrin compound and A3 Domain of Von Willebrand Factor (PDB: 1AO3) was recorded at -9.4 kcal/mol, indicating a very strong binding affinity. When compared to the range of ligand affinities commonly considered moderate (-7.0 to -8.0 kcal/mol), the value of -9.4 kcal/mol indicates that Quercitrin has a higher binding ability to the target site. RMSD analysis shows that in the best conformation (RMSD/ub = 0.0 Å and RMSD/lb = 0 Å), the interaction between Quercitrin and Domain A3 is very stable, indicating an optimal binding position.

Table 4. Quercitrin binding energy values on the A3 Domain of vWF (PDB: 1AO3)

Binding Affinity (kcal/mol)	RMSD/lb (Å)	RMSD/ub (Å)
-9.4	0	0.0
-9.4	1,402	2,317
-9.3	1,563	4.83
-9.0	1,823	7,775
-8.8	2,187	5,262
-8.6	2,758	9,208
-8.5	1,704	2,318
-8.2	2.87	8,184
-7.8	3,837	8,261

Alternative conformations with RMSD/lb = 1.402 Å and RMSD/ub = 2.317 Å still maintain fairly good stability, although not to the same degree as the main conformation. Conversely, conformations with higher RMSD/ub, for example 9.208 Å or 8.261 Å, show binding that tends to be less stable. Interpretation of these results indicates several potential conformational modes, only poses with low RMSD values can be considered relevant and potentially influence the biological activity of the target. Thus, based on its high binding affinity (-9.4 kcal/mol) and the presence of stable poses at low RMSD, Quercitrin shows potential as a candidate compound that modulates interactions in the A3 Domain of Von Willebrand Factor.

The chemical properties of Quercitrin as a flavonoid glycoside, where the sugar group can strengthen hydrogen bond formation and enhance polar interactions with key residues in the binding pocket, support the affinity and stability results found (Govindammal et al., 2022). Additionally, reports on Quercitrin's ability to enhance cellular protection and its relatively good bioavailability compared to the aglycone quercetin make it a relevant candidate for further development as a potential natural procoagulant (Wang et al., 2022).

Kaempferol

The results of testing the binding energy values of target proteins on Kaempferol compounds are as follows on table 5. Based on the test results, the binding energy between kaempferol compounds has a binding affinity value between -7.9 and -7.0 kcal/mol to the target protein A3 Domain of von Willebrand Factor (1AO3). The best interaction is shown at an energy of -7.9 kcal/mol with RMSD/lb and RMSD/ub = 0.0 Å, indicating a very stable bond without ligand position shift. In contrast, an energy of -7.0 kcal/mol shows a weaker interaction with an RMSD of 8.943 Å, while other variations at an energy of -7.9 kcal/mol also show positional shifts with an RMSD of up to 7.004 Å. This indicates that kaempferol has strong binding potential. However, the optimal stability of its interaction is greatly influenced by low RMSD conformations.

Table 5. Kaempferol binding energy values on the A3 Domain of vWF (PDB: 1AO3)

Binding Affinity (kcal/mol)	RMSD/lb (Å)	RMSD/ub (Å)
-7.9	0.0	0.0
-7.9	2,882	7,004
-7.9	2,265	6,138
-7.7	1.46	3,204
-7.4	2,942	7,571
-7.3	2,459	7,232
-7.2	2,594	3,854
-7.2	1,925	6.17
-7.0	4,255	8,943

Kaempferol is a flavonol that has been extensively studied for its anticancer, anti inflammatory, hepatoprotective, and cardioprotective activities. The main mechanisms of kaempferol are through the regulation of apoptosis and inhibition of angiogenesis. Comprehensive studies report that kaempferol plays a significant role in the prevention of degenerative diseases and is relevant in the development of flavonoid based drugs (Chen & Chen, 2013).

Neohesperidoside

Based on the test results, the binding affinity value between the neohesperidoside compound and the target protein A3 Domain of von Willebrand Factor (PDB: 1AO3) is in the range of -7.9 to -6.3 kcal/mol. This negative value indicates that the interaction between neohesperidoside and the target is relatively strong. The best interaction is

shown at a value of -7.9 kcal/mol with RMSD/lb and RMSD/ub = 0.0 Å, which indicates a stable bond. Conversely, a value of -6.3 kcal/mol indicates a weaker interaction with an increased RMSD of up to 6.11Å, while other variations such as -6.7 kcal/mol show a significant shift in the ligand. Although the ligand shift is still within a moderate range, this reinforces the conclusion that the more negative the binding affinity value and the lower the RMSD, the more stable the interaction between the ligand and the protein tends to be (Siagian et al., 2025).

Table 6. Neohesperidoside binding energy values on the A3 Domain of vWF (PDB: 1AO3)

Binding Affinity (kcal/mol)	RMSD/lb (Å)	RMSD/ub (Å)
-7.9	0.0	0.0
-7.5	1,993	6,807
-7.5	1,787	3,092
-7.2	1.77	3.66
-7.1	1,881	6,833
-6.8	1,659	6,722
-6.7	3,502	4,733
-6.5	3,308	4,882
-6.3	2,264	6.11

Furthermore, the findings in this study are supported by various previous in silico studies, which confirm that flavonoid glycoside compounds, including neohesperidoside, are capable of forming stable interactions with various target proteins, particularly enzymes, through a specific binding mechanism (Sugiharto et al., 2021). This is consistent with the finding that neohesperidoside from Biwa leaves can regulate the work of proteins that play a role in the blood clotting process. This consistency further strengthens the results obtained, where neohesperidoside derived from Biwa leaves shows the ability to interact with the functional domains of proteins involved in coagulation.

Drug Feasibility Analysis According to Lipinski's Rules (*Rules of Five*)

Based on the assessment using Lipinski's rules, there is a clear pattern. Coumarin, Phenolic Acid, and Kaempferol compounds meet all criteria without violation (0 violation), which means that theoretically they are more likely to be well absorbed by the body when ingested. Conversely, triterpenoid compounds have too large a molecular weight (>500 g/mol), while quercitrin and neohesperidoside have too many polar groups that can inhibit absorption. These differences indicate that compounds that comply with Lipinski's rules have physicochemical properties that are more conducive to becoming oral medications.

The Lipinski's Rules (Rules of Five), are empirical guidelines developed from observations of the physicochemical properties of oral drugs that have successfully reached clinical trials. These rules summarize four simple criteria, namely molecular weight ≤ 500 g/mol, hydrogen bond donors ≤ 5, hydrogen bond acceptors ≤ 10, and LogP ≤ 5, and state that molecules that violate more than one criterion tend to

experience problems with absorption or permeability after oral administration. Therefore, the Rules of Five are often used as an initial filter in the selection stage of drug discovery, but are not intended as a final determinant of pharmacological suitability (Lipinski et al., 2012).

Table 7. Results of drug feasibility analysis according to Lipinski's (Lipinski et al., 2012).

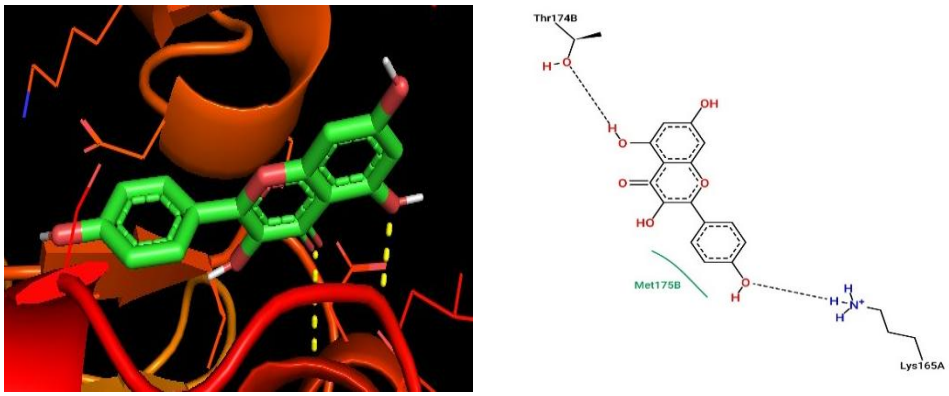
Compound	**Molecular Weight <500 (g/mol)**	**H-Bond acceptor <10**	**H-Bond donor <5**	**Log P <5**	**Molar Refractivity**	**TPSA ≤ 140 Å²**
Coumarin (Control)	146.14	2	0	1.75	42.48	30.21
Triterpenoid	552.76	7	3	2.97	148.27	129.51
Fenolic Acid	94.11	1	1	1.24	28.46	20.23
Quercitrin	448.38	11	7	1.60	109.00	190.28
Kaempferol	286.24	6	4	1.70	76.01	111.13
Neohesperidoside	326.30	10	7	0.64	66.96	169.30

Topological Polar Surface Area (TPSA) is a parameter that is very often used in ADME evaluation. Topological Polar Surface Area (TPSA) complements the Rules of Five assessment by providing a quick estimate of the polarity of a molecule's surface, namely the amount of area contributed by polar atoms such as oxygen and nitrogen as well as bound hydrogen. Because TPSA is directly related to a molecule's ability to passively permeate membranes, the threshold value often used in the literature is ≤ 140 Å² for adequate intestinal permeation. For penetration into the nervous system, TPSA can help explain why molecules that show good target affinity in silico are still predicted to have low oral absorption. TPSA calculations that consider sugar groups or phenolic substituents are very useful for assessing this issue in large natural compounds (Ertl et al., 2000).

In drug suitability assessment practice, the Rules of Five and TPSA should be used as a quick triage filter, then supplemented with additional criteria such as Veber's rules (observing rotatable bonds and TPSA), BDDCS/transporter analysis, and in vitro permeability prediction data (Caco-2). computational verification steps such as molecular dynamics simulations (to assess whether intramolecular hydrogen bonds cover the polar surface) and free energy calculations (MM-GBSA/MM-PBSA) are also recommended. For large natural compounds that violate the Rules of Five but show in silico activity, practical strategies include examining the possibility of active transport, derivatization or prodrug approaches to reduce TPSA, and/or considering local administration routes when oral administration is not realistic. In other words, the Rules of Five and TPSA are effective screening tools when combined with ADME data and chemical or formulation optimization strategies (Veber et al., 2002).

Visualization of Molecular Docking Results

Visualization of the 3D Structure of Kaempferol Ligand Interaction with the Target Protein



(a) **(b)**

Figure 1. Visualization of molecular docking of the Kaempferol compound with the protein Target Domain A3 von Willebrand Factor. **(a)** 3D structure of molecular binding simulation. **(b)** 2D structure of hydrogen bond and hydrophobic interactions.

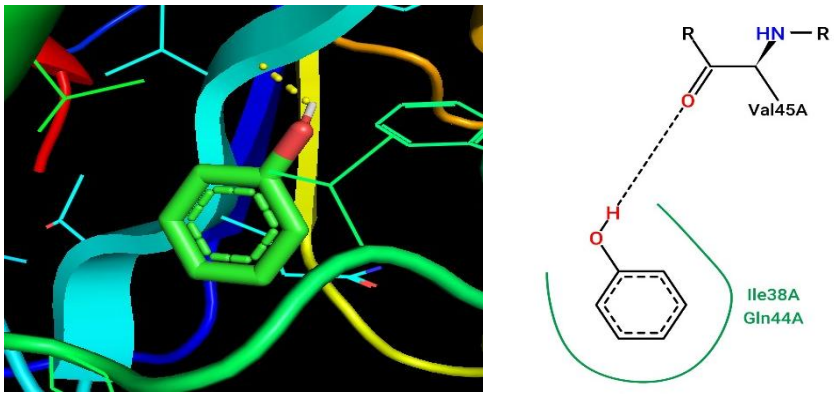
The 3D structure visualization of kaempferol docking was obtained using PyMOL software based on affinity score data and the prepared target protein structure. In the 3D view, the kaempferol ligand is visualized as a green aromatic ring bound to the protein site through a combination of polar and nonpolar interactions. In the polar region, dotted lines connect the phenolic or carbonyl group of kaempferol to the Thr174B residue, indicating the formation of hydrogen bonds between the –OH or =O group in the ligand and the –OH group in the threonine chain. Hydrogen bonds play a role as the main specific interaction in ligand-receptor binding, thus contributing significantly to the affinity of molecules to targets through electrostatic interactions between hydrogen donors and acceptors (Muttaqin, 2019). Additionally, a dotted line is also observed between the phenolic group of the ligand and the ammonium group ($–NH_3^+$) on Lys165A, indicating the presence of hydrogen bonds or additional electrostatic contributions. Meanwhile, hydrophobic contact is indicated by the proximity of the kaempferol aromatic ring surface to the Met175B residue. Overall, kaempferol forms a binding pattern involving at least two key hydrogen bonds accompanied by a number of hydrophobic interactions, thereby stabilizing its position in the protein binding pocket.

The 2D visualization confirms the interaction pattern between kaempferol and the protein, which includes a hydrogen bond between the –OH group of kaempferol and Thr174B, an electrostatic interaction or additional hydrogen bond between its phenolic group and the positively charged Lys165A, and a hydrophobic interaction between the aromatic ring of the ligand and the Met175B residue. In the 2D diagram, the kaempferol structure is represented by atom connecting lines following the standard CPK (Corey Pauling Koltun) color code. Hydrogen bonds are shown as specific, directional dotted lines, while hydrophobic contacts are marked with green arcs. Although weaker than hydrogen bonds, hydrophobic interactions collectively contribute to the stability of the complex through van der Waals forces and the

displacement of water from nonpolar surfaces, which is also consistent with the inherently stable characteristics of aromatic rings (Prakoso, 2024).

Visualization of the 3D Structure of Kaempferol Ligand Interaction with the Target Protein

The 3D visualization of molecular docking results was performed using PyMOL software by utilizing the score data of each ligand and the prepared protein structure. This image shows the ligand visualized as a simple green aromatic structure bound to the active site of the protein through a combination of polar and nonpolar interactions. Its harmonious position indicates compatibility with the morphology of the binding pocket. In the 3D image, dotted lines connect the phenolic acid hydroxyl group (–OH) with polar amino acid residues, indicating that hydrogen bonds play a very important role in stabilizing the ligand-protein complex. On the other hand, the proximity of the ligand's aromatic ring to the surrounding hydrophobic residues indicates the contribution of hydrophobic interactions to the overall stability of the binding. In phenolic compounds, the hydroxyl group can act as a hydrogen bond donor that interacts with the acceptor group on the protein (Fattara et al., 2024).



(a) **(b)**

Figure 2. Visualization of molecular docking of the Phenolic acid compound with the protein Target Domain A3 von Willebrand Factor. **(a)** 3D structure of molecular binding simulation. **(b)** 2D structure of hydrogen bond and hydrophobic interactions.

The 2D visualization maps in detail the interaction patterns at the atomic level between Phenolic Acid and important residues in the protein. The diagram shows the formation of hydrogen bonds marked by green dotted lines between the oxygen atom (–O) in the hydroxyl group of the ligand and the hydrogen atom in the amide group of the Gln44A residue. The green arcs in the diagram indicate hydrophobic interactions connecting the aromatic ring of the phenolic acid to the hydrophobic residues Val45A and Ile38A. The aromatic structure of the ligand structurally supports interactions with nonpolar residues around the active site (Irawan et al., 2025). This pattern indicates that phenolic acid binding is stabilized by two mechanisms simultaneously, namely hydrogen bonds that determine specificity and orientation, and hydrophobic interactions that increase complex stability through van der Waals forces.

CONCLUSION

Based on the in silico research conducted, it can be concluded that Biwa leaves (*Eriobotrya japonica*) contain bioactive compounds with potential as therapeutic adjuncts for Hemophilia A. Molecular docking simulations against the von Willebrand Factor A3 Domain (PDB: 1AO3) identified Kaempferol and Phenolic Acid as two promising candidates. Both compounds showed strong binding affinity and good binding pose consistency with free energy values (ΔG) of -7.9 kcal/mol and -4.4 kcal/mol, respectively. Molecular interaction analysis indicates that the binding is stabilized by a combination of specific hydrogen bonds with polar residues Thr174B and Lys165A and hydrophobic interactions with nonpolar residues Met175B and Val164A. Evaluation of drug suitability based on Lipinski's rules shows that Kaempferol meets the criteria for oral drugs, while Phenolic Acid is also suitable despite having moderate affinity. This study provides a new contribution by highlighting the potential modulation of the von Willebrand factor A3 domain using natural compounds from biwa leaves, a target and source of materials that have not been widely explored in the search for adjunctive therapies for hemophilia A. These computational findings provide a strong basis for the further development of Kaempferol and Phenolic Acid as candidate adjunctive therapies for Hemophilia A. Therefore, it is recommended to conduct further studies involving molecular dynamics simulations to assess stability under more realistic conditions, as well as in vitro and in vivo tests to confirm the procoagulant activity, safety, and pharmacokinetic profile (ADME) of both compounds.

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