

THE POTENTIAL INHIBITION OF XANTHINE OXIDASE BY PHENOLIC AND FLAVONOIDS DATE (*Phoenix dactylifera*) WITH MOLECULAR DOCKING METHOD

Akhmad Endang Zainal Hasan¹, Laksmi Ambarsari¹, Karichsa Hariana¹

^{1,2,3} Department of Biochemistry, IPB University

Corresponding Author: zainalhasan@apps.ipb.ac.id

Abstract: Dates is fruit of palm trees that mostly grow in the Middle East. Dates contain phenolic acids and flavonoids that have antioxidants and potentially inhibit the ability of xanthine oxidase. This study aims to identify the strength of the molecular interaction between flavonoid compounds and phenolic acids in dates with the xanthine oxidase enzyme. This research was conducted by site directed docking with *in-silico* method. The size of the center of retardation used in this research is $x = 26.569$, $y = 9.985$, and $z = 113.088$ and the retardation volume of $x = 14$, $y = 14$, and $z = 16$. Inhibition by flavonoid and phenolic acid compounds has produces good inhibition strength shown by Gibbs free energy which is negative. The best compound from the flavonoid and date palm phenolic acid group which has the best inhibitory power on the xanthine oxidase enzyme based on the analysis of the bonds formed is syringic acid. Based on the number of bonds formed between the xanthine oxidase enzyme and singingic acid, more bonds were formed than the standard ligand (allupurinol) without reducing the number of bonds formed between allupurinol and the xanthine oxidase enzyme.

Keywords: Dates, fenolic Acid, flavonoid, molecular docking, xanthine oxidase.

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1. INTRODUCTION

Xanthine oxidase (XOD) is an enzyme that has a homodimer with a molecular weight of 290 kDA. Each enzyme subunit consists of an N-terminal with two centers namely Fe-S, one coenzyme FAD, one C-terminal molybdopterin containing 4 redox centers (Enroth et al., 2000). XOD is an enzyme that catalyzes the oxidation of hypoxanthine to xanthine and xanthine to uric acid. Excess uric acid in human blood is a major factor in the onset of gout. These conditions can occur due to gene abnormalities or carrier traits, high consumption of purine-containing foods, and purine metabolism disorders (Murray et al. 2012).

Treatment of gout disease generally uses synthetic drugs, namely allopurinol. These drugs can inhibit the activity of the enzyme xanthine oxidase. However, the use of these drugs has side effects such as hypersensitivity syndrome, Steven Johnson syndrome, and renal

toxicity (Umamaheswari et al. 2011). If allopurinol is used for a long time, it can cause kidney failure, impaired liver function, and allergic reactions (Murata et al. 2009). The negative side effects caused by the use of these drugs are quite worrying, so a new, safer alternative is needed.

Natural products that have the ability to become xanthine oxidase inhibitors, one of which is sidaguri herb. These plants are reported to contain flavonoid compounds that can inhibit xanthine oxidase activity with greater inhibitory power than commercial antigout herbal products (Hidayat 2007). Earring plants are also reported to act as inhibitors of xanthine oxidase activity (Khairunnisa. 2013). The utilization of these two plants is still not optimal, because they cannot be consumed directly. A new alternative in the form of intake of food that can be consumed directly can be a promising breakthrough to prevent the disease.

Dates are fruits that can be consumed directly and contain compounds of phenolic acid, cinnamic acid, flavonoids, flavonols, sterols, procyanidins, carotenoids, anthocyanins, fiber, vitamins, and minerals (Ismail & Iryani, 2013). Siahpoosh et al. (2016) showed that the content of flavonoids, phenolic acids, and oligoproanthocyanidins in dates can be inhibitors of the action of the xanthine oxidase enzyme, but the molecular interactions of these compounds with the enzyme xanthine oxidase are still unknown.

Molecular interactions of flavonoid compounds and phenolic acids in dates with xanthine oxidase enzymes can be studied using the molecular anchoring method. The use of this method can provide benefits by narrowing the research focus, saving research costs, and streamlining research time (Sharma and Jha, 2010). Cao (2010) stated that the active site of the xanthine oxidase enzyme consists of two amino acids, namely Arg880 and Glu802. Molecular bonding with the compounds in the dates can be carried out in a directed manner to the active site of the enzyme. This study aims to identify the strength of the molecular interaction between flavonoid compounds and phenolic acids in dates with the xanthine oxidase enzyme. This research is expected to provide information in the form of compounds that have the best molecular interactions with the xanthine oxidase enzyme.

2. METHOD

2.1 Materials

The tools that will be used in the research are a computer with a specification of an Intel® Core™ i5 7200U processor, 4 GB of RAM (Random Access Memory) and a Microsoft® Windows™ 10 operating system. The software that will be used in the molecular anchoring preparation stage is AutoDock. Vina Tools (The Scripps Research Institute, USA), Marvin Sketch 6.0, and Discovery Studio 2016 Client. In addition, the software that will be used to visualize the tethering results in two dimensions is LigPlot+ 1.4.5. The materials used in this study were the three-dimensional structure of the xanthine oxidase enzyme protein, the two-dimensional structure of flavonoid compounds (Apigenin, Quercetin, Luteolin, Proanthocyanidin, Anthocyanins) and phenolic acids (Ferulic acid, Syringic acid, Vanillic acid, Protocatechuic acid and Coumaric acid). and its derivatives, namely Dactyliferic acid) in dates obtained from the site.

2.2 Ligand and Receptor Structure Preparation

The ligand used in this study were flavonoid compounds (apigenin, quercetin, luteolin, proanthocyanidin, anthocyanins) and phenolic acids (ferulic acid, syringic acid, vanillic acid, protocatechuic, and p-Coumaric acid and their derivatives, namely Dactyliphric acid) in dates (Al-Shwyeh, 2019). These ligand can be obtained from the PubChem Compound database in SDF (Structured Data File) format. The ligand structure was transformed into a three-dimensional

structure using Marvin Sketch 6.0 software and saved in PDB (Protein Data Bank) format. All ligand used were optimized using AutoDock Tools 1.5.6 software by adding hydrogen atoms and saved in PDBQT (Protein Data Bank, Partial Charge (Q), Atom Type (T) format.

The receptor used in this study was a protein (enzyme) xanthine oxidase with the code 1FIQ downloaded from the Protein Data Bank database via the <http://www.rcsb.org> page. The receptors used must have a maximum resolution of 2.5 and have a stable 3D structure. Protein preparation was carried out using the Discovery Studio Visualisizer 2016 software. Water molecules and ligand that were still attached to the protein were removed, then hydrogen atoms were added. After that, it is saved in the form of .pdb and the file is reopened using the AutoDockTools 1.5.6 program and then saved in the form of PDBQT. The method used is a modification of the method of Pratama et al. (2016).

2.2 Lipinski Solubility Analysis and Stability of Receptor Enzyme Structure

Before binding the ligand to the receptor, the ligand solubility analysis was carried out using Lipinski to determine the level of solubility of a ligand compound to be used. This analysis can be done through online access on the page <http://www.scfbio-iitd.res.in/software/drugdesign/lipinski.jsp>. The stability of the selected receptor can be analyzed using the Ramachandran plot. The method used is a modification of the method of Nogara et al. (2015).

2.3 Molecular Anchoring

Ligand and receptors that have been converted into PDBQT are stored in one folder on disk c. Prior to molecular anchoring, validation of the method to be used must first be carried out. Molecular docking was performed using Auto Dock Tools 1.5.6 and Auto Dock Vina software (Scripps Research Institute, USA). The Grid menu is selected to define the tethering coordinates and the Grid Box. The size and anchorage of the ligand used in site-directed docking were validated first using an inhibitor complexed in the crystal structure of the 1FIQ receptor, namely salicylic acid. Salicylic acid is a competitive inhibitor of the xanthine oxidase enzyme. All receptor and ligand documents with PDBQT format are included in the Vina folder. Furthermore, the "conf" document in the Vina folder is filled with data in the form of the document name of the receptor and ligand used, the name of the document resulting from molecular tethering, and the size and center of the anchoring coordinates obtained from validation.

The molecular docking process is carried out between the xanthine oxidase enzyme as a receptor with flavonoid compounds and phenolic acids in dates using the anchorage area (grid) adjusted to the anchoring area of the validation results, namely at positions 26.569, 9.985, and 113.088 (x, y, z) and the size of the anchorage. by 14, 14, 16 (x, y, z) using AutoDock Tools 1.5.6. Tethering is done using the cmd program. Programming commands are carried out up to the Vina folder, and entered with the command "C:\vina -config conf.txt -log log.txt" then enter. After the tethering process is complete, a document in the PDBQT format is generated. The document was converted to PDB using the Discovery Studio Visualisizer software. Another document produced is a "log" document in txt format, containing data on the change in the value of Gibbs free energy (ΔG). Then, the two documents are saved for analysis. the method used is a modification of the method of Singh et al. (2017).

2.4 Tether Result Analysis

The tethered ligand were opened using the Discovery Studio Visualisizer 2016 software. The ligand binding model with the selected receptor was the model with the lowest G and three-

dimensional (3D) visualization closest to the receptor area. The selected ligand model was combined with the receptor using the Discovery Studio Visualizer software. The result of combining the ligand with the receptor is stored in the form of .pdb and analyzed and visualized for energy and chemical bonds. Molecular interaction analysis in the form of two-dimensional (2D) analysis of hydrogen bonds and hydrophobic bonds was performed using Ligplot+ 1.4.5 software with an interaction radius of less than 5Å. The results of visualization of the test ligand and receptors are then compared with the results of visualization of the comparison ligand and receptors. The method used is a modification of the method of Pratama et al. (2016).

2. RESULT AND DISCUSSION

3.1. RESULT

3.1.1 *Ligand and Receptor Structure*

The ligand used in this study were flavonoid compounds and phenolic acids from dates fruit. Dates flavonoid compounds (apigenin, quercetin, luteolin, proanthocyanidin, anthocyanins) and date palm phenolic acids (ferulic acid, cinnamic acid, vanillic acid, Protocatechuic, and p-Coumaric acid and their derivatives, namely Dactylophric acid). The preparation of the ligand structure produces a 3D structure and contains polar hydrogen atoms. The addition of hydrogen atoms to the 3D structure is indicated by a white tube. The results of the ligand preparation are stored in pdbqt format.

The receptor used in this study is the xanthine oxidase enzyme with the code 1FIQ. The structure of the enzyme was downloaded from rcsb.org. The downloaded receptor structure contains complexed ligand and coenzymes and is shown in the red circle in Figure 1(a). The receptor structure also contains water molecules which are shown at points around the receptor. The receptor preparation resulted in a structure that was free of water molecules and ligand complexed at the receptor (Fig. 1(b)). The results of the receptor preparation are stored in pdb format and then converted into pdbqt format.



Figure 1 Structure of xanthine oxidase (Code 1FIQ) before preparation (a); after preparation (b). The red circle indicates that the receptor structure contains complexed ligands and coenzymes

3.1.2. Lipinski Solubility Analysis

Analysis of the solubility of the ligand used can be tested using Lipinski's rule. This rule serves to determine the permeability and ability of the ligand to be absorbed by the body. Ligand are considered to have good adsorption potential if they meet the two requirements of all these rules. Some of the parameters in these rules can be seen from the physicochemical characters related to the solubility of compounds in water and the permeability of compounds (Lipinski, 2004). The parameters are molecular weight <500g/mol, the number of hydrogen donors is less than 5, hydrogen acceptors is less than 10, and log P is less than 5. The results of the ligand solubility test using Lipinski's rule showed that the molecular weights of all test ligand had a molecular weight of <500g/ mole, except for proanthocyanidin which is 620g/mol. All ligand had a Log P value of less than 5, except for proanthocyanidins which had a Log P value of 5.8746. Ligand that have less than 5 hydrogen donors are anthocyanins, apigenin, p-Coumaric acid, ferulic acid, vanillic acid, Protocatechuic, and syringic acid compounds. Apart from that the ligand has more than equal to 5 hydrogen donors.

Compare with result, all ligands had less than 10 hydrogen acceptors (Table 1). All ligands to be used complied with Lipinski's rules, except for proanthocyanidins.

Tabel 1 Solubility analysis of test ligands with Lipinski's rule parameters

Test ligand	Structure	Molecular weight (g/mol)	Log P	Hydrogen Donor	Hydrogen Acceptor
Anthocyanins	C ₁₅ H ₁₁ O ₆ Cl	208	3.3806	0	1
Apigenin	C ₁₅ H ₁₀ O ₅	274	2.7309	4	5
Luteolin	C ₁₅ H ₁₀ O ₆ C ₁₅ H ₁₀ O ₇	290	2.0509	5	6
Quercetin	C ₉ H ₈ O ₃ C ₃₁ H ₂₈ O ₁₂	306	2.3961	6	7
p-Coumaric acid	C ₁₀ H ₁₀ O ₄ C ₈ H ₈ O ₄	163	0.1553	3	3
Proanthocyanidin	C ₇ H ₆ O ₄ C ₉ H ₁₀ O ₅	620	5.8746	7	1
Ferulic acid	C ₁₆ H ₁₆ O ₈	193	0.1639	1	4
Vanillic Acid		167	-0.2357	1	4
Protocatechuic acid		153	-0.5387	2	4
Syringic Acid		197	-0.2271	1	5
Dactylyphric Acid		353	1.9526	6	8

3.1.3. Stability of Xanthine Oxidase Enzyme Structure (1FIQ)

The stability of a macromolecule can be analyzed using the Ramachandran plot. The stability test is applied to show the similarity of a macromolecular crystal structure with its natural structure. The colored square, triangle, and cross symbols are visualizations of the amino acid residues of a protein on the Ramachandran plot. The results of the Ramachandran plot of the xanthine oxidase enzyme code 1FIQ showed that 93.5% (1057/1130) of amino acid residues were in the preferred region, 5.4% (61/1130) of amino acid residues were in the permitted region and 1.1% (13/1130) amino acid residues that are in the area that is not allowed (Figure 2). In addition to using the Ramachandran plot, the stability of a macromolecule can also be seen based on the resolution of a macromolecular crystal structure. Xanthine oxidase 1FIQ has a resolution of 2.5 (Enroth et al., 2000)

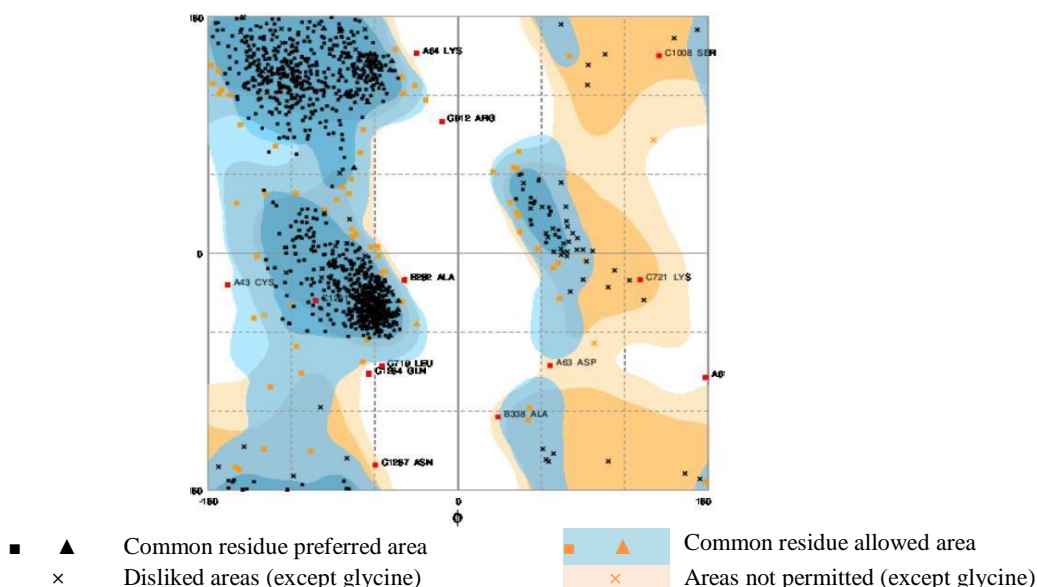


Figure 2 Diagram of Ramachandran Xanthine Oxidase 1FIQ

3.1.4. Tethering Method Validation on Vina Autodock Devices

Validation of the method was carried out by reattaching the complex inhibitor ligand to the xanthine oxidase (1FIQ) crystal structure. The inhibitory ligand used is salicylic acid. This ligand is used because it can be a competitive inhibitor of xanthine oxidase (Enroth et al. 2000). Validation is carried out by means of site targeted docking. The validation of the re-tethering can be seen in Figure 3, resulting in the center area $x=26,569$; $y=9,985$; $z=113,088$ and dimensions with a value of $x=14$; $y=14$; $z=16$ (Figure 3). The result of directed re-tethering has an affinity energy value of -7.3 kcal/mol, an RMSD value of l.b. (lower bound) of 1,019 and RMSD u.b. (upper bound) of 2,356.

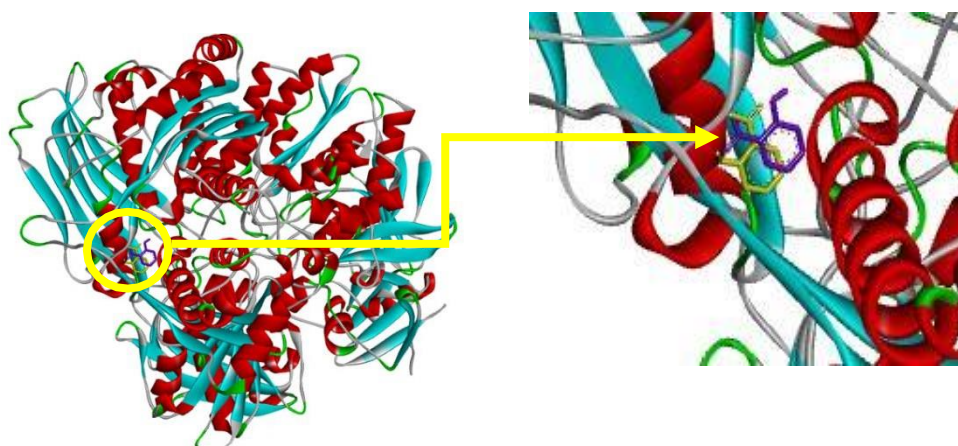


Figure 3 3D visualization of method validation results; The yellow color shows the interaction of salicylic acid (competitive inhibitor) with the initial xanthine oxidase and the purple color shows the interaction of salicylic acid with xanthine oxidase after redocking.

3.1.5. Gibbs free energy from binding of ligand and receptors

The binding of the ligand molecule produces several binding modes with different Gibbs free energies. Gibbs free energy is one of the parameters of the strength of the interaction between the ligand and the active site of the enzyme. Molecular docking of the test ligand in this study resulted in a Gibbs free energy value as shown in Figure 4. The comparison ligand produced a Gibbs free energy of -7.1. The anthocyanins ligand has the most negative Gibbs free energy value among all the test and comparison ligand, which is -7.3. The lowest Gibbs free energy value is shown in the syringic acid ligand, which is -4.7.

Another parameter used to see the strength of the interaction between the ligand and the receptor is the value of the inhibition constant (KI) (Table 2). The lower the resulting KI value indicates the greater the ability of the ligand to interact with the receptor. The value of the inhibition constant can be obtained from the equation $G=RT\ln Ki$. The R value is 1,986 cal/molK and the T value is 298 K. The highest inhibition constant value was obtained from the syringic acid ligand, which was 358.78. The lowest inhibition constant value was obtained from the anthocyanins ligand, which was 4.45. Based on the equation, the data obtained shows the smaller the Gibbs free energy, the higher the KI value.

3.1.6. Visualization of Ligand Binding Interaction with Receptors

A ligand can be attached to the receptor due to the interaction between the ligand and the amino acid residues on the receptor. Allopurinol is a drug used to inhibit the activity of xanthine oxidase. The result of binding allopurinol with xanthine oxidase enzyme produces 9 amino acid residues. Amino acid residues that play a role in binding Allopurinol with xanthine oxidase are used as benchmarks for the ability of flavonoid compounds and date phenolic acids as test ligand in inhibiting xanthine oxidase. The amino acid residues are Glu802, Arg 880, Leu1014, Phe1009, Leu873, Ala1078, Thr1010, Phe914, Ala1079. One of the visualizations of the bonding results between dactylic acid and xanthine oxidase compared to allopurinol can be seen in Figure 6. The red circle shows the amino acid residues that intersect between the test ligand used and the comparison ligand, allopurinol. The bonding residues that intersect between allopurinol and dactylifric acid are Thr1010, Arg880, Phe914, Ala1079, Ala1078, Glu802, Leu873, Leu1014, Phe1009.

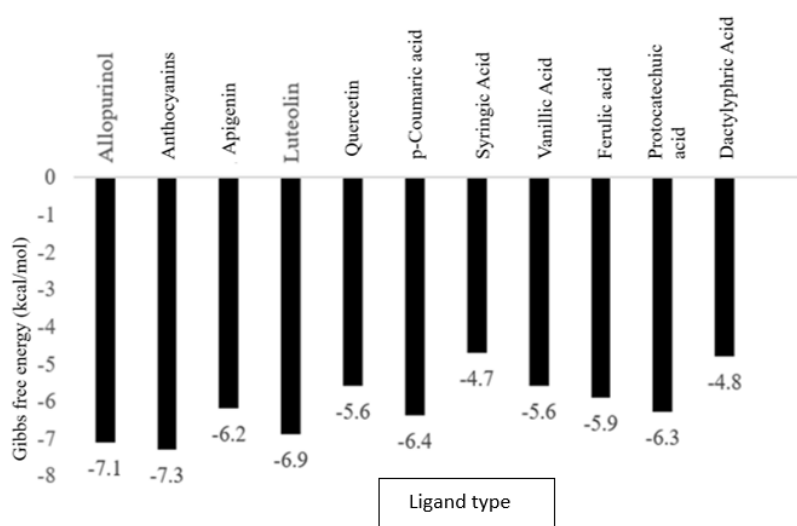


Figure 4 Gibbs free energy of bonding of the test and comparison ligand with the xanthine oxidase enzyme.

Table 2 Gibbs free energy and receptor binding KI between test and comparison ligand

Ligand	Gibbs free energy (kcal/mol)	Inhibition Constant (μ M)
Allopurinol	-7.1	6.24
Anthocyanins	-7.3	4.45
Apigenin	-6.2	28.53
Luteolin	-6.9	8.75
Quercetin	-5.6	78.54
p-Coumaric acid	-6.4	20.35
Syringic Acid	-4.7	358.78
Vanillic Acid	-5.6	78.54
Ferulic acid	-5.9	47.34
Protocatechuic acid	-6.3	24.1
Dactylyphric Acid	-4.8	303.07

The amino acid residues of other test ligands that play a role in the bonding process can be seen in Table 3. All the test ligands had almost the same residue slices. Among the amino acid residues that play a role in anchoring, there are amino acid residues that form hydrophobic bonds. Hydrophobic bonds are formed due to the presence of a phenol group belonging to the tested ligand interacting with the receptor. The number of hydrophobic bonds for each ligand can be seen in Table 3. The highest number of hydrophobic bonds formed was in the luteolin ligand as many as 14 bonds and the least hydrophobic bonds formed in the vanillic acid ligand with three bonds.

Table 3 Comparison of amino acid residues from the binding of the test ligand with the binding of the comparison ligand

Test ligand	Total Hydrophobic bond	Participated residue of Amino Acids	Binding site similarity (%)*
Allopurinol	8	Arg880, Thr1010, Phe1009, Leu1014, Phe914, Ala1079, Ala1078, Glu802, Leu873	-
Anthocyanins	13	Phe1009, Thr1010, Leu1014, Phe914, Ala1079, Ala1078, Glu802, Leu873, Leu648, Val1011, Glu1261, Ser876, Arg880.	9/9 (100)
Apigenin	9	Arg880, Thr1010, Phe914, Phe1009, Leu1014, Glu802, Ala1079, Ser876, Leu873, Glu1261, Val1011, Leu648.	8/9 (89)
Luteolin	14	Thr1010, Arg880, Phe914, Ala1079, Pro1076, Phe1009, Leu1014, Asn768, Thr803, Leu873, Glu802, Leu648, Lys 771, Val1011, Glu1261, Ser876	8/9 (89)

Quercetin	11	Thr1010, Arg880, Phe914, Ala1079, Val1011, Phe1009, Leu1014, Ser876, Leu873, Leu648, Glu802, Glu1261, Ser876, Ala1078, Pro1076, Gly799	9/9 (100)
p-Coumaric acid	8	Arg880, Phe914, Ala1079, Thr1010, Ser876, Phe1009, Leu873, Leu1014, Val1011, Glu802	8/9 (89)
Syringic Acid	8	Thr1010, Arg880, Phe914, Ala1079, Val1011, Phe1009, Leu1014, Leu873, Glu802, Ala1078, Ser1008, Glu1261	9/9 (100)
Vanillic Acid	3	Arg880, Phe914, Ala1079, Glu1261, Ala1078, Thr1010, Phe1009, Val1011, Glu802	7/9 (78)
Ferulic acid	7	Thr1010, Ser876, Phe914, Ala1079, Ala1078, Thr803, Phe1009, Val1011, Leu1014, Leu803, Glu802, Glu1261	8/9 (89)
Protocatechuic acid	8	Thr1010, Val1011, Phe914, Ala1079, Phe1009, Leu1014, Leu873, Glu802, Ser876, Arg880, Ser1008,	8/9 (89)
Dactylyphric Acid	11	Thr803, Thr1010, Arg880, Phe914, Ala1079, Ala1078, Glu1261, Glu802, Ser876, Leu873, Leu1014, Phe1009, Val1011, Pro1076	9/9 (100)

Binding Site Similarity (BSS) is the percentage of similarity of amino acid residues that play a role in the binding of the test ligand with amino acid residues in the binding of the comparison ligand as a control. The similarity of amino acid residues in the binding of the comparison ligand with the high test ligand was related to the inhibitory activity of xanthine oxidase. The absence of similarity in amino acid residues related to the test ligand and the comparison ligand proved that the test ligand could not inhibit the action of xanthine oxidase. The percentage of BSS for each test ligand anchorage can be seen in Table 3. The highest percentage of BSS was produced by anthocyanins, quercetin, syringic acid, and dactylic acid ligand, which was 100%. These ligand have similar amino acid residues that play a role in binding the same as amino acid residues that play a role in binding of receptors and comparison ligand. The lowest percentage of BSS produced by vanillic acid is 78%.

Other parameters used to observe the interaction between the ligand and the receptor include the distance and number of hydrogen bonds (Table 4). The hydrogen bonds formed at the molecular anchorage indicate the strength of the ligand interaction with the receptor used. The bond can be formed due to the presence of a hydroxyl group on the ligand attached to the receptor. Based on the results of the anchoring, the comparison ligand, allopurinol, produced a hydrogen bond of 2.93 Å. The test ligand for the flavonoid group in dates used in this study were anthocyanins, apigenin, luteolin, and quercetin ligand. Anthocyanins ligand cannot produce hydrogen bonds because they do not have a hydrogen donor. The apigenin ligand produces four hydrogen bonds with the closest distance of 2.32 and the furthest distance of 3.31. The luteolin ligand produces four hydrogen bonds with the closest distance of 2.5 and the furthest distance of 3. The quercetin ligand produces five hydrogen bonds with the closest

distance of 2.44 and the furthest distance of 3.01 .

The test ligand for the phenolic acid group in the dates used were p-Coumaric, syringic acid, vanillic acid, ferulic acid, protocatechute and dactylifric acid. P-coumarate ligand forms two hydrogen bonds with the closest distance of 3.02 and the farthest distance of 3.05. Cyrric acid ligand produces eight hydrogen bonds with the closest distance of 2.31 and the farthest distance of 3.06. The vanillic acid ligand produced the most hydrogen bonds, namely nine hydrogen bonds with the closest distance of 2.87 and the furthest distance of 3.3. The ferulic acid ligand formed five hydrogen bonds with the closest distance of 2.49 and the furthest distance of 3.2. The strong protocatechuic ligand produced four hydrogen bonds with the closest distance of 2.72 and the furthest distance of 3.26. The dactylic acid ligand produces three hydrogen bonds with the closest distance of 2.73 and the furthest distance of 2.98. The distance of the closest hydrogen bond that is formed on the results of docking with the receptor is the apigenin ligand of 2.32 . The farthest hydrogen bond distance formed was 3.31 in several ligand, namely apigenin, luteolin, and vanillic acid.

Table 4 The number and distance of hydrogen bonds formed at molecular bonding

Compound	Hydrogen Bond	
	Number of Bond	Bonding Distance (Å)
Allopurinol	1	0.15
Anthocyanins	0	0.00
Apigenin	4	0.11
		0.11
		0.14
		0.15
Luteolin	4	0.12
		0.13
		0.13
		0.15
Quercetin	5	0.11
		0.13
		2.88
		0.13
p-Coumaric acid	2	0.13
		0.13
Syringic Acid	8	0.11
		0.13
		0.13
		0.13
		0.13
		0.13
		0.14
Vanillic Acid	9	0.13
		0.13
		0.15

		0.15
		0.15
		0.13
		0.13
		0.14
		0.14
		0.15
		0.12
		0.15
Ferulic acid	5	0.13
		0.14
		0.14
		0.13
Protocatechuic acid	4	0.14
		0.13
		0.14
		0.13
Dactylyphric Acid	3	0.14
		0.15

3.2. DISCUSSION

3.2.1. Preparation of Ligand and Receptor Structure

The test ligand used in this study were flavonoid compounds (apigenin, quercetin, luteolin, proanthocyanidin, anthocyanins) and date palm phenolic acids (ferulic acid, syringic acid, vanillic acid, Protocatechuic, and p-Coumaric acid and their derivatives, namely Dactylyphric acid). Siahpoosh's research (2016) showed that the content of flavonoids, phenolic acids, and oligoproanthocyanidins in dates can inhibit the action of the xanthine oxidase enzyme. Phenolic acid derivatives consist of two types, namely hydroxycinnamic acid and hydroxybenzoic acid. Both of these derivatives were found in dates (Echegaray et al. 2020) and were used as test ligand to see their ability to inhibit the xanthine oxidase enzyme.

The comparison ligand used is Allopurinol, which is a commercial drug used to inhibit the formation of uric acid. Allopurinol is a derivative of xanthine which is a substrate for the enzyme xanthine oxidase. Before these ligand are used, preparation of the ligand structure must be carried out.

The preparation of the ligand Structure produces a 3D structure and contains polar hydrogen atoms. The addition of hydrogen atoms to the 3D structure is indicated by a white tube. The results of the ligand preparation are stored in pdbqt format. Compare with the results reported by other researchers, are the results or patterns the same.

The receptor used in this research is the xanthine oxidase enzyme. Xanthine oxidase is an enzyme that plays an important role in the formation of uric acid. This enzyme is an enzyme complex consisting of molybdenum, FAD, Fe₂S₂ as a redox reaction center. This enzyme also has two identical subunits facing each other, 1332 amino acid residues with a molecular weight of 290 kDa. The xanthine oxidase enzyme used with code 1FIQ was crystallized by Enroth et al. (2000) and has a resolution of 2.5. Cao et al. (2010) stated that the active sites of xanthine oxidase are Arg880 and Glu802. Water molecules are shown at points around the receptor. The complexed ligand at the receptor are shown in red circles in Figure 1(a). The receptor preparation resulted in

a receptor that was free of the water molecule and the ligand complex 1(b). Compare with the results reported by other researchers, are the results or patterns the same. The results of the receptor preparation are stored in pdbqt format. The pdbqt format on the ligand and receptor shows the presence of a partial charge on each atom (Syahputra et al., 2014).

3.2.2. *Lipinski Solubility Analysis*

The test ligand used in this study were flavonoid compounds (apigenin, quercetin, luteolin, proanthocyanidin, anthocyanins) and date palm phenolic acids (ferulic acid, syringic acid, vanillic acid, protocatechuic, and p-Coumaric acid and their derivatives, namely dactylyphric acid). Siahpoosh's research (2016) showed that the content of flavonoids, phenolic acids, and oligoproanthocyanidins in dates can inhibit the action of the xanthine oxidase enzyme. Phenolic acid derivatives consist of two types, namely hydroxycinnamic acid and hydroxybenzoic acid. Both of these derivatives were found in dates (Echegaray et al., 2020) and were used as test ligand to see their ability to inhibit the xanthine oxidase enzyme.

The comparison ligand used is Allopurinol, which is a commercial drug used to inhibit the formation of uric acid. Allopurinol is a derivative of xanthine which is a substrate for the enzyme xanthine oxidase. Before these ligand are used, preparation of the ligand structure must be carried out.

The preparation of the ligand structure produces a 3D structure and contains polar hydrogen atoms. The addition of hydrogen atoms to the 3D structure is indicated by a white tube. The results of the ligand preparation are stored in pdbqt format.

The receptor used in this research is the xanthine oxidase enzyme. Xanthine oxidase is an enzyme that plays an important role in the formation of uric acid. This enzyme is an enzyme complex consisting of molybdenum, FAD, Fe₂S₂ as a redox reaction center. This enzyme also has two identical subunits facing each other, 1332 amino acid residues with a molecular weight of 290kDa. The xanthine oxidase enzyme used with code 1FIQ was crystallized by Enroth et al. (2000) and has a resolution of 2.5. Cao et al. (2010) stated that the active sites of xanthine oxidase are Arg880 and Glu802. Water molecules are shown at points around the receptor. The complexed ligand at the receptor are shown in red circles in Figure 1(a). The receptor preparation, in a receptor that was free of the water molecule and the ligand complex 1(b). The results of the receptor preparation are stored in pdbqt format. The pdbqt format on the ligand and receptor shows the presence of a partial charge on each atom (Syahputra et al., 2014).

3.2.3. *Stability of Xanthine Oxidase (1FIQ) Enzyme Structure*

The stability of the receptor structure that will be used in the molecular docking process can be determined by two parameters, namely resolution and Ramachandran plot diagram. Macromolecular crystal resolution is considered good if the value is not more than 2.5 (Lu et al. 2009). The crystal structure of xanthine oxidase 1FIQ has a resolution of 2.5 (Enroth et al. 2000), therefore this value indicates that the structure of xanthine oxidase 1FIQ has a good and stable structure and can be used for molecular anchoring.

The plot of the Ramachandran diagram groups the amino acid residues that make up proteins based on the phi and psi angles of the enzyme (Ho and Bresseur 2005). The plot of the Ramachandran diagram shows three regions, namely the favorable region, the allowed region, and the disallowed region to be occupied by amino acid residues. A protein can be stable if the amino acids except glycine occupy the allowable area. The exception is the amino acid glycine because it does not have a side group so that C α can rotate freely (Ramachandran et al., 1963). The plot diagram of the macromolecular xanthine oxidase 1FIQ Ramachandran shows the results in the form of 93.5% amino acid residues in the preferred region, 5.4% of amino acid

residues in the permitted region and 1.1% amino acid residues in the not permitted region compare to previous reports (Figure 2). According to Ho and Brasseur (2005), protein can be said to be stable if the amino acid residue that occupies the area is not allowed to be less than 15%. Xanthine oxidase has good stability because the amino acid residue contained in the restricted area is less than 15%, so it can be used and produces accurate data on the molecular anchoring performed..

3.2.4. *Tethering Method Validation on Vina Autodock Devices*

Molecular docking is a procedure using a computer to predict the noncovalent bond between a ligand and a receptor. Ligand conformation and bond affinity can also be predicted using molecular anchoring methods (Trott and Olson 2010). Molecular tethering will be better and more accurate if the parameters match or approach the actual situation (Ferdian 2016), therefore the validation of the tethering method is carried out. This study validated using the enzyme xanthine oxidase with salicylic acid as a complex inhibitor with the code 1FIQ. Salicylic acid is known to be a competitive inhibitor for the xanthine oxidase enzyme (Enroth et al. 2000)

Validation is carried out with the directional belay method to determine the center of mooring and the volume of mooring accurately. The anchorage used is $x=26,569$, $y=9985$, and $z=113,088$. The anchoring volume is optimized by increasing and decreasing the size so that it covers the ligand and gets a small RMSD value. The resulting mooring volume is $x=14$, $y=14$, and $z=16$. The validation of the molecular anchoring results resulted in an affinity energy value of -7.3 kCal/mol, an RMSD value of l.b. (lower bound) of 1,019 and RMSD u.b. (upper bound) of 2,356. Negative affinity energy indicates that the ligand is attached to the receptor, so that the binding center and volume of the validation results can be used for molecular bonding using the test ligand.

3.2.5. *Gibbs free energy from binding of ligand and receptors*

Molecular docking was carried out using Autodock Vina software. The bonding results in the Gibbs free energy value of a bond. Gibbs free energy can be analyzed the strength of the interaction of the ligand with the receptor. Ligand and receptor interactions can occur if the resulting complex has a negative Gibbs free energy. The more negative the Gibbs free energy value, the more stable the interaction strength, so the better the ability of the ligand to inhibit the receptor. The change in the value of the Gibbs free energy is always directly proportional to the inhibition constant (Murray et al. 2012). The binding strength is indicated by the negative Gibbs free energy, which indicates that the reaction between the ligand and the receptor is spontaneous.

Based on the results of tethering, the value of Gibbs free energy resulting from molecular tethering indicates that the flavonoid and phenolic acid compounds in dates produce a negative Gibbs free value. This shows that the flavonoid and phenolic acid compounds in dates can bind to the xanthine oxidase enzyme spontaneously and strongly. In the proanthocyanidin compound, tethering cannot be carried out because the structure of this compound is larger than the gridbox from the validation results, therefore the proanthocyanidin compound cannot provide results from the tethering process.

The highest Gibbs free energy value produced in this study was anthocyanins compounds from the flavonoid group, namely -7.3 kcal/mol. This value is higher than allopurinol. This indicates that the binding interaction of anthocyanins compounds with xanthine oxidase is more spontaneous than allopurinol and other test ligand. The lowest Gibbs free energy value produced in this study was the syringic acid compound, -4.7 kcal/mol. However, syringic acid

can still act as a xanthine oxidase inhibitor because the value of Gibbs free energy produced is negative.

The Gibbs free energy value is directly related to the inhibition constant (KI), according to the equation $G=RT\ln Ki$. The higher the G value, the lower the KI value and the lower the ability of the ligand to interact with the enzyme. The lowest KI value is the anthocyanins compound of 4.45, compare for previous reports. Based on the value of Gibbs free energy and the value of the inhibition constant, anthocyanins compounds have the best ability to interact spontaneously with xanthine oxidase receptors.

3.2.6. Visualization and Interaction of Ligand Docking with Receptors

Visualization was carried out to see the results of the test ligand attachment and the comparison ligand with the receptor used. Visualization results in the form of amino acid residues that play a role in making contact between the ligand and the receptor. Amino acid residues are important components that play a role in the occurrence of binding results from binding the receptor with a ligand. The amino acid residues produced from bonding allopurinol with xanthine oxidase amounted to 9 residues, namely Arg880, Thr1010, Phe1009, Leu1014, Phe914, Ala1079, Ala1078, Glu802, Leu873 and attached to amino acid residues that play a role in the catalytic activity of xanthine oxidase, namely Arg880 (and Glu802). Cao et al. (2010). These results prove that allopurinol is a competitive inhibitor for the xanthine oxidase enzyme because it is attached to the active site of the enzyme.

The residue that plays a role in binding Allopurinol with xanthine oxidase is used as a benchmark for flavonoid compounds and date phenolic acid as test ligand in inhibiting xanthine oxidase. The amino acid residue of the test ligand that coincides with the comparison ligand indicates that the test ligand can play a role in inhibiting the action of the xanthine oxidase enzyme. The similarity of amino acid residues in the test ligand binding results can be used as a parameter to see the ability of the test ligand to inhibit the action of xanthine oxidase. The slice or similarity of amino acid residues between the test ligand and the comparison ligand is called Binding site similarity (BSS). In addition, the distance and number of hydrogen bonds formed can also be a parameter of the strength of the interaction between the ligand and the receptor.

Dates flavonoid compounds, namely anthocyanins, do not have an OH group, so they do not have a hydrogen donor, so that the tethering results do not form hydrogen bonds. The amino acid residues involved in hydrophobic bonding are Phe1009, Thr1010, Leu1014, Phe914, Ala1079, Ala1078, Glu802, Leu873, Leu648, Val1011, Glu1261, Ser876, Arg880. Anthocyanins are bound to the active site of xanthine oxidase, namely Arg880 and Glu802. Anthocyanins have a residual similarity percentage with allopurinol of 100%. This shows that anthocyanins can act as inhibitors of xanthine oxidase enzyme activity.

The apigenin ligand (C15H10O5) has 3 OH groups (Leopoldini et al. 2004) and has an H ion donor of 4 electrons. The presence of these hydrogen ions plays a role in forming hydrogen bonds with xanthine oxidase receptors (Leopoldini et al. 2004) The result of bonding with xanthine oxidase enzyme produces four hydrogen bonds in Ser876, Thr1010, and Glu1261 residues with the closest hydrogen bond distance of 2.32 in the oxygen molecule Ser876 with O to 5 in apigenin. The amino acid residues that form hydrophobic bonds in the binding of apigenin ligand to the receptor are Arg880, Phe914, Phe1009, Leu1014, Glu802, Ala1079, Leu873, Glu1261, Val1011, Leu648. Apigenin is bound to the active site of xanthine oxidase forming a hydrophobic bond to Arg880 and Glu802. Apigenin has a fairly large percentage of residue similarity with allopurinol, namely 89%. This shows that apigenin can form a strong interaction with xanthine oxidase and inhibit the activity of the receptor.

Luteolin (C15H10O6) has four OH groups. The structure of luteolin is similar to that of

apigenin, the difference between the two is the OH at the B ring position. Luteolin has an H ion donor of five electrons. When bonded with xanthine oxidase, 4 hydrogen bonds are formed on the amino acid residue Thr1010 with the closest distance of 2.50 Å. Other residues that play a role in the binding of luteolin ligand with xanthine oxidase are Arg880, Phe914, Ala1079, Pro1076, Phe1009, Leu1014, Asn768, Thr803, Leu873, Glu802, Leu648, Lys 771, Val1011, Glu1261, Ser876. Luteolin forms hydrophobic bonds with the active site of the enzyme, namely at the amino acid residues Arg880 and Glu802. Luteolin has a similar percentage of amino acid residues with allopurinol of 89%. Based on the tethering data, luteolin ligand can act as a xanthine oxidase inhibitor in forming uric acid.

Quercetin has five OH groups and a hydrogen ion donor of six hydrogen bonds. The bonding of quercetin with xanthine oxidase forms five hydrogen bonds. The amino acid residue that forms hydrogen bonds is the amino acid Thr1010 and the active site of the enzyme is Arg880. Quercetin produces a percentage similarity of amino acid residues with allopurinol of 100%. The amino acid residues that form hydrophobic bonds in the mooring are Phe914, Ala1079, Val1011, Phe1009, Leu1014, Ser876, Leu873, Leu648, Glu802, Glu1261, Ser876, Ala1078, Pro1076, Gly799.

Based on the data, quercetin can form a stronger bond because it is attached to the active site of the Arg880 enzyme with the closest bond distance of 2.44 .

The results of the tethering of phenolic acid group compounds, namely P-coumarate has two OH groups, has two hydrogen donors, and bonding with xanthine oxidase forms two hydrogen bonds with bond distances of 3.02 and 3.05 . The amino acid residues that form hydrogen bonds in the bonding of P-Coumarate with xanthine oxidase are Ser876 and Arg880. Other amino acid residues that form hydrophobic bonds are Phe914, Ala1079, Thr1010, Phe1009, Leu873, Leu1014, Val1011, Glu802. The amino acid residues were similar to allopurinol binding results of 89%. P-Coumarate ligand can bind more stable and stronger because it forms hydrogen bonds with the active site of the enzyme Arg880 with a bond distance of 3.02 .

P-Coumaric acid has two OH bonds, has one hydrogen donor, and forms eight hydrogen bonds with xanthine oxidase. The amino acid residues that form hydrogen bonds in the bonding of P-Coumarate with xanthine oxidase are Thr1010, Ser1008, Arg880 and Glu802. Other amino acid residues that form hydrophobic bonds are Phe914, Ala1079, Val1011, Phe1009, Leu1014, Leu873, Ala1078, Ser1008, Glu1261. The amino acid residues were similar to allopurinol binding results of 100%. P-Coumarate ligand can bind more stable and stronger because it forms hydrogen bonds with both active sites of the enzyme, namely Glu802 with a bond distance 2.42 and Arg880 with a bond distance of 2.80 and 3.31. Based on the tethering data, syringic acid can be an inhibitor in inhibiting xanthine oxidase in forming uric acid.

Vanillic acid has two OH bonds, has one hydrogen donor, and forms nine hydrogen bonds with the closest bond distance of 2.87Å. The amino acid residues that form hydrogen bonds in the bonding of vanillic acid with xanthine oxidase are Arg880, Glu802, Ala1079, Thr1010, Glu1261, and Val1011. Other amino acid residues forming hydrophobic bonds are Phe914, Ala1078, Phe1009. The amino acid residues were similar to allopurinol binding results of 78%. Vanillic acid ligand can bind stably and strongly because they form the most hydrogen bonds and form hydrogen bonds with the active sites of the enzymes Glu802 and Arg880 with bond distances of 2.95 and 3.07.

Ferulic acid has two OH bonds, has one hydrogen donor and forms five hydrogen bonds. The amino acid residues that form hydrogen bonds in the binding of ferulate with xanthine oxidase are Thr1010, Val1011, Glu1261, and Ala1079. Other amino acid residues that form hydrophobic bonds are Ser876, Phe914, Ala1078, Thr803, Phe1009, Leu1014, Leu803, and Glu802. The amino acid residues were similar to allopurinol binding results of 89%. Based on the tethering data, ferulic acid ligand can bind to the xanthine oxidase enzyme and become an

inhibitor of uric acid formation.

Protocatechuic acid have three OH bonds, two hydrogen donors, can form four hydrogen bonds with xanthine oxidase. The amino acid residues that form hydrogen bonds in the binding of protocatechuic acid with xanthine oxidase are Arg880, Glu802, and Thr1010. Other amino acid residues that form hydrophobic bonds are Val1011, Phe914, Ala1079, Phe1009, Leu1014, Leu873, Ser876, Ser1008. The amino acid residues were similar to allopurinol binding results of 89%. Protocatechuic acid ligand can bind more stable and stronger because they form hydrogen bonds with both active sites of the enzyme Arg880 with a bond distance of 2.85Å and Glu802 with a bond distance of 2.72Å.

Dactyliphric acid has 5 OH bonds, six hydrogen donors, and can form three hydrogen bonds with xanthine oxidase with bond distances of 2.73 Å, 2.87 Å, and 2.98 Å. The amino acid residues that form hydrogen bonds in the bonding of dactyliphric acid with xanthine oxidase are Ser876, Glu1261, and Glu802. Other amino acid residues that form hydrophobic bonds are Thr803, Thr1010, Arg880, Phe914, Ala1079, Ala1078, Leu873, Leu1014, Phe1009, Val1011, and Pro1076. The amino acid residues were similar to allopurinol binding results of 100%. The dactylic acid ligand can bind more stable and stronger because it forms hydrogen bonds with the active site of the enzyme Glu802 with a bond distance of 2.98 .

Based on the results of the analysis of these parameters, the best compounds from the flavonoid and phenolic acid groups of dates in inhibiting the activity of the xanthine oxidase enzyme were syringic acid compare with previous reports.

4. CONCLUSION

The best compound from the flavonoid and date palm phenolic acid group which has the best inhibitory power on the xanthine oxidase enzyme based on the analysis of the bonds formed is syringic acid. Based on the number of bonds formed between the xanthine oxidase enzyme and syringic acid, more bonds were formed than the standard ligand (allupurinol) without reducing the number of bonds formed between allupurinol and the xanthine oxidase enzyme.

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