BIOLOGY, MEDICINE, & NATURAL PRODUCT CHEMISTRY ISSN 2089-6514 (paper)
Volume 14, Number 2, October 2025 | Pages: 1247-1256 | DOI: 10.14421/biomedich.2025.142.1247-1256 ISSN 2540-9328 (online)

Molecular Dynamic Study of Java Cardamom (*Wurfbania compacta*) Leaf Compounds Targeting Xanthine Oxidase for Antihyperuricemia

Ali Nofriyaldi[1]*, Nitya Nurul Fadilah[1], Selvy Isnaeni[2]
[1]Pharmacy Department, Faculty of Health Science, Perjuangan Tasikmalaya University,
[2]Agrotechnolgy Departement, Faculty of Agriculture, Perjuangan Tasikmalaya University
Jl. Peta No.177 Tasikmalaya, Telp. (0265) 326058 Indonesia.

Corresponding author*
alinofriyaldi13@gmail.com

Manuscript received: 25 September, 2025. Revision accepted: 27 November, 2025. Published: 12 December, 2025.

Abstract

Hyperuricemia is a disorder marked by elevated concentrations of uric acid in the bloodstream, which can trigger gout and other metabolic complications. Conventional therapies such as allopurinol are effective but often cause side effects. This study aims to explore the potential of bioactive compounds from cardamom leaves (*Wurfbania compacta*) as antihyperuricemic agents through an in silico approach. The methods used include mining phytochemical data from the literature, predicting pharmacokinetic properties and toxicity (ADMET), molecular docking as well as molecular dynamic. The screening results show that the compound with the most stable interaction from cardamom (*Wurfbania compacta*) with receptors that play a role in inhibiting xanthine oxidase enzyme in silico is the compound kaempferol with a Binding energy value of -8.1 kcal/mol and Ki 1.15 uM (micromolar). ADMET analysis indicates that the main candidate has a good pharmacokinetic profile and low toxicity potential. Based on the results of the RMSD and RMSF molecular dynamic analysis, the kaempferol compound shows a stable level of interaction and has 3 amino acid similarities with the comparison drug allopurinol, namely THR1010, VAL1011 and ALA1078, so it has the potential to be used as a candidate antihyperuricemia drug. These findings suggest that cardamom leaves have prospects as a source of natural antihyperuricemia compounds. Further in vitro and in vivo studies are needed to verify the biological activity and safety of its use.

Keywords: Antihyperuricemia; Java cardamom leaves; In Silico; Molecular Dynamic; Xanthine Oxidase.

INTRODUCTION

Hyperuricemia is a health condition characterized by elevated levels of uric acid in the blood. This condition often does not show any signs or symptoms during its early stages. Typically, uric acid levels are considered high when they exceed 7.0 mg/dL in men or 6.0 mg/dL in women (Sovia et al., 2025). Hyperuricemia, which means having high levels of uric acid in the blood, is connected to several health issues like gout, heart problems, kidney diseases, metabolic syndrome, and diabetes (Du et al., 2024). A crucial enzyme involved in producing uric acid is called xanthine oxidase (XO). This enzyme helps convert hypoxanthine into xanthine, and then into uric acid. Blocking XO is a proven method used to treat high levels of uric acid in the blood (Liu et al., 2021).

While traditional XO inhibitors like allopurinol and febuxostat work well, they often cause unwanted side effects, leading to increased exploration of natural products as potentially safer options (Mehmood et al., 2019). Recent research has shown that bioactive compounds from plants, such as flavonoids, polyphenols, and peptides, have the ability to inhibit xanthine oxidase and reduce high uric acid levels. These findings are backed by both in vitro assays and in silico molecular modeling techniques (Dirgantara et al., 2022). One of the plants that contains flavonoids is cardamom. There are several types of cardamom, one of which is Javanese cardamom (*Wurfbainia compacta*),, whose boiled fruit extract is empirically believed to be used as an antihyperuricemic agent. The flavonoid derivative from Javanese cardamom fruit, in silico, is a candidate that plays a role as an antihyperuricemic, namely the compound 3-cyclohexene-1-methanol (Nofriyaldi et al., 2025). In the realm of medicinal plants, cardamom, has been used for centuries in different cultures for its healing benefits. Cardamom leaf extract was found to have strong antifungal activity against different fungal plant pathogens and also exhibited antimicrobial effects against both types of bacteria, which are Gram-positive and Gram-negative that cause human infections, and exhibited antihyperglycemic effects by reducing glucose absorption and mimicking the action of insulin (Mekky et al., 2023). The bioactive properties of cardamom leaves have not been thoroughly studied, especially

regarding their ability to inhibit XO and their potential use in treating hyperuricemia.

Advances in computational pharmacology, particularly in molecular docking and molecular dynamics (MD) simulations, have made it possible to accurately model how phytochemicals interact with target enzymes such as XO. Advances in computational pharmacology, particularly in molecular docking and molecular dynamics (MD) simulations, have made it possible to accurately model how phytochemicals interact with target enzymes such as XO (Mekky et al., 2023). This study seeks to examine the ability of phytochemical compounds found in the leaves of *Wurfbainia compacta* to act as natural inhibitors of xanthine oxidase, using a combined in silico method that includes molecular docking and molecular dynamics simulation. The results could help in identifying new plant-based treatments for hyperuricemia that offer better safety.

MATERIALS AND METHODS

Materials

ChemDraw Ultra 8.0 from Cambridge soft Corporation 2003 8 versions, Marvin Sketch from ChemAxon 1999 23.1 version, Molegro Molecular View 2008 2.5 version, AutoDock from Molecular Graphics Laboratory Tools 1.5.6 version, BIOVIA Discovery Studio and Desmond (Dassault Systemes) were utilized. Web servers include RCSB PDB, PDBsum, and PreADMET. A personal computer with Linux Ubuntu 18.04.5 LTS 64-bit, Processor Intel® CoreTM i5-8400 CPU @ 2.80GHz x 6, Memory 7,7 GiB, Graphics GeForce GTX 970/PCIe/SSE2, GNOME 3.28.2, Disk 245,1 GB. Plant material and protein: 50 cardamom leaves compounds from the literature were used, and a receptor protein with PDB ID code 3NVW and allopurinol were used as positive control drugs.

Procedures

Receptor Identification

Receptors were obtained from the website https://www.rcsb.org/. A receptor with a resolution of less than 2Å and a natural ligand similar in structure to the reference drug was chosen. The receptor was then examined on the http://www.ebj.ac.uk/pdbsum site to confirm that it aligned with the Ramachandran plot (Ruswanto et al., 2018).

Docking Validations

Docking validation was performed to develop a reliable method by repeatedly docking the original ligand to the target protein using AutoDockTools (Sari et al., 2020).

Ligand Preparation

The ligands were protonated by adding protons (H) and simulating a specific pH level to match the pH of the human body, which is 7.4. The resulting file was saved in .mrv format. The next step involved conducting a conformational search, and the file was then saved in .mol2 format. It was imported into the Molegro Molecular Viewer, and subsequently, the molecule was exported in .pdb format (Sagitasa et al., 2021).

Figure 1. 2D visualization of the redocking results of receptor 3NVW

Prediction of Pharmacokinetic and Toxicity

Toxicity and pharmacokinetic tests were conducted using the PreADMET website. The results included analysis of the ames test, carcinogenity in mice, carcinogenity in rats, HIA, Caco-2, and PPB parameters were reviewed (Mardianingrum et al., 2021).

Drug Scan

A drug screening process was conducted using Lipinski's program to assess how similar a compound is to a reference drug based on the pharmacokinetic processes that take place in the body. The parameter examined during this stage was Log p (Rahmawaty et al., 2022).

Molecular Docking and Visualization

The process was conducted using AutoDock to dock the test ligand onto the target receptor, with the grid dimensions based on the validation results for the x, y, and z axes, which were 38.017, 20.172, and 18.698, respectively (Dermawan et al., 2019). The outcomes were presented as binding energy (ΔG) and inhibition constant (Ki), followed by visualization to examine the possible interactions between the ligand and the receptor (Ruswanto et al., 2023).

Molecular Dynamic Simulations

The simulation process was conducted on the selected compound based on the docking results with the receptor using the Desmond software to investigate the stability between the compound and the receptor. The process began with preparing the test compound and the solvation stage, where the ligand-receptor complex was dissolved in TIP3P-type water. Neutralization was then carried out by adding Na^+ and Cl^- ions to ensure the system reached a neutral state, mimicking the physiological conditions of the human body. Next, minimizing was performed to reduce the potential energy and produce a more stable conformation by avoiding atomic clashes during the formation or breaking of hydrogen bonds. Following this, heating was applied, with temperature and pressure adjusted to match the normal human body temperature so that the protein complex could function normally during the simulation. Equilibration was then performed, where the system reached a constant state of temperature, volume, and pressure. The simulation was run for 100 nanoseconds using an NPT ensemble (Zubair et al., 2020). The results

of the simulation were analyzed using RMSD, RMSF, and protein-ligand residue contact plots.

RESULTS AND DISCUSSION

Result of Receptor Identification

In this study, a receptor with the PDB code 3NVW was used, which was downloaded from the web server https://www.rcsb.org, because this receptor has a resolution value of 1.6 Å and has a natural ligand structure similar to the comparator drug. The receptor with PDB code 3NVW has a value of most favored regions of 90.0% and a value of disallowed regions of 0.2%, which means it meets the criteria and can be used as a target protein for the binding of molecules against antihyperuricemia activity. The receptor with PDB code 3NVW has an active site of hydrogen bonds with amino acids namely GLU802, ARG880, THR1010, and MOS1328. Meanwhile, for the hydrophobic binding interactions with amino acids namely LEU873, SER876, PHE914, PHE1009, VAL1011, LEU1014, ALA1078, and ALA1079. More clear interactions can be seen in Figure 1.

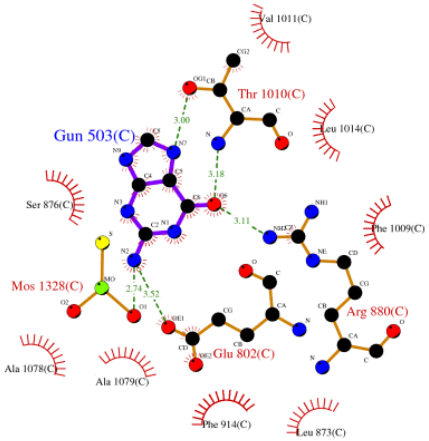

Figure 2. Active Side of Xanthine Oxidase Receptor.

Result of Docking Validation

Docking validation is performed using the AutoDockTools application, by re-docking the natural ligand with the prepared receptor. The purpose of this validation is to obtain a valid method, which is said to be valid if the receptor has an RMSD value < 2 Å. This is because the smaller the RMSD value, the more similar the position of the docked natural ligand is to that of the natural crystallographic ligand. Based on the docking validation results in Table 1, the receptor 3NVW is considered valid because the obtained RMSD value is < 2 Å, which is 0.27 Å, with a binding energy of -6.88 kkal/mol, and in the Gridbox setup, x = 38.017, y = 20.172, and z = 18.698.

The results from redocking are then visualized using the BIOVIA Discovery Studio 2017 application to observe the interactions between the ligand and receptor, allowing for the identification of the interactions and the amino acid residues involved in the ligand-receptor complex.

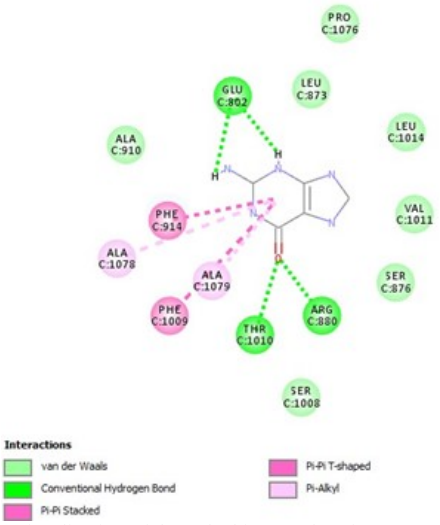

Figure 3. 2D visualization of the redocking results of receptor 3NVW.

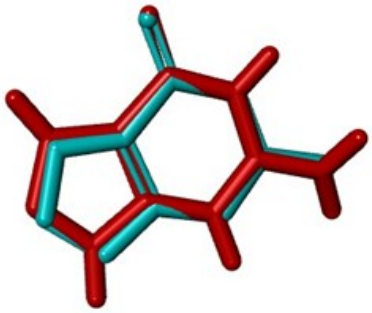

Figure 4. Overlay results before docking (red) and after docking (teal).

Table 1. Docking Validation Results.

PDB code	*Grid Box*			RMSD (Å)	*Binding Energy* (kkal/mol)
	X	Y	Z		
3NVW	38.017	20.172	18.698	0,27	-6.88

Result of Ligand Preparation

The test compound from cardamom was first prepared by conducting protonation and conformational analysis using the MarvinSketch application. the compounds prepared included 15 compounds such as 5-Hydroxy-3,7,4'-trimethoxyflavone, +/-.-2-Methoxy-3,8-dioxocephalotax-1-ene, Erucyl amide, Linolenic acid, Phytol, Hexadecanoic acid, Neophytadiene, Carvestrene, Carvol, 1,8-Cineole, Kaempferol, Quercetin, Luteolin, Pelargonidin, and Allopurinol. The purpose of the protonation stage is to adjust the pH of the test compound to the physiological pH of the human body, which is 7.4. Next, the conformation is performed to maintain the position of the molecule that is most stable when interacting with the active site of the receptor.

Result of Pharmacokinetic and Toxicity

The parameters analyzed include the Ames test which aims to demonstrate the mutagenic potential of compounds. Additionally, it analyzes oral rat acute toxicity (LD50) and Hepatotoxicity. The results of the toxicity tests can be seen in Table 2. Furthermore, 9 test compounds and a comparator drug that passed the toxicity test were continued with pharmacokinetic testing.

Table 2. Toxicity and pharmacokinetic Test Results.

No	Compounds name	Toxicity test			Phamacokinetic Test		
		Ames toxicity	LD50 (mol/kg)	Hepatotoxicity	CaCo-2	HIA (%)	PPB (%)
1	5-Hidroxy-3,7,4'-trimetoksiflavon	No	2.639	No	23.67	96.48	82.47
2	2-Methoxy-3,8-dioxocephalotax-1-ene	Yes	2.36	Yes	-	-	-
3	Erucyl amide	No	1.863	No	35.78	95.56	100
4	Linolenic acid	No	1.624	Yes	-	-	-
5	Phytol	No	1.546	No	37.62	100	100
6	Hexadenoic acid	No	1.595	No	27.52	98.29	100
7	Neophytadiene	No	1.48	No	23.15	100	100
8	Carvestrene	No	1.847	No	23.63	100	100
9	Carvol	No	1.781	No	47.74	100	58.04
10	1,8-Cineole	No	2.013	No	21.89	100	100
11	Kaempferol	No	2.317	No	9.49	83.92	79.38
12	Quercetin	Yes	2.551	No	-	-	-
13	Luteolin	Yes	2.709	No	-	-	-
14	Pelargonidin	Yes	2.38	No	-	-	-
15	Allopurinol	No	2.424	No	16,52	78,26	3,11

Description:

: Not qualified

: Qualified

Caco-2: < 4 low permeability, 4-70 moderate permeability, > 70 high permeability. HIA: 0-20% low absorption, 20-70% moderate absorption, 70-100% high absorption. PPB < 90% weak binding, > 90% strong binding.

Result of Drug scan

Drug scan was performed using the Lipinski's program to analyze the physicochemical properties of cardamom. The physicochemical properties are analyzed to observe the similarity of a compound to oral drugs through the pharmacokinetic processes that occur in the body. A compound is considered good if it meets 2 or more criteria of the Lipinski's rule, which are molecular weight < 500g/mol, Log P <5, hydrogen bond donors <5, hydrogen bond acceptors <10, and molar refractivity between 40-130 (Yusuf et al., 2022).

Table 3. Drug Scan Results.

No	Compounds name	Molecular Weight (g/mol)	Log P	Hydrogen Bond Donor	Hydrogen bond acceptor	Molar Refractory
1	5-Hidroksi-3,7,4'-trimetoksiflavon	328	2,9	1	6	86,5
2	Carvol	150	2,48	0	1	46,3
3	Kaempferol	285	0,75	3	6	70,87
4	Allopurinol	136	0,05	2	4	33,34

Result of Molecular Docking and Visualization

Based on the docking results in Table 4, it can be seen that there are several compounds with lower Binding energy (ΔG) and inhibition constant (Ki) values compared to the reference drug (allopurinol). Subsequently, 2 compounds were selected which have low Binding energy (ΔG) and inhibition constant (Ki) values and meet the toxicity, pharmacokinetics, and Lipinski's test criteria, namely the compound 5-Hydroxy-3,7,4'-trimethoxyflavone with a Binding energy value (ΔG) of -8.89 kcal/mol and an inhibition constant (Ki) of 306.54 nM, as well as the compound kaempferol with a Binding energy of -8.1 kcal/mol and Ki of 1.15 µM. Subsequently, visualization was performed using the reference drug and these selected compounds.

Table 4, Molecular Docking Results.

No	Compounds name	*Binding energy* (kcal/mol)	Ki	Run
1	5-Hidroksi-3,7,4'-trimetoksiflavon	-8,89	306,54 nM	23
2	Carvol	-7,22	5,09 uM	21
3	Kaempferol	-8,1	1,15 uM	70
4	Allopurinol	-5,83	53,39 µM	

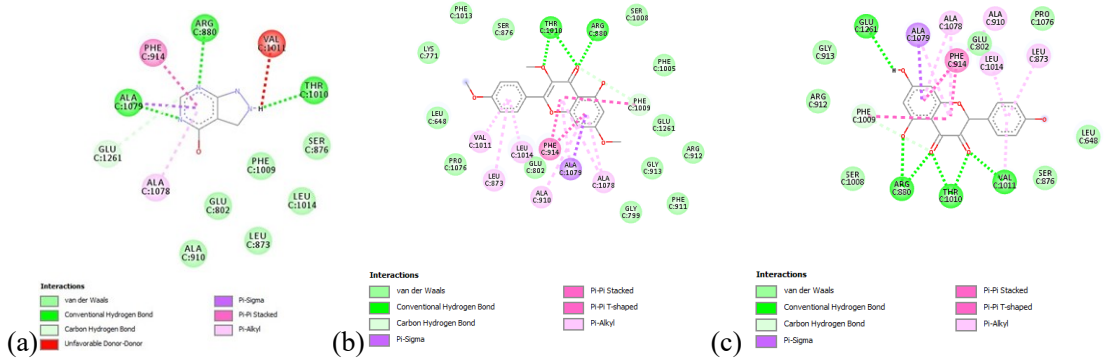

Figure 5 Visualization of the Interaction of Receptor 3NVW with compounds (a) Allopurinol, (b) 5-Hydroxy-3,7,4'-trimethoxyflavone, and (c) Kaempferol.

In Figure 4 and Table 5, the visualization results show that the reference drug, allopurinol, forms hydrogen bonds with the amino acid residues ALA1079, ARG880, THR1010, and GLU1261. In the compound 5-Hydroxy-3,7,4'-trimethoxyflavone, hydrogen bonds are formed with the amino acid residues ARG880, THR1010, PHE1009, and there are similarities with allopurinol, namely ARG880 and THR1010. Meanwhile, in the kaempferol compound, hydrogen bonds are formed with the amino acid residues ARG880, THR1010, VAL1011, GLU1261, PHE1009, and it shares 3 amino acid residues with allopurinol, namely ARG880, THR1010, and GLU1261. In addition to the amino acid residues involved in hydrogen bonds, there are also amino acid residues involved in hydrophobic bonds. In allopurinol, the amino acid residues that participate in hydrophobic bonding are PHE914 and ALA1078. In the compound 5-Hydroxy-3,7,4'-trimethoxyflavone, the residues that contribute to hydrophobic bonding are ALA1078, ALA1079, PHE914, ALA910, LEU1014, LEU873, VAL1011, and has 2 amino acid residues that are the same as allopurinol, namely PHE914 and ALA1078.

Table 5. Results of the visualization of the interaction bonds between 5-Hydroxy-3,7,4'-trimethoxyflavone, Kaempferol, and allopurinol towards the receptor.

No	Compounds name	Hydrogen bond	Hydrophobic bonds
1	5-Hidroksi-3,7,4'-trimetoksiflavon	ARG880, THR1010, PHE1009	ALA1078, ALA1079, PHE914, ALA910, LEU1014, LEU873, VAL1011
2	Kaempferol	ARG880, THR1010, VAL1011, GLU1261, PHE1009	ALA1078, ALA1079, PHE914, ALA910, LEU1014, LEU873
3	Allopurinol	ALA1079, ARG880, THR1010, GLU1261	PHE914, ALA1078, VAL1010

Result of Molecular Dynamic Simulation

The selected test compounds are 5-Hydroxy-3,7,4'-trimethoxyflavone and kaempferol, along with the comparator drug (allopurinol), and will proceed to the molecular dynamics simulation stage using Desmond software. The result can be seen in Figure 5.

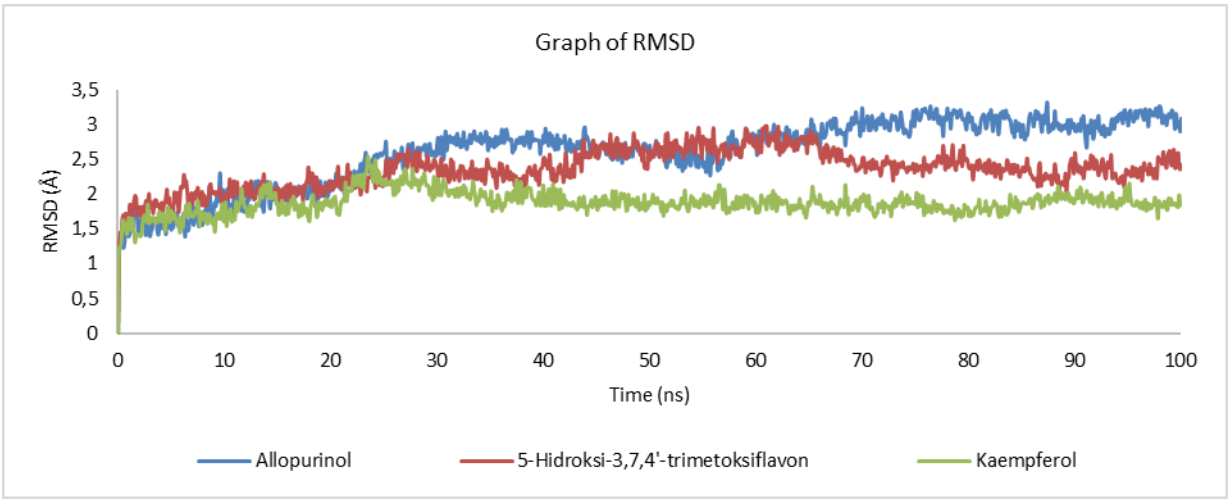

Figure 6. RMSD Value Graph of Allopurinol, 5-Hydroxy-3,7,4'-trimethoxyflavone and Kaempferol.

Based on the figure 5 indicate an increase. The RMSD value for allopurinol starts stabilizing at 30-50 ns with an RMSD value of ± 2.7 Å, then fluctuates and stabilizes again from 60-80 ns with an RMSD value of ± 3 Å, after which it fluctuates again and stabilizes at 93-100 ns with an RMSD value of ± 3 Å. For the compound 5-Hydroxy-3,7,4'-trimethoxyflavone, it starts stabilizing at 30-40 ns with an RMSD value of ± 2.3 Å, then fluctuates and stabilizes again from 44-58 ns with an RMSD value of ± 2.5 Å, fluctuates again, and stabilizes at 70-77 ns and 80-85 ns with an RMSD value of ± 2.4 Å. For the kaempferol compound, it starts stabilizing at 40-56 ns with an RMSD value of ± 1.9 Å, then fluctuates and stabilizes again at 57-64 ns with an RMSD value of ± 1.8 Å, then fluctuates again and stabilizes at 95-100 ns with an RMSD value of ± 1.8 Å.

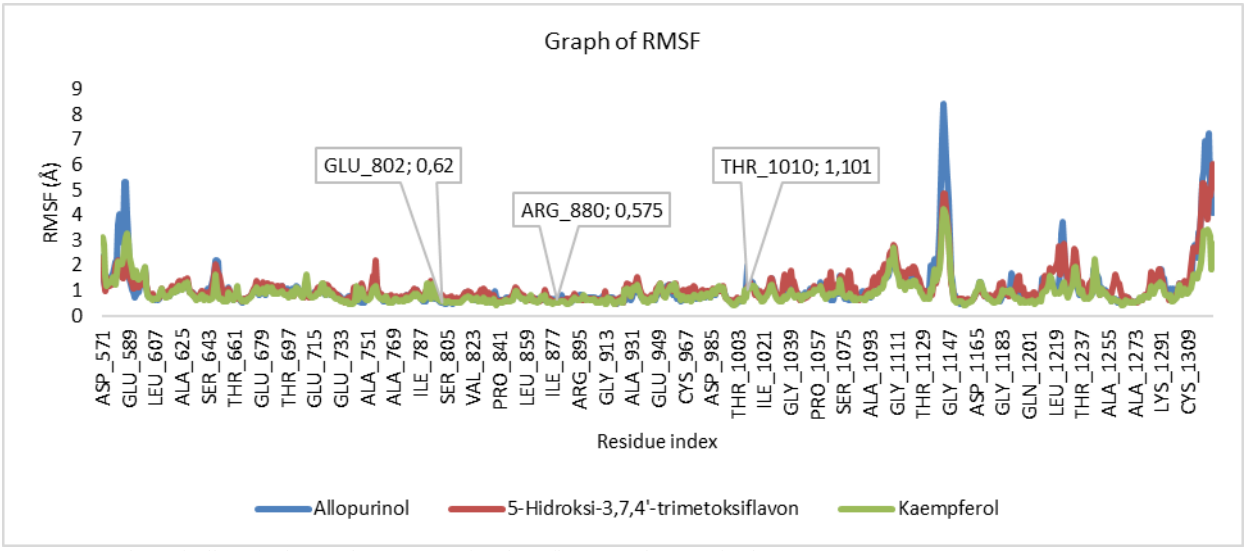

Figure 7. RMSF values of Allopurinol, 5-Hydroxy-3,7,4'-trimethoxyflavone and Kaempferol.

The Root Mean Square Fluctuation parameter or RMSF is analyzed to determine the fluctuations of ligand interactions with amino acids during the simulation[15]. Based on the RMSF analysis results in Figure 7, it can be seen that the fluctuations of the compounds Allopurinol, 5-Hydroxy-3,7,4'-trimethoxyflavone, and Kaempferol show almost identical fluctuation movements. Based on the analysis results in Table 6, the compound 5-Hydroxy-3,7,4'-trimethoxyflavone has 2 amino acid similarities with allopurinol as a comparison, which are GLU802 and SER876. Meanwhile, the compound kaempferol has 3 amino acid similarities with allopurinol, namely THR1010, VAL1011, and ALA1078.

Table 6. Amino acids from molecular dynamics.

No	Compounda name	Molecular Dynamic Result
1	5-Hidroksi-3,7,4'-trimetoksiflavon	**GLU802**, **SER876**, PHE914, ARG880, ALA1079, SER1080, GLU1261
2	Kaempferol	ARG880, SER1008, **THR1010**, **VAL1011**, **ALA1078**, ALA1079, GLU1261
3	Allopurinol	**GLU802**, **SER876**, **THR1010**, **VAL1011**, LYS771, **ALA1078**

Discussion

Protein preparation with the aim of optimizing ligand-receptor interactions by separating water molecules and standard ligands from the receptor. This separation of water molecules is performed to prevent the formation of hydrogen bonds between the ligand and water molecules. If the separation of water molecules is not carried out, the binding process will become increasingly complex, resulting in longer time requirements and the presence of one ligand will hinder other ligands from binding to the active site. Separation is also carried out on natural ligands present on the receptor to be used in the docking validation process.

Based on the visualization results in Figure 2, it can be seen that the interaction of the 3NVW receptor has hydrogen bonds with the amino acid residues ARG880, THR 1010, and GLU 802. Meanwhile, there are hydrophobic bonds with the amino acid residues PHE914, ALA1078, ALA1079, and PHE1009. Based on the overlay results in Figure 3b, it can be seen that the position of the natural ligand from redocking, which is turquoise in color, is similar to the position of the natural ligand in crystallography, which is red.

Based on Table 2, it can be seen that there are 9 test compounds that passed the toxicity test. In the LD50 parameter, all compounds are predicted to have a high LD50 value. In addition, for the parameter of hepatotoxicity, the compounds are not toxic to the liver. Furthermore, 9 test compounds and a comparator drug that passed the toxicity test were continued with pharmacokinetic testing.

Protonation aims to adjust the pH of the test compound to the physiological pH of the human body, which is 7.4. Subsequently, conformation is carried out to maintain the position of the most stable molecule when interacting with the active site of the receptor. Toxicity testing on test compounds to observe the toxic effects of a substance on biological systems. In the LD50 parameter, all compounds are predicted to have high LD50 values. According to (Thahara et al., 2022), the higher the LD50 value, the lower the toxicity, indicating that the compound is safe.

In pharmacokinetic testing, ADME (Absorption, Distribution, Metabolism, and Excretion) was analyzed using the website https://preadmet.webservice.bmdrc.org/. The parameters observed included Human colon adenocarcinoma (Caco-2), Human Intestinal Absorption (HIA), and Plasma Protein Binding (PPB). The results of the pharmacokinetic testing can be seen in Table 2 wich states that all test compounds have moderate permeability or the ability to penetrate the intestinal epithelial cell barrier moderately, has an HIA value between 70-100% which means it has a high absorption capability, A PPB value > 90% indicates an inactive nature. Meanwhile, the other 3 test compounds have values < 90%, which means they are loosely bound to plasma proteins, allowing them to act on the target and produce a biological response.

Human colon adenocarcinoma (Caco-2) is a parameter that indicates permeability capability used to determine the transfer of drugs through intestinal epithelial cells derived from human colorectal adenocarcinoma with dual transport pathways in vitro (Chintha et al., 2020). Based on Table 2, it can be seen that all test compounds have moderate permeability or the ability to penetrate the intestinal epithelial cell barrier moderately. The administration of drugs can be challenging because the human digestive tract is very complex and has a number of physiological barriers that affect drug delivery. These challenges include poor drug solubility, poor drug stability, and low drug permeability across the mucosal barrier (Pasaribu, 2024).

The Human Intestinal Absorption (HIA) parameter is used to predict the drug absorption process in the intestine by looking at the results of adding bioavailability to absorption, which is evaluated from the excretion ratio through bile, feces, and urine (Monikasari et al., 2023). Based on Table 3, it can be seen that all test compounds have HIA values between 70-100%, which means they have high absorption capacity. The Plasma Protein Binding (PPB) parameter shows the ability of compounds to bind to plasma proteins. Compounds that are strongly bound to plasma proteins will be inactive; only free and unbound drugs (free-bound) can act on targets, resulting in a biological response and can enter the elimination process (Rahmawaty et al., 2022). As seen in Table 2, 6 compounds have PPB values > 90%, meaning they are inactive. In contrast, the other 3 test compounds have values < 90%, which means they are free-bound to plasma proteins, allowing them to act on targets and produce a response. From the analysis of these three parameters, it can be concluded that the 3 test compounds have good pharmacokinetic properties in the body.

The molecular weight parameter relates to the ability of a compound to cross biological membranes during the distribution process. Compounds with a molecular

weight <500 g/mol can easily penetrate biological membranes, while compounds with a molecular weight >500 g/mol will have difficulty crossing the cell membrane, thereby hindering the distribution process. The Log P parameter or Logarithm of the Partition Coefficient indicates a compound's ability to dissolve in biological fluids. A good Log P value is less than 5, as higher Log P values indicate greater hydrophobicity in the compound. Hydrophobic compounds are typically more toxic because they stay longer in the lipid bilayer and spread throughout the body, which reduces their ability to specifically bind to the intended target enzyme. On the other hand, a Log P value that is too low means the compound is more hydrophilic and may not be able to pass through the lipid bilayer effectively (Kelutur et al., 2020).

The number of hydrogen bond donors and acceptors is related to the biological activity of a drug. The greater the number of hydrogen bonds, the higher the energy required for the absorption process to occur. The molar refractivity parameter is the total polarizability value of a drug compound, where non-polar compounds will form momentum that causes them to bind with receptors, whereas polar ones play a role in the elimination of metabolic residues from the body (Frimayanti et al., 2021). According to Table 3, it can be seen that all tested compounds meet all of Lipinski's parameters, so all tested compounds can be considered as candidates for oral drugs. The oral dosage forms include tablets, capsules, syrups, and lozenges (Nurhikma et al., 2024).

Molecular Docking is the process of anchoring a molecule to a receptor through computer representation. The parameters analyzed from this process are the Binding energy (ΔG) and Inhibition constant (Ki) (Ikhlas et al., 2023). Binding energy and the inhibition constant are related to binding affinity. The lower the binding affinity, the less energy is required for the compound to bind or interact with the target receptor. A low free binding energy value indicates that the formed ligand-protein binding complex will be more stable. If the ligand's binding to the receptor is more stable, it can be predicted that its activity will also be greater (Faqiha et al., 2022). The free binding energy value is related to the Ki value; the lower the free binding energy, the lower the Ki value (Fakih et al., 2021). A lower Ki value means a smaller concentration of molecules is required to inhibit the target receptor (Kartika & Ruswanto, 2021).

This visualization is performed to understand the interactions that occur between ligands and receptors. The interactions analyzed are hydrogen bonds and hydrophobic interactions, as both types of bonds can influence the physicochemical properties of drugs and the stability of the conformation that occurs. Hydrogen bonds are bonds that occur between hydrogen atoms and N, O, or F atoms (Vinsiah & Fadhillah, 2018). Therefore, hydrogen bonds contribute to the affinity of a molecule for a target protein. If there are many hydrogen bonds accompanied by hydrophobic bonds, it can be said that the interaction between the ligand and receptor is a strong interaction. Hydrophobic bonds are interactions between amino acids on the ligand and receptor that help maintain the binding conformation (Zubair et al., 2020). Hydrophobic bonds combine polar areas of the drug molecule with polar areas of the biological receptor. Hydrophobic interactions can affect the stability of the bond between the compound and the receptor.

In allopurinol, the amino acid residues that play a role in hydrophobic bonding are PHE914 and ALA1078. In the compound 5-Hydroxy-3,7,4'-trimethoxyflavone, the amino acid residues that contribute to hydrophobic bonding are ALA1078, ALA1079, PHE914, ALA910, LEU1014, LEU873, VAL1011, and it has 3 amino acid residues that are the same as allopurinol, namely ALA1078, PHE914, and VAL1011. In the kaempferol compound, the amino acid residues involved in hydrophobic bonding are ALA1078, ALA1079, PHE914, ALA910, LEU1014, LEU873, and it has 2 amino acid residues that are the same as allopurinol, which are PHE914 and ALA1078. The similarity in the amino acid residues of hydrogen bonds and hydrophobic bonds between the test compound and the comparator (allopurinol) indicates that the test compound has similar activity to allopurinol which is capable of inhibiting the activity of the enzyme xanthine oxidase (Dari, 2022).

This RMSD analysis aims to compare how the position of the ligand-receptor complex changes over time during the simulation process. The RMSD results for the compounds betagirin and 3-Cyclohexene-1-methanol along with allopurinol indicate an increase in RMSD values, which suggests that the protein structure has started to open and the ligand is beginning to search for a suitable binding location on the receptor (Muttaqin, 2019). Based on the RMSD analysis results, the compound Kaempferol has more stable interactions compared to the compound 5-Hydroxy-3,7,4'-trimethoxyflavone and allopurinol because it has a lower RMSD value and the stability of that compound tends to be more constant.

The Root Mean Square Fluctuation parameter or RMSF is analyzed to determine the fluctuations of ligand interactions with amino acids during the simulation[15]. Based on the RMSF analysis results in Figure 7, it can be seen that the fluctuations of the compounds Allopurinol, 5-Hydroxy-3,7,4'-trimethoxyflavone, and Kaempferol show almost identical fluctuation movements. In the compound 5-Hydroxy-3,7,4'-trimethoxyflavone, the amino acid residues that experienced the highest fluctuations were THR1319, GLY1320, and LYS1326, while the lowest fluctuations were observed in the amino acid residues ILE1001, ALA919, and ALA881. In the compound Kaempferol, the highest fluctuations occurred in the amino acid residues GLU1143, THR1144, and ASN1145, while the lowest fluctuations were seen in the amino acid residues CYS999, ILE1000, and ILE1001.

Conversely, in the reference drug Allopurinol, the highest fluctuations were observed in the amino acid residues PHE1142, GLU1143, and THR1144, while the lowest fluctuations occurred in the amino acid residues ILE1000, ILE1001, and ARG804.

Amino acid residues with low fluctuation will have low flexibility and demonstrate more stable binding interactions, allowing them to play a role in the active site of ligand-receptor binding. Meanwhile, amino acid residues with high fluctuation will have high flexibility and show less stable interactions because the position of these amino acids undergoes many changes during dynamic molecular simulation (Mardianingrum et al., 2021). Based on the analysis results, GLU802, ARG880, and THR1010 are important amino acid residues or constitute the binding active site in the xanthine oxidase receptor. Figure 6 shows that the residues GLU802, ARG880, and THR1010 have low fluctuation and do not exhibit high flexibility during the simulation process. Hence, it can be said that the test compound can provide activity as an antagonist against the xanthine oxidase receptor.

The compatibility of amino acids with the comparative drug is one of the parameters in molecular dynamic studies. The more amino acids that match with the comparative drug, it can be assumed that the compound has an inhibitory ability that is almost the same or even better than that of the comparative drug (Frimayanti et al., 2021). In addition to the RMSD and RMSF plots, the stability of the compound is also supported by the interactions between the compound and the receptor. Based on the analysis results in Table 6, the compound 5-Hydroxy-3,7,4'-trimethoxyflavone has 2 amino acid similarities with allopurinol as a comparison, which are GLU802 and SER876. Meanwhile, the compound kaempferol has 3 amino acid similarities with allopurinol, namely THR1010, VAL1011, and ALA1078. Based on this, the compound kaempferol has the potential to be a candidate for antihyperuricemia drugs and is assumed to be able to bind to receptors and be stable due to having many amino acid similarities with allopurinol as a comparative drug.

CONCLUSION

The compound with the most stable interaction from java cardamom (*Wurfbania compacta*) leaf with receptors that play a role in inhibiting xanthine oxidase enzyme in silico is the compound kaempferol with a Binding energy value of -8.1 kcal/mol and Ki 1.15 uM (micromolar). Kaempferol has a stable interaction based on RMSD and RMSF analysis and shares three amino acids with allopurinol, namely THR1010, VAL1011, and ALA1078, so it can be said to have potential as a candidate for antihyperuricemia drugs. Therefore, further research related to in vitro and in vivo tests needs to be carried out to determine the actual biological conditions that occur in the body before binding to the target receptor that causes hyperuricemia.

Acknowledgements: The authors gratefully acknowledge the support of Ministry of Education and Culture in providing funds. We also thank the research team and Perjuangan University of Tasikmalaya for their support.

Authors' Contributions: Ali Nofriyaldi designed the study. Nitya Nurul Fadilah & Selvy Isnaeni carried out the laboratory work. Ali Nofriyaldi analyzed the data and wrote the manuscript. All authors read and approved the final version of the manuscript

Competing Interests: The authors declare that there are no competing interests.

Funding: This funding is provided by the Ministry of Education and Culture under the Fundamental Research scheme.

REFERENCES

Chintha, C., Carlesso, A., Gorman, A. M., Samali, A., & Eriksson, L. A. (2020). Molecular modeling provides a structural basis for PERK inhibitor selectivity towards RIPK1. *RSC Advances*, *10*(1), 367–375. https://doi.org/10.1039/C9RA08047C

Dari, D. W. (2022). Uji Potensi Senyawa Metabolit Sekunder Tanaman Putri Malu (Mimosa pudica L.) Sebagai Inhibitor Xanthine Oxidase Secara In Silico. *Jurnal Ilmu Kefarmasian*, *3*(2).

Dermawan, D., Sumirtanurdin, R., & Dewantisari, D. (2019). Molecular Dynamics Simulation of Estrogen Receptor Alpha Against Andrografolid as Anti Breast Cancer. *Indonesian Journal of Pharmaceutical Science and Technology*, 65–76. https://doi.org/10.24198/ijpst.v6i2.18168

Dirgantara, S., Insanu, M., & Fidrianny, I. (2022). Evaluation of xanthine oxidase inhibitory, antioxidative activity of five selected Papua medicinal plants and correlation with phytochemical content. *Pharmacia*, *69*(4), 965–972. https://doi.org/10.3897/pharmacia.69.e91083

Du, L., Zong, Y., Li, H., Wang, Q., Xie, L., Yang, B., Pang, Y., Zhang, C., Zhong, Z., & Gao, J. (2024). Hyperuricemia and its related diseases: Mechanisms and advances in therapy. *Signal Transduction and Targeted Therapy*, *9*(1), 212. https://doi.org/10.1038/s41392-024-01916-y

Elis Nurul Ikhlas, Lina Rahmawati Rizkuloh, & Richa Mardianingrum. (2023). Analisa In Silico Senyawa Biji Lada Hitam (Piper nigrum L.) Terhadap Aktivitas Antioksidan. *JURNAL RISET RUMPUN ILMU KESEHATAN*, *2*(2), 301–321. https://doi.org/10.55606/jurrikes.v2i2.1815

Fakih, T. M., Wisnuwardhani, H. A., Dewi, M. L., Ramadhan, D. S. F., Hidayat, A. F., & Prayitno, R. (2021). Simulasi Dinamika Molekuler Senyawa Asam Ferulat dan Turunannya dari Kulit Buah Nanas (Ananas comosus) sebagai Inhibitor Enzim Tirosinase. *Jurnal Sains Farmasi & Klinis*, *8*(2), 208. https://doi.org/10.25077/jsfk.8.2.208-220.2021

Faqiha, A. F., Indrawijaya, Y. Y. A., Suryadinata, A., Amiruddin, M., & Mutiah, R. (2022). Potensi Senyawa Nitazoxanide dan

Arbidol sebagai Antivirus SARS-CoV-2 terhadap Reseptor NSP5 (7BQY dan 2GZ7) dan ACE2 (3D0G dan 1R4L). *Journal of Food and Pharmaceutical Sciences*, 570–583. https://doi.org/10.22146/jfps.3393

Frimayanti, N., Djohari, M., & Khusnah, A. N. (2021). Molekular Docking Senyawa Analog Kalkon sebagai Inhibitor untuk Sel Kanker Paru-Paru A549. *JURNAL ILMU KEFARMASIAN INDONESIA*, *19*(1), 87. https://doi.org/10.35814/jifi.v19i1.765

Kartika, C., & Ruswanto, R. (2021). *Studi In Silico Perbandingan Aktivitas Antikanker Payudara dari Senyawa Kuersetin dengan Derivatisasi Kuersetin terhadap Protein HER-2*.

Kelutur, F. J., Mustarichie, R., & Umar, A. K. (2020). Virtual Screening Kandungan Senyawa Kipas Laut (Gorgonia mariae) sebagai Antiasma. *ALCHEMY Jurnal Penelitian Kimia*, *16*. https://doi.org/10.20961/alchemy.16.2.39965.199-210

Liu, N., Xu, H., Sun, Q., Yu, X., Chen, W., Wei, H., Jiang, J., Xu, Y., & Lu, W. (2021). The Role of Oxidative Stress in Hyperuricemia and Xanthine Oxidoreductase (XOR) Inhibitors. *Oxidative Medicine and Cellular Longevity*, *2021*(1), 1470380. https://doi.org/10.1155/2021/1470380

Mardianingrum, R., Bachtiar, K. R., Susanti, S., Aas Nuraisah, A. N., & Ruswanto, R. (2021). Studi In Silico Senyawa 1,4-Naphthalenedione-2-Ethyl-3-Hydroxy sebagai Antiinflamasi dan Antikanker Payudara. *ALCHEMY Jurnal Penelitian Kimia*, *17*(1), 83. https://doi.org/10.20961/alchemy.17.1.43979.83-95

Mehmood, A., Ishaq, M., Zhao, L., Safdar, B., Rehman, A., Munir, M., Raza, A., Nadeem, M., Iqbal, W., & Wang, C. (2019). Natural compounds with xanthine oxidase inhibitory activity: A review. *Chemical Biology & Drug Design*, *93*(4), 387–418. https://doi.org/10.1111/cbdd.13437

Mekky, R. H., Hegazy, M. M., Afifi, W. M., Mostafa, A. E., Abbass, H. S., & Abd-Elraouf, M. (2023). Composition and Functional Properties of Cardamom Leaves. In M. F. Ramadan (Ed.), *Cardamom (Elettaria cardamomum): Production, Processing and Properties* (pp. 157–178). Springer International Publishing. https://doi.org/10.1007/978-3-031-35426-7_12

Monikasari, H., Mardianingrum, R., & Bachtiar, K. R. (2023). Kajian Dari Senyawa Umbi Bawang Putih (Allium sativum L.) terhadap Interaksi Aktif sebagai Analgetik-Antiinflamasi Secara in Silico. *Jurnal Farmasi Sandi Karsa*, *9*(2), 60–73. https://doi.org/10.36060/jfs.v9i2.96

Muttaqin, F. Z. (2019). STUDI MOLECULAR DOCKING, MOLECULAR DYNAMIC, DAN PREDIKSI TOKSISITAS SENYAWA TURUNAN ALKALOID NAFTIRIDIN SEBAGAI INHIBITOR PROTEIN KASEIN KINASE 2-α PADA KANKER LEUKEMIA. *Pharmacoscript*, *2*(1), 49–64. https://doi.org/10.36423/pharmacoscript.v2i1.241

Nofriyaldi, A., Rahmawati, N. A., Nurzaman, M. H., Fadilah, N. N., & Isnaeni, S. (2025). In Silico Study of Java Cardamom (Amomum compactum) Fruit Derivative Compounds as Antihyperuricemia. *Tropical Journal of Natural Product Research*, *7*(9). https://doi.org/10.26538/tjnpr/v9i7.10

Nurhikma, E., Jannah, S. R. N., Wijaya, D. P., Zulham, Z., Astari, C., Nofriyaldi, A., Purnamasari, N., & Indalifiany, A. (2024). *Farmasetika* (1st ed.). Widina Media Utama.

Pasaribu, E. (2024). Biofarmasetika Obat Sediaan Oral. In *Biofarmasetika*. Media Sains Indonesia.

Rahmawaty, A., Cahyani, F. R., Safitri, N., Hapitria, E. N., & Megantara, S. (2022). UJI IN SILICO KANDUNGAN SENYAWA TANAMAN ANGGUR (Vitis vinifera L.) UNTUK KANDIDAT OBAT ANTI HIPERLIPIDEMIA. *Majalah Farmasi dan Farmakologi*. https://doi.org/10.20956/mff.v26i2.19859

Ruswanto, R., Mardianingrum, R., Nofianti, T., Pratita, A. T. K., Naser, F. M., & Siswandono, S. (2023). Design and computational study of the thiourea–cobalt(III) complex as an anticancer candidate. *Journal of Pharmacy & Pharmacognosy Research*, *11*(3), 499–516. https://doi.org/10.56499/jppres23.1622_11.3.499

Ruswanto, T., Nofianti, T., Mardianingrum, R., Lestari, A., & Sepriliani, T. (2018). Desain dan Studi In Silico Senyawa Turunan Kuwanon-H sebagai Kandidat Obat Anti HIV Design and In Silico Study of Kuwanon-H as Anti HIV Drug Candidate. *Jurnal Kimia VALENSI*, *4*(1).

Sagitasa, S., Elizabeth, K., Sulaeman, L. I., Rafasafly, A., Syafra, D. W., Kristande, A., & Muchtaridi, M. (2021). Studi In Silico Senyawa Aktif Daun Singawalang (Petiveria alliacea) Sebagai Penurun Kadar Glukosa Darah Untuk Pengobatan Penyakit Diabetes Melitus Tipe-2. *Chimica et Natura Acta*, *9*(2). https://doi.org/10.24198/cna.v9.n2.34083

Sari, I. W., Junaidin, J., & Pratiwi, D. (2020). STUDI MOLECULAR DOCKING SENYAWA FLAVONOID HERBA KUMIS KUCING (Orthosiphon stamineus B.) PADA RESEPTOR α-GLUKOSIDASE SEBAGAI ANTIDIABETES TIPE 2. *Jurnal Farmagazine*, *7*(2), 54. https://doi.org/10.47653/farm.v7i2.194

Sovia, F., A-Adawiyah, R., & Yuliana, T. P. (2025). Evaluation of the Effectiveness of Renggak Leaf Extract (Amomum dealbatum Roxb.) as an Antihyperuricemic in Mice. *Journal of Pharmaceutical and Sciences*, *8(2)*, 1082–1090. https://doi.org/10.36490/journal-jps.com

Thahara, C. A., Rizarullah*, R., Atika, R. A., & Wahab, A. (2022). Potensi Pendekatan in Silico Sebagai Penghambat Aktivitas Protein Protease Utama SARS-CoV-2 dari Tiga Senyawa Tanaman Obat Jahe Merah. *Jurnal IPA & Pembelajaran IPA*, *6*(3), 207–218. https://doi.org/10.24815/jipi.v6i3.24914

Vinsiah, R., & Fadhillah, F. (2018). Studi Ikatan Hidrogen Sistem Metanol-Metanol dan Etanol-Etanol dengan Metode Molekular Dinamik. *Sainmatika: Jurnal Ilmiah Matematika dan Ilmu Pengetahuan Alam*, *15*(1), 14. https://doi.org/10.31851/sainmatika.v15i1.1739

Yusuf, M. J., Widiyana, A. P., & Damayanti, D. S. (2022). *STUDI IN SILICO AFINITAS SENYAWA AKTIF BIJI KACANG MERAH*.

Zubair, M. S., Maulana, S., & Mukaddas, A. (2020). Penambatan Molekuler dan Simulasi Dinamika Molekuler Senyawa Dari Genus Nigella Terhadap Penghambatan Aktivitas Enzim Protease HIV-1: Molecular Docking and Molecular Dynamics Simulation of Compounds from Nigella Genus on Protease HIV-1 Enzyme Inhibitors. *Jurnal Farmasi Galenika (Galenika Journal of Pharmacy) (e-Journal)*, *6*(1), 132–140. https://doi.org/10.22487/j24428744.2020.v6.i1.14982